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Founded in 1964 by Prof. Dr. José Fernandes Pontes

IBEPEGE
Instituto Brasileiro de Estudos e Pesquisas de Gastroenterologia e Outras Especialidades

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Publication

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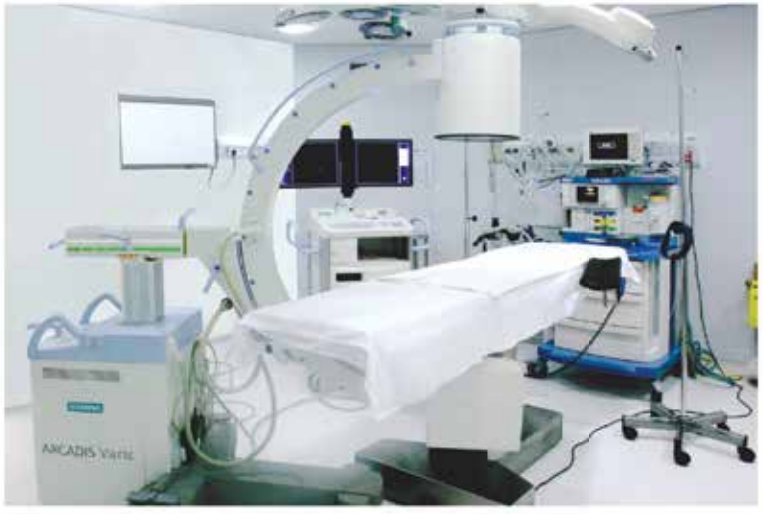
FBG
Federação Brasileira de Gastroenterologia

SOBED
Sociedade Brasileira de Endoscopia Digestiva

SBNPE
Sociedade Brasileira de Nutrição Parenteral e Enteral

SBMDN
Sociedade Brasileira de Motilidade Digestiva e Neurogastroenterologia

SBH
Sociedade Brasileira de Hepatologia



O IGESP, um hospital geral com perfil cirúrgico que é referência no atendimento de alta complexidade, agora também vai ser referência em modernidade.

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UMA ESTRUTURA MODERNA QUE REFLETE A NOSSA VOCAÇÃO: O CUIDADO COM A VIDA.

Uma empresa do grupo

Trasmontano
Saúde

Entyvio*
vedolizumabe

Apresentando **Entyvio*: o primeiro e único tratamento biológico** seletivo aprovado para pacientes com **Doença de Crohn (DC) e Retocolite Ulcerativa (RCU).**[1]

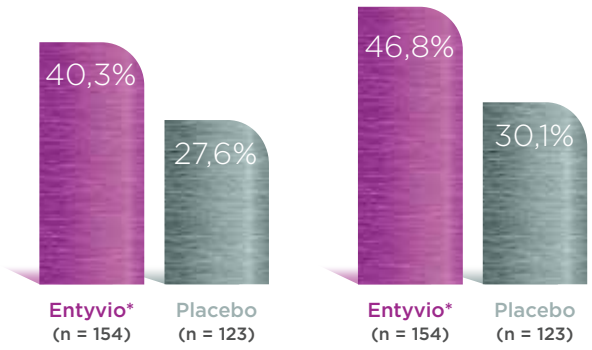
TRATAMENTO COM PRECISÃO[1]

Novo tratamento que fornece remissão duradoura para pacientes com DC e RCU.[1]

ENTYVIO* OFERECE REMISSÃO DURADOURA NA DOENÇA DE CROHN[2,3]

RESPOSTA CLÍNICA NA INDUÇÃO[2]

Subpopulação virgem de tratamento anti-TNFα[2]

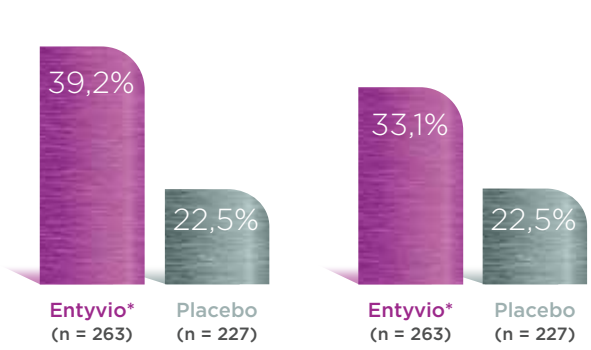


Valor de p frente ao placebo[a]: 0,032 | 0,006

a = os valores de p são unicamente para fins exploratórios.

Adaptado de: Sandborn WJ, *et al.* Digital oral poster session presented at: 9th Congress of ECCO; 2014 Feb 20-22; Copenhagen, Denmark. DOP073.

Subpopulação com falha ao tratamento com anti-TNFα[2]

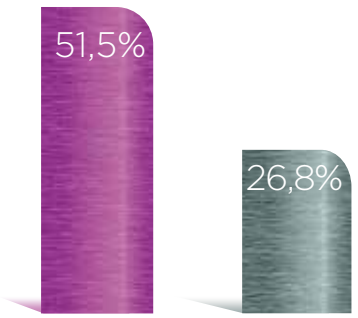


0,0001 | 0,005

Adaptado de: Sandborn WJ, *et al.* Digital oral poster session presented at: 9th Congress of ECCO; 2014 Feb 20-22; Copenhagen, Denmark. DOP073.

REMISSÃO CLÍNICA NA MANUTENÇÃO[3]

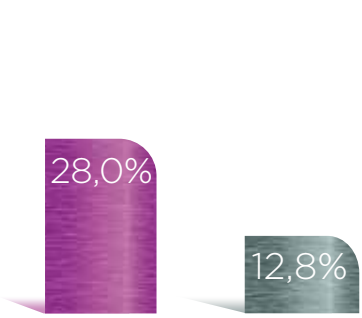
Subpopulação virgem de tratamento com anti-TNFα[3]



IC95% (8,9, 40,6)

Adaptado de: Hanauer S, *et al.* Am J Gastroenterol. 2012;107(Suppl1): A1542.

Subpopulação com falha ao tratamento com anti-TNFα[3]



IC95% (3,0 27,5)

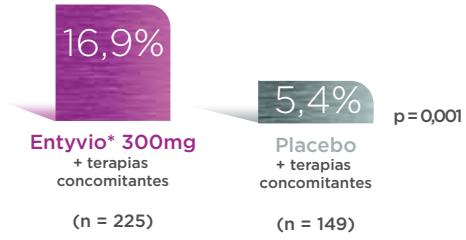
Adaptado de: Hanauer S, *et al.* Am J Gastroenterol. 2012;107(Suppl1): A1542.

ENTYVIO* OFERECE EFICÁCIA DURADOURA NA RCU[4,5]

REMISSÃO E RESPOSTA CLÍNICA NA INDUÇÃO[4]

Remissão Clínica

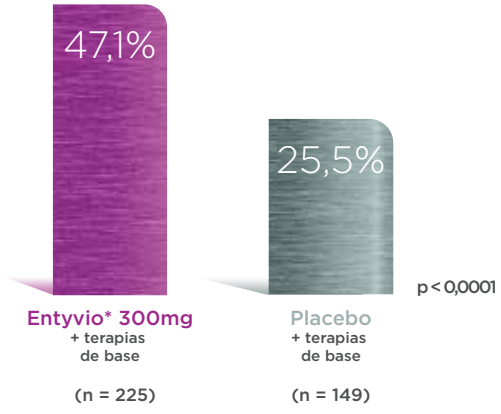
Semana 6[4]



Adaptado de: Feagan BG, *et al.* N Engl J Med. 2013;369(8):699-710.

Resposta Clínica

Semana 6[4]

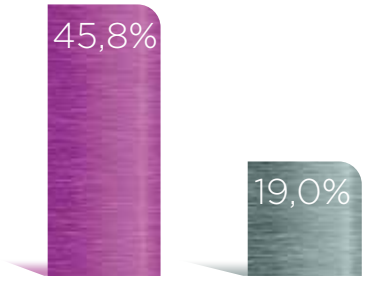


Adaptado de: Feagan BG, *et al.* N Engl J Med. 2013;369(8):699-710.

REMISSÃO CLÍNICA NA MANUTENÇÃO[5]

Subpopulação virgem de tratamento com anti-TNFα[5]

Semana 52

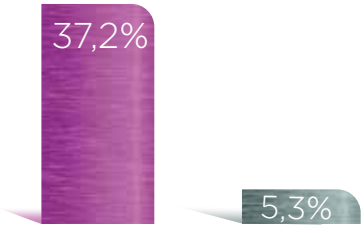


IC95% (12,4, 41,2)

Adaptado de: Feagan B, *et al.* Am J Gastroenterol. 2012;107(S1):S609–S610. Abstract 1522.

Subpopulação que falhou ao tratamento com anti-TNFα[5]

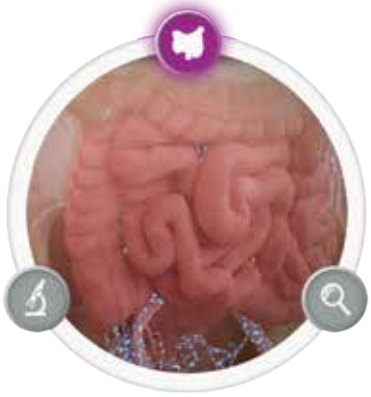
Semana 52



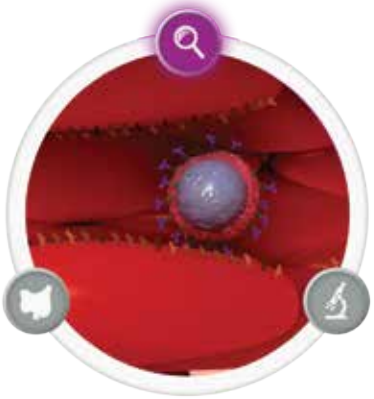
IC95% (15,8, 48,0)

Adaptado de: Feagan B, *et al.* Am J Gastroenterol. 2012;107(S1):S609–S610. Abstract 1522.

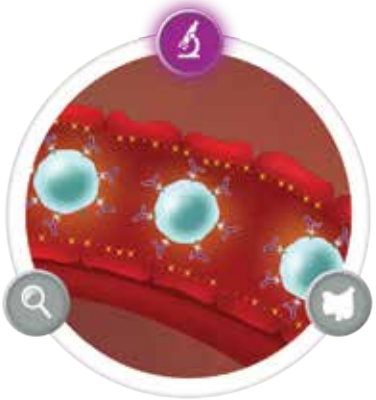
ENTYVIO* ATUA DE MANEIRA SELETIVA NO LOCAL DA INFLAMAÇÃO INTESTINAL[1,6]



Biológico seletivo; diferente dos tratamentos atuais.[1*]



Entyvio* liga-se especificamente à integrina $\alpha 4\beta 7$ encontrada nas células T que migram preferencialmente para o trato gastrointestinal inibindo a adesão à MAdCAM-1.[1,6*]



Restrição do tráfego linfocitário e redução da inflamação intestinal.[1,6*]

PERFIL DE SEGURANÇA[6,7]

- **Taxas relatadas de infecções graves e infecções similares ao placebo[6]**
- Taxas de descontinuação devido a eventos adversos similares em todos os braços de tratamento[6]
- Baixa incidência de reações relacionadas à infusão[6]
- Baixa taxa de imunogenicidade[6]
- Dados não sugerem risco aumentado para malignidade[6]
- 2830 pacientes avaliados em estudo de segurança[7]

*Imagens desenvolvidas pela Takeda.

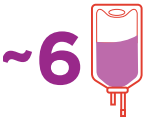
Entyvio* vedolizumabe

Entyvio*
vedolizumabe

DOSE PADRÃO PARA TODOS OS PACIENTES:
INFUSÃO INTRAVENOSA 300mg[6]

Ajustes baseados no peso não são necessários.[6]

30 minutos de administração. Infusão de frasco único nas semanas 0, 2 e 6 e depois a cada 8 semanas.[6]



Infusões por ano após a indução.[6]

- Pacientes recebem uma dose de 300mg em intervalos regulares de 8 semanas.[6]
- Para pacientes que apresentarem diminuição na resposta, a dose pode ser ajustada para intervalos de 4 semanas.[6]

Referências bibliográficas: 1) Poole RM. Vedolizumab: first global approval. Drugs. 2014;74(11):1293-303. **2)** Sandborn WJ, *et al.* Efficacy of induction treatment with vedolizumab for patients with Crohn's disease who have experienced tumour necrosis factor antagonist failure or are tumour necrosis factor antagonist naive. Digital oral poster session presented at: 9th Congress of ECCO; 2014 Feb 20-22; Copenhagen, Denmark. DOP073. **3)** Hanauer S, *et al.* Vedolizumab Maintenance Therapy for Crohn's Disease: results of GEMINI II, a randomized, placebo-controlled, double-blind, multi-centre phase 3 trial. Am J Gastroenterol. 2012;107 (Suppl 1):A1542. **4)** Feagan BG, *et al.* Vedolizumab as induction and maintenance therapy for ulcerative colitis. N Engl J Med. 2013;369(8):699-710. **5)** Feagan B, *et al.* Vedolizumab Maintenance Therapy for Ulcerative Colitis: Results of GEMINI I, a Randomized, Placebo-Controlled, Double-Blind, Multicenter Phase 3 Trial. Am JGastroenterol. 2012;107(S1):S609–S610. Abstract 1522. **6)** Entyvio* [Bula]. São Paulo: Takeda Pharma Ltda. **7)** Colombel JF, *et al.* The safety of vedolizumab for ulcerative colitis and Crohn's disease. Gut. 2016. pii: gutjnl-2015-311079. doi: 10.1136/gutjnl-2015-311079. [Epub ahead of print].

Entyvio* - vedolizumabe. USO INTRAVENOSO USO ADULTO. Indicações: Entyvio* é indicado para o tratamento de pacientes adultos com: -Colite ulcerativa moderada a grave na fase ativa que apresentaram uma resposta inadequada, perda de resposta ou são intolerantes ao tratamento convencional ou a um antagonista de fator de necrose tumoral alfa (TNF-α). - Doença de Crohn moderada a grave na fase ativa que apresentaram uma resposta inadequada, perda de resposta ou são intolerantes ao tratamento convencional ou a um antagonista de fator de necrose tumoral alfa (TNF-α). **Contraindicações:** Entyvio* é contraindicado para pacientes com hipersensibilidade ao vedolizumabe ou a qualquer um dos excipientes do produto. Entyvio* é contraindicado na presença de infecções ativas graves, tais como tuberculose, septicemia, citomegalovírus, listerioses e infecções oportunistas, como leucoencefalopatia multifocal progressiva (LMP). **Cuidados e advertências:** Em estudos clínicos foram relatadas reações relacionadas à infusão e reações de hipersensibilidade, sendo a maioria delas de gravidade leve a moderada. Infecções: O tratamento com Entyvio* não deve ser iniciado em pacientes com infecções ativas graves até que as infecções sejam controladas, e os médicos devem considerar a suspensão do tratamento em pacientes que desenvolvem uma infecção grave durante o tratamento crônico com Entyvio*. Entyvio* é contraindicado em pacientes com tuberculose ativa. Alguns antagonistas de integrina e alguns agentes imunossupressores sistêmicos foram associados com leucoencefalopatia multifocal progressiva (LMP). Nenhum caso de LMP foi relatado em estudos clínicos com vedolizumabe. Uso anterior e concomitante de produtos biológicos: Não há dados disponíveis de estudos clínicos do vedolizumabe para pacientes previamente tratados com natalizumabe ou rituximabe. Uso durante a gravidez e a lactação - Categoria B de Risco na Gravidez - Este medicamento não deve ser utilizado por mulheres grávidas sem orientação médica ou do cirurgião-dentista. As mulheres em idade fértil devem usar métodos contraceptivos adequados para evitar a gravidez e o seu uso deve ser mantido durante pelo menos 18 semanas após o último tratamento com Entyvio*. Lactação: Não se sabe se o vedolizumabe é excretado no leite humano ou absorvido sistemicamente após a ingestão. **Interações medicamentosas:** Não foram conduzidos estudos de interação. O vedolizumabe foi estudado em pacientes adultos com colite ulcerativa e doença de Crohn com administração concomitante de corticosteroides, imunomoduladores (azatioprina, 6-mercaptopurina e metotrexato) e aminosalicilatos. As análises da farmacocinética da população sugerem que a administração concomitante de tais agentes não teve efeito clinicamente significativo na farmacocinética do vedolizumabe. O efeito do vedolizumabe na farmacocinética dos medicamentos comumente coadministrados não foi estudado. Vacinações: As vacinas vivas, em particular vacinas vivas orais, devem ser usadas com cautela durante o tratamento com Entyvio*. **Reações adversas:** A proporção de pacientes que descontinuaram o tratamento devido a eventos adversos foi de 9% para os pacientes tratados com vedolizumabe e 10% para os pacientes tratados com placebo. Nos estudos combinados do GEMINI I e II, as reações adversas que ocorreram em ≥ 5% dos pacientes foram náusea, nasofaringite, infecção do trato respiratório superior, artralgia, febre, fadiga, cefaleia, tosse. Reações relacionadas à infusão foram relatadas em 4% dos pacientes que estavam recebendo vedolizumabe. **Atenção: este produto é um medicamento novo e, embora as pesquisas tenham indicado eficácia e segurança aceitáveis, mesmo que indicado e utilizado corretamente, podem ocorrer eventos adversos imprevisíveis ou desconhecidos. Nesse caso, notifique os eventos adversos pelo Sistema de Notificações em Vigilância Sanitária - NOTIVISA, disponível em www.anvisa.gov.br/hotsite/notivisa/index.htm ou para a Vigilância Sanitária Estadual ou Municipal. Posologia:** - Colite ulcerativa A dose recomendada é 300 mg de Entyvio*, administrada por infusão intravenosa nas Semanas 0, 2 e 6 e depois a cada oito semanas. Em pacientes que responderem ao tratamento com Entyvio*, o uso de corticosteroides pode ser reduzido e/ou interrompido – à critério médico. - Doença de Crohn A dose recomendada é 300 mg de Entyvio*, administrada por infusão intravenosa nas Semanas 0, 2 e 6 e depois a cada oito semanas. Os pacientes com doença de Crohn que não apresentarem resposta podem se beneficiar de uma dose de Entyvio* na Semana 10 (veja ADVERTÊNCIAS E PRECAUÇÕES). Nos pacientes que responderem, continuar o tratamento a cada oito semanas a partir da Semana 14. MS – 1.0639.0271 **SE PERSISTIREM OS SINTOMAS, O MÉDICO DEVERÁ SER CONSULTADO. MEDICAMENTO SOB PRESCRIÇÃO MÉDICA.** *Marca depositada por Takeda Pharma Ltda. INC. ENT_1014_0715_VPS.

Contraindicação: Hipersensibilidade a qualquer dos componentes do medicamento.
Interação medicamentosa: Não foram conduzidos estudos de interação.

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Gastro-Imuno

Takeda

Tecta* 40mg

pantoprazol magnésico di-hidratado

Maior alívio dos sintomas da DRGE, após 8 semanas, quando comparado ao esomeprazol.[1]

24 horas[1-4]

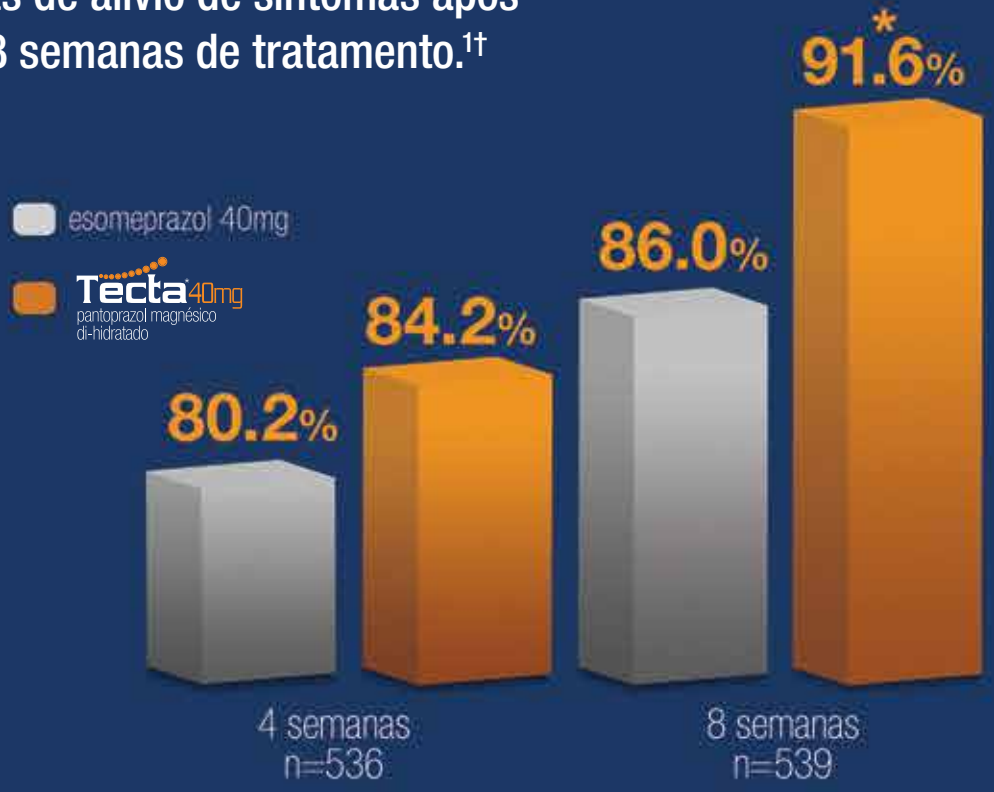
Potência[2]

POSOLOGIA NA DRGE:[3]

1x ao dia, 40mg

O alívio dos sintomas com Tecta* 40mg foi significantemente maior quando comparado ao esomeprazol após 8 semanas.[1]

Taxas de alívio de sintomas após 4 e 8 semanas de tratamento.[1†]



†ReQuest GI < 1,73 *p<0,05

Adaptado de: Moraes-Filho JP, *et al.* Aliment Pharmacol Ther. 2014;39(1):47-56.

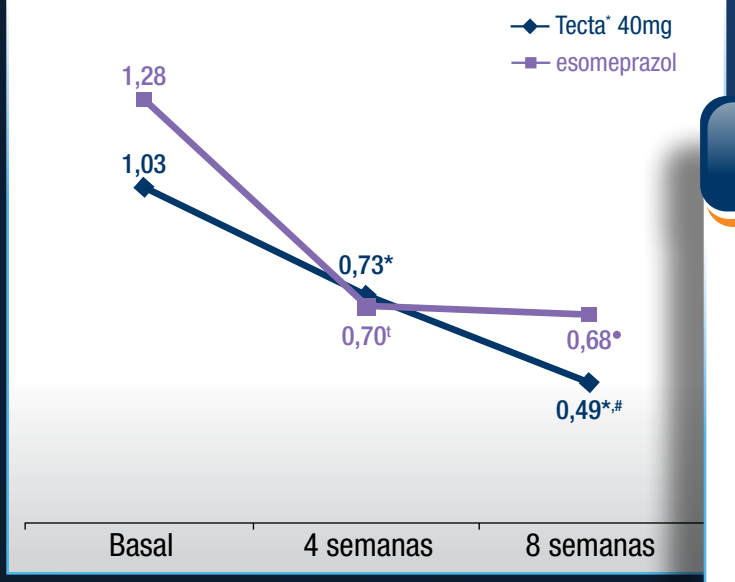
O alívio dos sintomas está diretamente associado à satisfação do paciente.[1]

94,8% dos pacientes tratados com Tecta* 40mg declararam estar muito satisfeitos com o tratamento.[1]

Os DISTÚRBIOS DE SONO reduziram significantemente a frequência e intensidade de 4 para 8 semanas apenas no tratamento com Tecta* 40mg.[1]

SINTOMAS NOTURNOS.

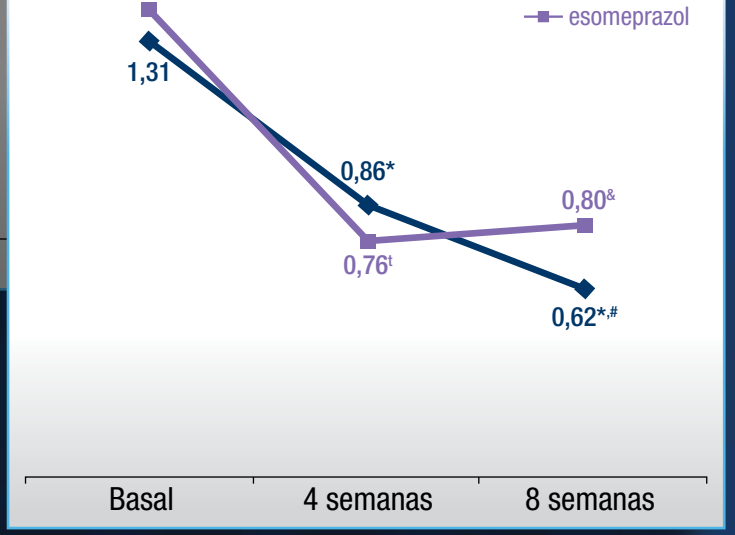
FREQUÊNCIA



População ITTe
n=563
4 sem. x basal
*p=0,0030 tp<0,0001
4 sem. x 8 sem.
#p=0,0001 • p< 0,0001

Adaptado de: Moraes-Filho JP, *et al.* Aliment Pharmacol Ther. 2014;39(1):47-56.

INTENSIDADE



População ITTe
n=563
4 sem. x basal
*p=0,0452 tp= 0,0162
4 sem. x 8 sem.
#p<0,0001 &p= 0,0130

Adaptado de: Moraes-Filho JP, *et al.* Aliment Pharmacol Ther. 2014;39(1):47-56.

Tecta* 40mg

pantoprazol magnésico di-hidratado

POSOLOGIA NA DRGE:[3]

1x ao dia, 40mg

Maior alívio dos sintomas da DRGE, após 8 semanas, quando comparado ao esomeprazol.[1]

Contraindicação: hipersensibilidade conhecida ao pantoprazol, benzimidazóis substituídos ou aos demais componentes da fórmula. **Interação medicamentosa:** o uso concomitante com alta dose de metotrexato pode elevar e prolongar os níveis séricos de metotrexato e/ou seus metabólitos, levando possivelmente à toxicidade do metotrexato.

TECTA* 40 mg - pantoprazol magnésio di-hidratado. USO ORAL. USO ADULTO. ACIMA DE 18 ANOS. Apresentação: Comprimidos gastrorresistentes de 40 mg. **Indicações:** TECTA* 40 mg é indicado para o tratamento das esofagites de refluxo moderadas ou graves e dos sintomas de refluxo gastroesofágico. Também é indicado para tratamento intermitente de sintomas de acordo com a necessidade (on demand). **Contraindicações:** TECTA* não deve ser usado em casos de hipersensibilidade conhecida ao pantoprazol, benzimidazóis substituídos ou aos demais componentes da fórmula. **Este medicamento é contraindicado na faixa etária de 0 a 18 anos. Categoria B de risco na gravidez: Este medicamento não deve ser utilizado por mulheres grávidas sem orientação médica ou do cirurgião-dentista. Advertências e Precauções:** Na presença de qualquer sintoma de alarme (como significativa perda de peso não intencional, vômitos recorrentes, disfagia, hematêmese, anemia ou melena) e quando houver suspeita ou presença de úlcera gástrica, deve-se excluir a possibilidade de malignidade, já que o tratamento com pantoprazol pode aliviar os sintomas e retardar o diagnóstico. Em terapia de longo prazo, especialmente quando o tratamento exceder 1 ano, os pacientes devem ser mantidos sob acompanhamento regular. Infecções Gastrointestinais: O tratamento com IBP pode estar associado a um risco aumentado de infecção por *Clostridium difficile, Salmonella e Campylobacter.* Fratura óssea: O tratamento prolongado com inibidores da bomba de próton (IBP) pode estar associado a um aumento do risco de osteoporose relacionada a fraturas no quadril, pulso ou coluna. Hipomagnesemia: Hipomagnesemia tem sido raramente relatada em pacientes tratados com IBP por pelo menos três meses (na maioria dos casos, após um ano de terapia). Gravidez e lactação: Categoria B de risco na gravidez. Pacientes idosos: Não é necessário o ajuste de dose. Insuficiência renal: Não é necessário o ajuste de dose em pacientes com insuficiência renal. Insuficiência hepática: a dose diária de 20 mg de pantoprazol não deve ser excedida em pacientes com insuficiência hepática grave. Inibidores da Protease do HIV: A coadministração de pantoprazol não é recomendada com inibidores da protease do HIV para os quais a absorção é dependente do pH do ácido intragástrico tais como o atazanavir, nelfinavir. Metotrexato: O uso concomitante com alta dose de metotrexato pode elevar e prolongar os níveis séricos de metotrexato e/ou seus metabólitos. Influência na absorção de vitamina B12: Em pacientes com a Síndrome de Zollinger-Ellison e outras patologias hipersecretórias que necessitam de tratamento a longo prazo, o pantoprazol, assim como todos os medicamentos IBPs, pode reduzir a absorção de vitamina B12 (cianocobalamina) devido a hipo ou acloridria. **Interações medicamentosas:** Não se esperam diferenças nas interações medicamentosas entre o pantoprazol magnésico e o pantoprazol sódico. Como os demais membros de sua classe, TECTA* pode alterar a absorção de medicamentos cuja biodisponibilidade dependa do pH do suco gástrico, como cetoconazol e itraconazol. Outros estudos de interações: Pantoprazol é extensamente metabolizado no fígado via enzimas do citocromo P450 (CYP2C19 e CYP3A4). Os estudos de interação com fármacos que também são metabolizados com estas vias, como a carbamazepina, diazepam, glibenclamida, nifedipino, fenitoína e um contraceptivo oral contendo levonorgestrel e etinilestradiol, não se observaram interações clínicas significativas. Os resultados de uma série de estudos de interação demonstraram que o pantoprazol não afeta o metabolismo de substâncias ativas metabolizados por CYP1A2 (tais como cafeína, teofilina), CYP2C9 (tais como piroxicam, diclofenaco, naproxeno), CYP2D6 (tais como metoprolol), CYP2E1 (como o etanol), e não interfere com a glicoproteína-P relacionada à absorção de digoxina. Não houve interações com administração concomitante de antiácidos. Estudos de interação também foram realizados administrando pantoprazol concomitantemente com os respectivos antibióticos (claritromicina, metronidazol, amoxicilina) e nenhuma interação clinicamente relevante foi encontrada. Clopidogrel: A administração concomitante do pantoprazol e clopidogrel em indivíduos saudáveis não teve efeito clinicamente importante na exposição ao metabólito ativo do clopidogrel ou inibição plaquetária induzida pelo clopidogrel. Não é necessário qualquer ajuste da dose de clopidogrel quando administrado com uma dose aprovada de pantoprazol. Anticoagulantes cumarinicos (femprocumona ou varfarina): não afeta a farmacocinética da varfarina, femprocumona ou o INR (tempo de protrombina do paciente/média normal do tempo de protrombina). O consumo de alimentos não interfere nas ações de TECTA* no organismo. **Reações adversas:** O pantoprazol (a substância ativa) é muito bem tolerado, de modo que a maioria dos eventos adversos observados tem sido leve e transitória. Reações incomuns: Distúrbios do sono, cefaleia, diarreia, náusea/vômito, inchaço e distensão abdominal, dor e desconforto abdominal, boca seca, constipação, aumento nos níveis de enzimas hepáticas, tontura, prurido, exantema, erupções cutâneas e erupções, astenia, fadiga e mal estar. Reações raras: agranulocitose, hipersensibilidade (incluindo reações e choque anafiláctico), hiperlipidemias, alterações de peso, depressão , distúrbios de paladar, distúrbios visuais (visão turva), aumento nos níveis de bilirrubina, urticária, angioedema, artralgia, mialgia, ginecomastia, elevação da temperatura corporal, edema periférico. Reações muito raras: leucopenia, trombocitopenia, pancitopenia, desorientação. Atenção: este produto é um medicamento novo e, embora as pesquisas tenham indicado eficácia e segurança aceitáveis, mesmo quando indicado e utilizado corretamente, podem ocorrer eventos adversos imprevisíveis ou desconhecidos. 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Nos casos com esofagite não cicatrizada ou com sintomas persistentes recomenda-se um período adicional de quatro semanas. Os sintomas recorrentes poderão ser controlados administrando-se um comprimido de TECTA* 40 mg ao dia, quando necessário *("on demand")*, de acordo com a intensidade dos sintomas. Considerar a mudança para terapia contínua nos casos em que os sintomas não puderem ser devidamente controlados por terapia *"on demand"*. Em casos isolados de esofagite de refluxo, a dose diária pode ser aumentada para dois comprimidos ao dia, particularmente nos casos de pacientes refratários a outros medicamentos antiulcerosos. MS – 1.0639.0256. AO PERSISTIREM OS SINTOMAS, O MÉDICO DEVERÁ SER CONSULTADO. MEDICAMENTO SOB PRESCRIÇÃO. TC40_0814_0315_VPS.

Referências Bibliográficas: 1) Moraes-Filho JP, *et al.* Randomised clinical trial: daily pantoprazole magnesium 40 mg *vs.* esomeprazole 40 mg for gastro-oesophageal reflux disease, assessed by endoscopy and symptoms. Aliment Pharmacol Ther. 2014;39(1):47-56. **2)** Hein J. Comparison of the efficacy and safety of pantoprazole magnesium and pantoprazole sodium in the treatment of gastro-oesophageal reflux disease: a randomized, double-blind, controlled, multicentre trial. Clin Drug Investig. 2011;31(9):655-64. **3)** Tecta* [Bula]. São Paulo: Takeda Pharma Ltda. **4)** Morales-Arambula M, *et al.* Nighttime GERD: prevalence, symptom intensity and treatment response to a 4 week treatment with 40 mg of pantoprazole magnesium O.D. A report from the GERD Mexican Working Group. Gastroenterology. 2009;136(5 supl 1):A428.

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Manipulation of the intestinal microbiota: the medicine revolution of the 21st century

Quilici FA. Manipulation of the intestinal microbiota: the medicine revolution of the 21st century. Arq Gastroenterol. 2017;54(2):83-4.

Bacteria usually co-exists with humans for millennium, and can be found in our bodies in many places, most of them in the gastrointestinal tract. They constitute the human microbiome formed by a great variety and diversity of microorganisms. It is estimated that 70% of these microorganisms are concentrated in the intestine, particularly in the colon, forming the intestinal microbiota, with up to 10^{11} bacteria per milliliter. It shelties about 3 million microbial genes, corresponding to 150 times the human genome. The set formed by microbial cells and genes added to human cells and genes creates the concept of "superorganism".

Before birth, there are no bacteria present in the digestive tract, but in childbirth, their colonization happens quickly. The intestinal microbiota begins to settle from 2 to 3 years of age and, from there, remains relatively stable. However, this stability can be influenced by diet, diseases, use of medications (mainly antibiotics) and aging. This microbiota is composed of commensal bacteria (native to the host) or temporary bacteria (passing through the body). Both can be beneficial, potentially harmful or pathogenic to humans. The beneficial bacteria interact positively with the human immune system, provoking a competitive inhibition with the pathogens bacteria, keeping the intestinal microbiota in a balance called **eubiosis**. The genre of bacteria with the greatest health benefit are *Bifidobacterium* and *Lactobacillus*.

Recent researches suggests that the normal intestinal microbiota is not simply a collection of microorganisms but reflects an interrelationship between different genre of bacteria that possibly work together to benefit the host. It is now believed that the existence of a large diversity of microorganisms in the gastrointestinal tract is important and beneficial for the host. A healthy microbiota plays an important role in digestion and absorption of nutrients, production of vitamins, maintenance of the structural and functional integrity of the intestine, protection against pathogens and modulation of the inflammatory response through the interaction of the microbiota and the immune system (gut associated lymphoid tissue – GALT).

The balance of the intestinal microbiota is a dynamic process, constantly exposed to factors that alter the quantity and diversity of its bacteria, which may be positive or negative.

These studies[1,5-8] have also shown that particularities of the modern lifestyle associated with intrinsic factors of the host, such as unbalanced feeding, excessive consumption of alcohol, smoking, stress, frequent use of laxatives, gastric protectors, antibiotics, gestation, old age, frequent constipation or diarrhea, contribute to negative changes in the microbiota. This favors the increase of the pathogenic bacteria in relation to the beneficial ones, installing an imbalance called **dysbiosis.**

Current studies[2-4] have shown the presence of dysbiosis in patients with certain diseases such as inflammatory bowel disease (IBD), irritable bowel syndrome (IBS), gastrointestinal infections, antibiotic-associated diarrhea (AAD), pseudomembranous colitis, celiac disease, colorectal cancer, type I and II diabetes, obesity, atopy and asthma, rheumatoid arthritis, and some neurological diseases. However, it is not yet known whether this change in the intestinal microbiota causes all or part of the disease, or whether the change is a consequence of the disease itself.

However, one of the main goals of these researches is to determine how to intervene or even to positively manipulate the composition of the intestinal microbiota favoring a greater quantity and diversity of beneficial bacteria, seeking the cure of various diseases. This positive intervention is possible with the practice of healthy living habits associated with adequate diet, consumption of prebiotics, probiotics and even the transplantation of feces microbiota.

At the beginning of the 21st century, researches seem to indicate that there will be another revolution in medicine, the possibility of manipulating the intestinal microbiota, opening new horizons for us humans in the ancient, primitive and cruel war between health and disease.

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Quilici FA. O manejo da microbiota intestinal: a revolução médica do século XXI. Arq Gastroenterol. 2017;54(2):83-4.

REFERENCES

1. Ganesh BP, Versalovic J. Luminal Conversion and Immunoregulation by Probiotics. Front Pharmacol. 2015;6:269.
2. Martinello F, Roman CF, Souza PA. Effects of probiotic intake on intestinal bifidobacteria of celiac patients. Arq Gastroenterol. 2017;54:85-90.
3. Martins CP, Chaves CHA, Castro MGB, Gomes IC, Passos MCF. Prevalence of small intestine bacterial overgrowth in patients with gastrointestinal symptoms. Arq Gastroenterol. 2017;54:91-5.
4. McClave SA, Taylor BE, Martindale RG, Warren MM, Johnson DR, Braunschweig C, et al. Guidelines for the Provision and Assessment of Nutrition Support Therapy in the Adult Critically Ill Patient: Society of Critical Care Medicine (SCCM) and American Society for Parenteral and Enteral Nutrition (A.S.P.E.N.). JPEN J Parenter Enteral Nutr. 2016;40:159-211.
5. Montalban-Arques A, De Schryver P, Bossier P, Gorkiewicz G, Mulero V, Gatlin DM 3rd, Galindo-Villegas J. Selective manipulation of the gut microbiota improves immune status in vertebrates. Front Immunol. 2015;6:512.
6. Moraes AC, Silva IT, Almeida-Pititto Bd, Ferreira SR. [Intestinal microbiota and cardiometabolic risk: mechanisms and diet modulation]. [Article in Portuguese]. Arq Bras Endocrinol Metabol. 2014;58:317-27.
7. Patel R, DuPont HL. New Approaches for Bacteriotherapy. Clin Infect Dis. 2015;60 (Suppl 2):S108-21.
8. Schippa S, Conte MP. Dysbiotic Events in Gut Microbiota: Impact on Human Health. Nutrients. 2014;6:5786-805.

ORIGINAL ARTICLE

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Effects of probiotic intake on intestinal bifidobacteria of celiac patients

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ABSTRACT – Background – Healthy individuals exhibit a significantly higher concentration of faecal bifidobacteria in comparison to celiac patients. Even though there are potential benefits in probiotic usage, they have been little explored as an adjunctive therapy in celiac disease. **Objective –** This study aimed at the comparison of faecal bifidobacteria concentration and pH among celiac patients and healthy subjects before and after the daily intake of 100 g of yogurt containing probiotic for a thirty-day period. **Methods –** Feces from 17 healthy subjects and 14 celiac patients were analyzed, in which stool culture was performed for the isolation and quantification of faecal bifidobacteria. Furthermore, Gram's method was employed for the microscopic analysis of the colonies, while the identification of the *Bifidobacterium* genus was made through determination of the fructose-6-phosphate phosphoketolase enzyme. Faecal pH was measured using a calibrated pHmeter. **Results –** Faecal bifidobacteria concentration before probiotic consumption was significantly higher in healthy individuals ($2.3x10^8 \pm 6.3x10^7$ CFU/g) when compared to celiac patients ($1.0x10^7 \pm 1.7x10^7$ CFU/g). Faecal pH values did not show a significant difference. After the daily consumption of probiotic-containing yogurt both groups showed a significant increase in the concentration of faecal bifidobacteria, but healthy subjects presented significantly higher bifidobacteria concentrations ($14.7x10^8 \pm 0.2x10^8$ CFU/g) than the celiac group ($0.76x10^8 \pm 0.1x10^8$ CFU/g). The obtained pH values from both groups were not significantly different, being 7.28±0.518 for the celiac patients and 7.07±0.570 for healthy individuals after the probiotic intake. **Conclusion –** The probiotic supplementation significantly increased the number of bifidobacteria in the feces of celiac patients, although it was not sufficient to reach the concentration found in healthy individuals prior to its consumption.

HEADINGS – Probiotics. Celiac disease. Bifidobacterium. Hydrogen-ion concentration. Feces, microbiology. Microbial colony count.

INTRODUCTION

Patients with celiac disease (CD) have an intolerance to the polipeptide fragments of gluten, mediated by T lymphocytes. Gluten is a water-insoluble substance found in wheat flour, rye, barley and oats[30]. CD depends on genetic, immunological and environmental factors and it is characterized by total or partial atrophy of the intestinal villi and consequent poor absorption of nutrients[6,9,27]. Its prevalence in Brazil is shown to be 1/214[3].

CD diagnosis must be based on clinical, histopathological (gold standard) and serological examinations[3,27]. There are few studies on the intestinal microbiota role in CD, even though gliadin (a gluten peptide) and microorganisms similarly activate pro-inflammatory routes[12]. The information about the intestinal microbiota of celiac patients is mainly obtained from a stool sample examination[15].

Healthy subjects present a significantly higher concentration of bifidobacteria when compared to celiac patients, while faecal pH seems to remain the same in both situations[8].

The only effective and possible treatment for CD is dietary, throughout the exclusion of gluten from the diet, which allows the remission of symptoms and the restoration of the regular mucosa[1]. Without treatment, CD has a high morbi-mortality rate, with risks of developing complications such as anemia, infertility, osteoporosis and cancer, being the most prevalent the intestinal lymphoma[27]. Alternative treatments are also available and can be used simultaneously as palliatives, for instance, the use of probiotics, mainly *Lactobacillus* and *Bifidobacterium*[7,15,24].

The presence of bifidobacteria in the gastrointestinal tract seems to suffer variations throughout life and it is associated with beneficial effects to health, including the re-composition of the intestinal microbiota, the growth inhibition of pathogenic bacteria, regeneration of the epithelial barrier and anti-inflammatory effects[11,21,25,26,32]. Some species have the capacity of inhibiting the increased permeability induced by gliadin, weakening its cytotoxic effect and the host autoimmune response[10]. Smecuol et al. showed that celiac patients on a gluten diet had experienced beneficial effects related to gastrointestinal tract symptoms (such as constipation and gastroesophageal reflux) when consuming bifidobacteria in capsules before meals[28]. Other beneficial effects of bifidobacteria consumption with a gluten diet have been described, such as the reduction of human α-defensin 5 (HD-5) and paneth cells[20].

Bifidobacterium and *Lactobacillus* are widely used in several food products, such as yogurt, milk, cheese and dietetic supplements, upon which research has been increasing. Even though there are potential benefits in their usage, probiotics have been

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poorly explored as an adjunctive therapy in CD[17,32]. In this context, the hypothesis is that the intestinal microbiota of patients with controlled CD can be restored by the daily intake of probiotic-containing yogurt. The results of this study will allow the analysis of the necessity and efficacy of supplementation with probiotics to restore the intestinal microbiota equilibrium, with consequent reduction of gastrointestinal complications and infections, improving the quality of life of celiac patients.

METHODS

Study design

The Ethics Committee for Studies with Humans of the Federal University of Santa Catarina, Brazil, approved the experimental protocol for this study (number 772, 2010). The participants with CD were recruited in the local Association of Celiac People in Brazil (Associação dos Celíacos do Brasil – ACELBRA) during its monthly meetings. All celiac patients were on a controlled stage of the disease during the study, *i.e*., they were on a gluten free diet, without signals and symptoms of CD. The non-celiac participants were randomly recruited from the population.

Volunteers were submitted to a clinical and sociodemographic questionnaire and the research started by collecting the first stool sample to quantify bifidobacteria and measure faecal pH. Afterwards, each volunteer consumed one unit of probiotic-containing yogurt (100g) from Piá Essence, PIÁ®, Nova Petrópolis-RS) per day, having eaten in the fasting state at morning, during one month. The yogurt delivery was made weekly. After 30 days of consumption, feces were collected again in order to quantify bifidobacteria and measure faecal pH.

Exclusion criteria

The following exclusion criteria for the participation in the study were adopted: individuals with suspicion or diagnosis of autoimmune diseases; suspicion or diagnosis of diabetes; lactose intolerance; allergy to any excipient present in the yogurt; individuals who consumed products containing prebiotics and/or probiotics three months prior to the beginning of research, and individuals who presented fever, diarrhea and/or vomit three months prior to the beginning or during study.

Determination of faecal bifidobacteria content and pH

For the isolation and quantification of bifidobacteria and measurement of faecal pH, participants collected stool samples, which were sent to the laboratory and analyzed within 8 h after collection[13,29].

Feces aliquot (1 g) from each volunteer was diluted in 9 mL of distilled and deionized sterile water for the measurement of faecal pH in pHmeter PHTEK®. Another feces aliquot (1 g) from each stool sample was diluted in 9 mL of phosphate buffer. The mixture was homogenized five times using the anaerobic technique. From this dilution (10^{-1}), serial fold dilutions up to 10^{-7} were prepared. The stock phosphate buffer was previously prepared with 34 g of KH_2PO_4 in 500 mL of distilled and deionized water, having the pH adjusted to 7.2 with NaOH 1 N and the volume completed to 1 L with distilled water, being subsequently sterilized in an autoclave at 121°C during 18 minutes. For the dilution of the stool sample, the phosphate buffer was diluted a thousand times from the stock solution.

The culture media used for isolation of bifidobacteria was the RCA (Reinforced Clostridial Agar, DifcoTM BD) supplemented with antibiotics (nalidixic acid 2%, polymyxin B sulfate 0.85%, kanamycin sulfate 0.5%, iodoacetic acid 0.5%, 2,3,5-triphenyltetrazolium chloride 0.5% and amphotericin B 0.001%)[14].

The spread-plating of 100 µL from each dilution was prepared and the plates were incubated at 37°C for 72 hours under anaerobic conditions[14] using a commercial anaerobic atmosphere generation system (Anaerobac from Probac®), followed by counting of bifidobacteria colonies in the plates containing between 30 and 300 colony-forming units (CFU). For the confirmation of the *Bifidobacterium* genus, Gram staining was made, as well as catalase proof and fructose-6-phosphate phosphoketolase (F6PPK) reaction, as stated by Orban & Patterson (2000), for all isolated colony types[18].

The results from the bifidobacteria quantification were presented as CFU per gram of feces (CFU/g). To obtain the results, the number of CFU counted in each plate was multiplied by its respective dilution factor and corrected for the sample volume spread. They are expressed as mean ± standard deviation (n=17 for the control group and n=14 for the celiac group).

Determination of yogurt bifidobacteria content and pH

All lots of the yogurt Piá Essence donated were analyzed for the isolation and quantification of bifidobacteria and measurement of pH. One pot containing 100 g of yogurt was randomly selected from each lot and 1 g was diluted in 9 mL of distilled and deionized sterile water for measurement of pH in pHmeter PHTEK® previously calibrated.

Another yogurt aliquot (1 g) was also diluted in 9 mL of phosphate buffer. Serial dilutions were made from this solution as for the feces analysis. The spread-plating from each dilution, the counting of colonies, the confirmation of the genus and the expression of the results were made as previously described for the stool samples.

Statistical analysis

The statistical analysis was performed using the program GraphPad Prism® version 5.0 from 2007. For data distribution analysis, the D'Agostino normality test and Pearson Omnibus Normality Test were employed. Spearman's correlation coefficient was employed to verify the correlation among the bifidobacteria concentration, faecal pH and volunteers' ages. Wilcoxon test was used for the comparison of the results between groups. A significance level of 5% (P<0.05) was adopted for all tests.

RESULTS

The yogurt package informs that each 100 g of yogurt contains 10^8 CFU of *Lactobacillus acidophilus* and *Bifidobacterium lactis*. Amongst the yogurt lots available to the volunteers, the average concentration of bifidobacteria was $6.67x10^8 \pm 10.3x10^8$ CFU/g of yogurt. The average yogurt pH was 4.28±0.15 and there was a significant correlation between the bifidobacteria concentration and yogurt pH (P=0.0121). There was growth of Gram-positive bacillus colonies in every lot of yogurt, being all catalase negative and showing F6PPK activity.

Amongst the 17 healthy control individuals, 10 were female and seven were male, aged between 18 and 58 years (average of 26 years old). This group of individuals did not have any relatives with CD.

From the group of 14 celiac patients, 10 were female and four were male, with ages ranging from 18 to 60 years, being the average being 38 years old. The prevalence of CD in their families was

higher in first (father, mother and siblings) and second (grandparents, aunts, uncles and cousins) degree relatives. Two (14.4%) volunteers had first-degree celiac relatives, three (21.4%) had first and second-degree celiac relatives, one (7.1%) could not answer and eight (57.1%) did not have celiac relatives. The average age in which the diagnosis was made was 36 years old, where 100% of the patients had the small intestine biopsy done for confirmation of CD. A relation between faecal bifidobacteria concentration and age was not observed in any of the groups, either celiac or healthy subjects.

Seven (50%) of the 14 celiac patients received drug therapy after CD diagnosis, being calcium therapy the most prevalent in 57% of them, mainly related to women 30 years old or older.

During the stool sample examination from the volunteers, bifidobacteria colonies were observed, presenting round shape, smooth surface, pink to wine color and small to medium size. The colony morphology was similar between both groups, celiac and control (Figure 1). Bifidobacteria appeared in the form of short and long Gram-positive bacilli, with or without bifurcated ends V or Y-shaped, and as Gram-positive coccobacilli (Figure 2).

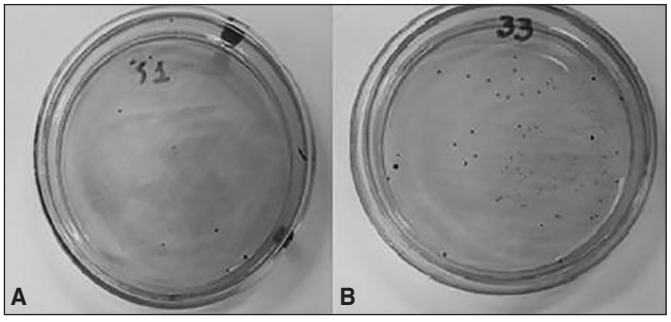

FIGURE 1. Bifidobacteria colonies in Reinforced Clostridial agar media supplemented with antibiotics. (A) Celiac patient plate. (B) Control subject plate.

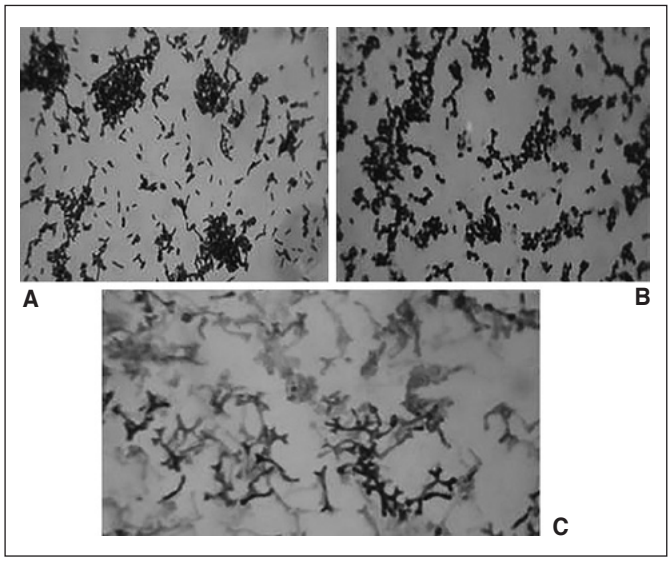

FIGURE 2. Micromorphology of bifidobacteria colonies stained by Gram method in optical microscopy in 1000 times increase. (A) Short Gram-positive bacilli, isolated, in pairs or grouped. (B) Gram-positive cocobacilli, isolated, in pairs or grouped. (C) Long Gram-positive bacilli with bifurcated ends V or Y-shaped.

The results of bifidobacteria quantification in the stool samples are shown in Figure 3. Healthy individuals presented a significantly higher concentration of bifidobacteria ($2.3x10^8 \pm 6.3x10^7$ CFU/g) before the probiotic-containing yogurt intake when compared to the celiac group ($1.0x10^7 \pm 1.7x10^7$ CFU/g) (Figure 3). Celiac patients presented, in average, 83% less bifidobacteria than healthy individuals. Still, celiac faecal pH (7.19±0.521) was not significantly different from the faecal pH of the control group (7.18±0.522).

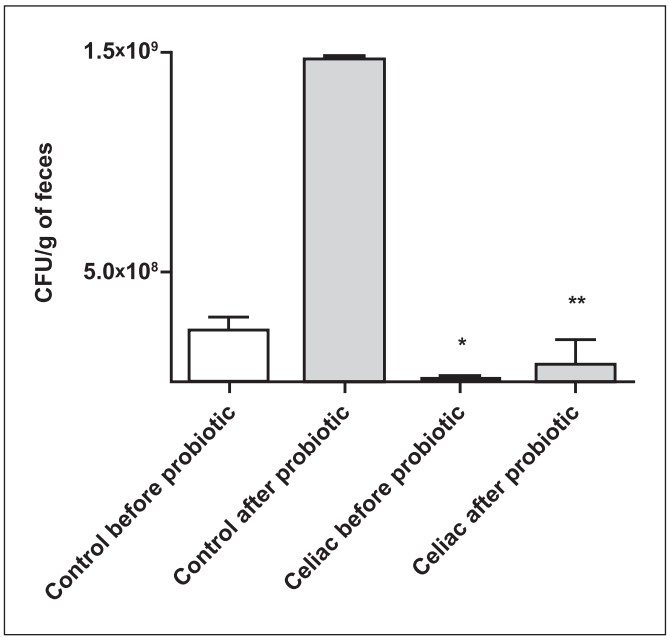

FIGURE 3. Number of colony forming units (CFU) of bifidobacteria per gram of feces from control and celiac groups, before and after probiotic intake. Results are expressed as mean ± standard deviation (control group n=17 and celiac group n=14). * $P<0.05$ nonparametric *t* test, when compared to the respective control group before probiotic intake or ** when compared to the respective control group after probiotic intake.

After the daily intake of 100 g of probiotic-containing yogurt for 30 days, healthy individuals presented a significantly higher bifidobacteria concentration ($14.7x10^8 \pm 0.2x10^8$ CFU/g) than celiac patients ($0.76x10^8 \pm 0.1x10^8$ CFU/g) (Figure 3). However, faecal pH of celiac patients (7.28±0.518) did not show significant difference from the faecal pH of healthy individuals (7.07±0.570) after the yogurt intake.

DISCUSSION

Several probiotic supplements can be found on the market; meanwhile it is still hard to find gluten free products for celiac patients. In this context, the product options for this research were limited. Amongst the companies for which support was requested, only PIÁ®, Nova Petrópolis-RS, provided the products. The average bifidobacteria concentration provided for the research participants ($6.67x10^8 \pm 10.3x10^8$ CFU/g of yogurt) is enough to bring benefits to their health, according to Vinderola & Reinheimer[31].

A number of factors can affect probiotic bacteria viability in yogurts. High carbohydrate concentrations added to the product before its fermentation can inhibit the bacteria, leading to long periods of fermentation and an underdevelopment of acidity[16].

Oliveira & Damin[16] found that the number of probiotic bacteria remained stable for at least seven days of storage. However, in this study volunteers consumed the probiotics up to their expiration date, which simulates the acquisition of products commercialized for the general population. A yogurt of a lot provided for the volunteers was randomly tested six days after its expiration date, in which a bifidobacteria concentration of $1.74x10^6$ CFU/g of yogurt was found. Coupled with the likely concentration of *Lactobacillus*, this would still be a probiotic food and bring benefits to people's health[19], including celiac patients.

The largest number of female celiac patients in this study is consistent with literature, which shows a higher prevalence of CD in women[3]. About 30% of celiac patients evaluated in this study have a relative with CD, which is similar to a study made with patients from Association of Celiac People in Brazil, section from Santa Catarina, (ACELBRA-SC) in 2004, revealing that 27% of associates had relatives with CD[3]. This data reinforces the idea that genetic determinants of CD are associated with environmental factors[15]. It is important to note that 100% of the celiac patients who participated in the research had the intestinal biopsy done for their diagnosis, which is recommended by the literature[30].

The poor intestinal absorption of most nutrients resulting from the inflammatory response on CD can explain why most celiac patients reported having osteoporosis and osteopenia[3,30]. It also explains why most participants of this research have replenished calcium and vitamin D after CD diagnosis. The supplementation with probiotic-containing yogurt could bring not only the benefits from the probiotics for celiac patients but also a greater amount of calcium absorbed from their diet.

The mechanisms of action of probiotics have not been completely elucidated, even though many have been suggested and possibly operate individually or associated[32]. There is evidence that probiotics have antimicrobial action, compete for limited nutritional resources from the intestinal microbiota, block adhesion of pathogens in the intestinal mucosa and have antitoxin effects of pathogens[22]. Bifidobacteria can also benefit people's health by lowering intestinal pH through the production of short chain fatty acids (acetate and lactate), thus inhibiting pathogenic bacteria growth. This is a digestive system self-mechanism for population control and selectivity of bacterial colonization[13]. Indeed, a significant correlation between faecal pH and bifidobacteria concentration was not seen in this study.

Macro and micromorphology of bifidobacteria colonies found in the stool samples were similar in both groups and were as described in the literature. However, the results show a significant lower quantity of bifidobacteria CFU per gram of feces of celiac patients than in the control group. Some studies show that allergic children and patients with atopic diseases are frequently colonized by a reduced number of bifidobacteria when compared to healthy children, showing a close relationship between bifidobacteria concentration and host immune disorders[5].

Nadal et al.[15] reported an imbalance in the intestinal biota of celiac children, especially the reduction of faecal *Bifidobacterium* spp. concentration. Similarly, Collado et al.[4] have reported that celiac children with active or inactive disease had inferior bifidobacteria counting than control groups for both analyzed samples, either feces or intestinal biopsy specimens. Therefore, this imbalance seems to be independent on the activity of the disease. This explains the lower bifidobacteria concentration found in feces of adult celiac patients in this study, all in a controlled phase of CD.

The results found in this study for bifidobacteria concentration without probiotic consumption show a significantly higher bifidobacteria count in healthy subjects when compared to celiac patients, which is consistent with literature[8]. Even after probiotic consumption, the faecal bifidobacteria count in celiac patients from this study has not reached the counting in healthy individuals without probiotic consumption (Figure 3).

The values of faecal pH for both groups before probiotic intake had no significant difference, having them remained very similar even after probiotic intake. These results suggest that the higher faecal bifidobacteria concentration after probiotic consumption did not increase intestinal fermentation, which would lower the pH and ease bifidobacteria growth[13]. However, it is worth noting that the pH from the control group was slightly more acidic than the pH from the celiac patients. The increase in bifidobacteria count favors the lowering of faecal pH due to the fermentation done by these bacteria[13]. The results of pH values from both groups, celiac and control, suggest that the smaller amount of bifidobacteria in the intestine of celiac patients is probably not related to faecal pH, but to the pathogenesis of CD. Thus, the relationship between bifidobacteria counting and CD has yet to be elucidated.

The maintenance of pH values before and after probiotic ingestion may be related to time or quantity/concentration of the daily-consumed probiotic, being suggested that probiotic effects are dose-dependent[19]. However, the recommended dose by the literature was consumed in this study, which is between 10^6 e 10^{11} CFU/day, depending on the desired effect[22].

In order to have the metabolism and intestinal content reflected in feces, variables must be taken into account, including intestinal motility, total fiber ingestion, intestinal secretion, and duration of dietetic intervention. Because of that, faecal pH may not exactly reflect colon pH. In fact, Bouhnik et. al.[2] have not considered the faecal pH as a good indicator of intestinal acidification, since it has not changed after ingestion of nondigestible carbohydrates by 200 healthy volunteers, despite the increase in the number of faecal bifidobacteria.

Although the healthy intestinal microbiota remains to be defined, there are many diseases related to its imbalance. In most cases, there is no information yet if microbiota imbalance has a triggering role or if it is a disease consequence. Anyway, both relationships lead to the hypothesis that an intervention to restore the microbiota to the healthiest state could mitigate the disease. The consumption of properly selected probiotics could be used with such role[23].

There is indication, amongst research to elucidate activity of bifidobacteria, that intestinal microbiota change can influence the typical inflammatory reactions in CD in a specie-specific way[4]. Therefore, it is thought that bifidobacteria has a great therapeutic potential, and manipulation of intestinal biota, as with probiotic supplementation, might improve quality of life of celiac patients.

However, it should be noted that the inclusion of a small number of participants, the evaluation of pH and bifidobacteria contents during a short period of time and the availability of molecular methods, more accurate to evaluate the intestinal microbiota, may be considered limitations of the present study. Therefore, we suggest that additional studies should be performed in order to evaluate all the aspects regarding intestinal microbiota and probiotic supplementation in CD.

It is still not clear why celiac patients who are in a controlled phase of the disease – i.e., on a gluten free diet, with restored intestinal villi and with no symptoms –, present less bifidobacteria.

CONCLUSION

The results obtained in the present study allow the conclusion that there is a lower bifidobacteria count in the intestinal microbiota of celiac patients, even when they are on a gluten free diet and consuming probiotic-containing food, when compared to the control group. This disturbance is independent on the faecal pH.

Supplementation with probiotics increased the number of faecal bifidobacteria, which reflects its intestinal concentration. Further research must be performed in order to evaluate the equilibrium of other bacteria (for instance, the pathogenics); to verify how long bifidobacteria count remains elevated after probiotic consumption; to correlate small intestine biopsy results with bifidobacteria concentration, since celiacs were on a gluten free diet; and evaluate if the microbiota imbalance was due to gluten contamination in food.

In summary, this information will help develop specific dietetic recommendations to celiac patients based on their microbiota composition.

Authors' contributions

Martinello F: survey execution. Roman CF: writing and translation of text. Souza PA: statistical analysis and writing of text.

Martinello F, Roman CF, Souza PA. Efeitos do consumo de probióticos sobre as bifidobactérias intestinais de pacientes celíacos. Arq Gastroenterol. 2017;54(2):85-90.

RESUMO – Contexto – Indivíduos saudáveis apresentam uma concentração de bifidobactérias fecais significativamente maior em comparação a pacientes celíacos. Apesar de haver benefícios potenciais no uso de probióticos na doença celíaca, estes têm sido pouco explorados como uma terapia adjuvante. **Objetivo –** Este estudo objetivou a comparação do pH e concentração fecal de bifidobactérias entre pacientes celíacos e indivíduos saudáveis antes e após o consumo diário de 100 g de iogurte contendo probiótico por um período de 30 dias. **Métodos –** Foram analisadas fezes de 17 pessoas saudáveis e 14 pacientes celíacos, tendo sido realizada a coprocultura para o isolamento e quantificação de bifidobactérias fecais. Além disso, o método de Gram foi empregado na análise microscópica das colônias, enquanto a identificação do gênero *Bifidobacterium* foi feita através da determinação da enzima frutose-6-fosfato fosfocetolase. O pH fecal foi medido usando um pHmetro calibrado. **Resultados –** A concentração de bifidobactérias fecais antes do consumo do iogurte probiótico foi significativamente maior em indivíduos saudáveis ($2.3x10^8 \pm 6.3x10^7$ UFC/g) quando comparada aos celíacos ($1.0x10^7 \pm 1.7x10^7$ CFU/g). Por outro lado, o pH fecal de ambos os grupos não apresentou diferença significativa. Após o consumo diário de iogurte contendo probiótico, ambos os grupos tiveram um aumento significativo na concentração de bifidobactérias fecais, entretanto indivíduos saudáveis apresentaram concentrações de bifidobactérias significativamente maiores ($14.7x10^8 \pm 0.2x10^8$ UFC/g) do que o grupo celíaco ($0.76x10^8 \pm 0.1x10^8$ UFC/g). Os valores de pH obtidos de ambos os grupos não foram significativamente diferentes, sendo de 7.28±0.518 para os pacientes celíacos e de 7.07±0.570 para os indivíduos saudáveis após o consumo do probiótico. **Conclusão –** A suplementação com probiótico aumentou significativamente o número de bifidobactérias nas fezes dos pacientes celíacos apesar de não ter sido suficiente para alcançar a concentração encontrada em indivíduos saudáveis antes do consumo de probióticos.

DESCRITORES – Probióticos. Doença celíaca. Bifidobacterium. Concentração de íons de Hidrogênio. Fezes, microbiologia. Contagem de colônia microbiana.

REFERENCES

1. Accomando S, Cataldo F. The global village of celiac disease. Dig Liver Dis. 2004; 36:492–8.
2. Bouhnik Y, Raskine L, Simoneau G, Paineau D, Bornet F. The capacity of short-chain fructo-oligosaccharides to stimulate faecal bifidobacteria: a dose-response relationship study in healthy humans. Nutr J. 2006;5:8.
3. Cassol CA, Pellegrin CP, Wahys MLC, Pires MMS, Nassar SM. [Clinical profile of Santa Catarina members of Brazilian Celiac Association]. Arq Gastroenterol. 2007;44:257-65.
4. Collado MC, Calabuig M, Sanz Y. Differences between the fecal microbiota of coeliac infants and healthy controls. Curr Issues Intest Microbiol. 2007;8:9–14.
5. Collado MC, Donat E, Ribes-Koninckx C, Calabuig M, Sanz Y. Imbalances in faecal and duodenal *Bifidobacterium* species composition in active and non-active coeliac disease. BMC Microbiol. 2008; 8:232.
6. Di Cagno R, Rizzello CG, Gagliardi F, Ricciuti P, Ndagijimana M, Francavilla R, et al. Different fecal microbiotas and volatile organic compounds in treated and untreated children with celiac disease. Appl Environ Microbiol. 2009;75:3963-71.
7. Fontana L, Bermudez-Brito M, Plaza-Diaz J, Munoz-Quezada S, Gil A. Sources, isolation, characterisation and evaluation of probiotics. Br J Nutr. 2013; 109 Suppl 2:S35-50.
8. Golfetto L, Senna FD, Hermes J, Beserra BTS, França FS, Martinello F. Lower bifidobacteria counts in adult patients with celiac disease on a gluten-free diet. Arq Gastroenterol. 2014;51:139-43.
9. Ivarsson A, Hernell O, Nyström L, Persson LA. Children born in the summer have increased risk for coeliac disease. J Epidemiol Community Health. 2003;57:36–9.
10. Laparra JM, Sanz Y. Bifidobacteria inhibit the inflammatory response induced by gliadins in intestinal epithelial cells via modifications of toxic peptide generation during digestion. J Cell Biochem. 2010; 109:801-7.
11. Leahy SC, Higgins DG, Fitzgerald GF, Sinderen DV. Getting better with bifidobacteria. J Appl Microbiol. 2005;98:1303-15.
12. Medina M, Palma GD, Ribes-Koninckx C, Calabuig M, Sanz Y. *Bifidobacterium* strains suppress in vitro the pro-inflammatory milieu triggered by the large intestinal microbiota of coeliac patients. J inflamm. 2008;5:1-13.
13. Mohan R, Koebnick C, Schildt J, Mueller M, Radke M, Blaut M. Effects of *Bifidobacterium lactis* Bb12 Supplementation on Body Weight, Fecal pH, Acetate, Lactate, Calprotectin, and IgA in Preterm Infants. Pediatr Res. 2008;64:418-22.
14. Muñoa FJ, Pares R. Selective medium for isolation and enumeration of *Bifidobacterium* spp. Appl Environ Microbiol. 1988;54:1715-8.
15. Nadal I, Donant E, Ribes-Koninckx C, Calabuig M, Sanz Y. Imbalance in the composition of the duodenal microbiota of children with celiac disease. J Med Microbiol. 2007;56:1669–74.
16. Oliveira MN, Damin MR. [Effect of total solids and sucrose contents on acidity, firmness and viability of yogurt and probiotic bacteria in fermented milk]. Ciênc Tecnol Aliment. 2003;S23:172-6. Available from http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0101-20612003000400032.
17. OMG. Organização Mundial de Gastroenterologia. Guias Práticas da OMGE: Probióticos e Prebióticos. 2008. Available from: http://www.serdigital.com.br/gerenciador/clientes/biologicus/arquivos/25.pdf.
18. Orban JI, Patterson JA. Modification of the phosphoketolase assay for rapid identification of bifidobactérias. J Microbiol Methods. 2000;40:221-4.
19. Pérez N, Iannicelli JC, Girard-Bosch C, González S, Varea A, Disalvo L. Effect of probiotic supplementation on immunoglobulins, isoagglutinins and antibody response in children of low socio-economic status. Eur J Nutr. 2010; 49:173–9.

20. Pinto-Sánchez MI, Smecuol EC, Temprano MP, Sugai E, González A, Moreno ML et al. Bifidobacterium infantis NLS super strain reduces the expression of [alpha]-defensin-5, a marker of innate immunity, in the mucosa of active celiac disease patients. J Clin Gastroenterol. 2016. [Epub ahead of print].
21. Pokusaeva K, Fitzgerald GF, Sinderen DV. Carbohydrate metabolism in Bifidobacteria. Genes Nutr. 2011;6:285-306.
22. Saad SMI. Probióticos e prebióticos: o estado da arte. Rev Bras Cienc Saude. 2006;42:1-16. Available from: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S1516-93322006000100002&lng=en.
23. Sanders M E. Impact of probiotics on colonizing microbiota of the gut. J Clin Gastroenterol. 2011;45:S115-9.
24. Sanz, Y. Novel perspectives in celiac disease therapy. Mini Rev Med Chem. 2009;9:359–67.
25. Satokari RM, Vaughan EE, Smidt H, Saarela M, Matto J, de Vos WM. Molecular approaches for the detection and identification of bifidobacteria and lactobacilli in the human gastrointestinal tract. Syst Appl Microbiol. 2003;26:572–84.
26. Shanahan F. Probiotics in perspective. Gastroenterology. 2010;139:1808-12.
27. Silva TSG, Furlanetto TW. Diagnóstico de doença celíaca em adultos. Rev Assoc Med Bras. 2010;56:122-6. Available from: http://www.scielo.br/scielo.php?script=sci_arttext&pid=S0104-42302010000100027&lng=en.
28. Smecuol E, Hwang HJ, Sugai E, Corso L, Chernavsky AC, Bellavite FP et al. Exploratory, randomized, double-blind, placebo-controlled study on the effects of Bifidobacterium infantis natren life start strain super strain in active celiac disease. J Clin Gastroenterol. 2013;47:139-47.
29. Thitaram SN, Siragusa GR, Hinton AJr. Bifidobacterium-selective isolation and enumeration from chicken caeca by a modified oligosaccharide antibiotic-selective agar medium. Lett Appl Microbiol. 2005;41:355-60.
30. Van Heel DA, West J. Recent advances in coeliac disease. Gut. 2006;55:1037-46.
31. Vinderola CG, Reinheimer JA. Enumeration of *Lactobacillus casei* in the presence of *Lactobacillus acidophilus* and lactic starter in fermented dairy products. Int Dairy J. 2000;10:271-5.
32. WHO. World Health Organization Food and Agriculture Organization of the United Nations: Joint FAO/WHO expert consultation on evaluation of health and nutritional properties of probiotics in food including powder milk and live lactic acid bacteria. 2001. Available from: ftp://ftp.fao.org/docrep/fao/009/a0512e/a0512e00.pdf.

ORIGINAL ARTICLE

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Prevalence of small intestine bacterial overgrowth in patients with gastrointestinal symptoms

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ABSTRACT – Background – Small intestine bacterial overgrowth is a heterogeneous syndrome characterized by an increase in the number and/or the presence of atypical microbiota in the small intestine. The symptoms of small intestine bacterial overgrowth are unspecific, encompassing abdominal pain/distension, diarrhea and flatulence. Due to the increased cost and complexity for carrying out the jejunal aspirate, the gold standard for diagnosis of the syndrome, routinely the hydrogen (H_2) breath test has been used, utilizing glucose or lactulose as substrate, which is able to determine, in the exhaled air, the H_2 concentration produced from the intestinal bacterial metabolism. However, due to a number of individuals presenting a methanogenic microbiota, which does not produce H_2, the testing on devices capable of detecting, concurrently, the concentration of exhaled H_2 and methane (CH_4) is justified. **Objective** – This study aimed to determine the prevalence of small intestine bacterial overgrowth in patients with digestive symptoms, through a comparative analysis of breath tests of H_2 or H_2 and CH_4 associated, using glucose as substrate. **Methods** – A total of 200 patients of both sexes without age limitation were evaluated, being directed to a Breath Test Laboratory for performing the H_2 test (100 patients) and of exhaled H_2 and CH_4 (100 patients) due to gastrointestinal complaints, most of them patients with gastrointestinal functional disorders. **Results** – The results indicated a significant prevalence of small intestine bacterial overgrowth in the H_2 test and in the test of exhaled H_2 and CH_4 (56% and 64% respectively) in patients with gastrointestinal symptoms, and higher prevalence in females. It found further that methane gas was alone responsible for positivity in 18% of patients. **Conclusion** – The data found in this study is consistent with the findings of the current literature and underscores the need for using devices capable of capturing the two gases (exhaled H_2 and CH_4) to improve the sensitivity and hence the accuracy of small intestine bacterial overgrowth diagnosis in daily medical practice.

HEADINGS – Bacterial growth. Small intestine. Breath tests. Hydrogen. Methane.

INTRODUCTION

Small intestine bacterial overgrowth (SIBO) is a heterogeneous syndrome, which can be characterized by an increase in the number and/or presence of an atypical microbiota in the small intestine[5,15]. It is known that although the gold standard for the diagnosis of overgrowth is still the presence of 1 x 10^5 to 10^6 colony forming units/mL (cfu/mL) in the jejunal aspirate, this definition has been challenged in the present scenario[2,5]. Khoshini and collaborators suggest that healthy individuals rarely have higher bacterial counts than 1 x 10^3 cfu/mL, which should be the new threshold for definition of the syndrome[8].

The real prevalence of this syndrome is not well known due to difficulties in defining and thus the very detection of SIBO[2]. However, studies suggest that SIBO is a very common clinical condition, especially in patients with impaired gastrointestinal motility or anatomical abnormalities in the digestive tract[5]. Thus, a high prevalence of overgrowth was observed in patients with functional gastrointestinal disorders, inflammatory bowel disease, liver diseases, pancreatic diseases, celiac disease, diabetes mellitus and neuromuscular diseases[3,12]. Furthermore, SIBO has been associated with certain clinical conditions including rosacea, hepatic encephalopathy, obesity, gastroparesis, Parkinson's disease, fibromyalgia, among many others[14,19,21]. It is believed that qualitative and quantitative alterations of the intestinal microbiota (dysbiosis) occur in all of these clinical conditions associated with chronic symptoms determining SIBO[5,14]. Thus, the bacterial overgrowth is almost always a secondary condition, and, if the underlying problem is not addressed and treated properly (which is not always possible), the chance of SIBO recurrence is very high, even after antibiotic therapy[16]. In such cases, the need for repeated cycles of antibiotics is frequent to improve the intestinal symptomatology[9].

The examination considered the gold standard for SIBO diagnosis is the jejunal aspirate culture, which is not routinely performed because it requires a complex technique and is costly[15]. In clinical practice, the most widely used method is the Hydrogen (H_2) breath test which has good sensitivity and specificity for the diagnosis[5,15,21]. This indirect and non-invasive test that uses glucose or lactulose as

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a substrate, is able to determine in the exhaled breath, the concentration of H_2 produced from the intestinal bacterial metabolism. However, approximately 8 to 27% of individuals do not produce H_2, due to the presence of methanogenic microbiota, which results in a significant percentage of false negative results[1,7,18]. For this reason, there has been a recommendation to perform the test in modern equipment capable of detecting, concomitantly, the concentration of exhaled H_2 and methane (CH_4), making it more sensitive and more acurate[5,15,21]. Most studies point out that the diagnostic accuracy of the breath test using glucose is higher compared to that using lactulose[20].

Breath tests capable of identifying exhaled gases begin with acquiring the jejunal sample (baseline) for the measurement of H_2 and/or CH_4 in the exhaled air[6]. After determining the baseline value, which is generally less than 10 parts per million (ppm), the patient ingests the carbohydrate that will be tested on the recommended dose (20 grams of lactulose in 250 ml of water and 25 to 50 g glucose in 250 mg of water)[21]. The test will be considered normal if a significant increase in H_2 and/or CH_4 concentration does not occur in the expired air (less than 12 ppm increase over the baseline value). It is important to observe and analyze possible symptoms that patients may present during the test[5,21].

SIBO treatment is empirical, recommending the use of broad spectrum antibiotics for 10 to 14 days (quinolone, metronidazole, Amoxycillin, tetracycline). The antibiotic of choice considered is rifaximin, which has low systemic absorption and minimal collateral effects[13,16,17]. This drug has been shown to be more effective and safer in many studies, but is not yet available in Brazil[12]. Thus, it is recognized that, currently, the emerging need to develop a systematic and consensual approach to the diagnosis and treatment of SIBO[16,19].

This study aimed to determine the prevalence of SIBO in patients with digestive symptoms, through a comparative analysis of breath tests using H_2 or H_2 and CH_4. It is intended to infer whether, in fact, the tests on devices able to detect both gases in exhaled air (H_2 and CH_4) are cost-effective and necessary for the diagnosis of SIBO.

METHODS

A retrospective observational study was made, that evaluated the data and the results of breath tests using H_2 or H_2 and CH_4 performed in a Breath Test Laboratory in Belo Horizonte (Cemad). This is the only service that has the breath test device able to detect exhaled H_2 and CH_4 in this city (GastroCH4ECK™). The project was approved by the Ethics Committee of the Medical Sciences Faculty Research in the resolution number 46290015.9.0000.5134.

The patients who underwent these tests and from which will be made the interpretation of the results, were sent to the laboratory due to presenting gastrointestinal symptoms and clinical suspicion of SIBO. Spreadsheets were constructed detailing the results of breath tests using only H_2 and the H_2 and CH_4. The results confirmed or disproved the SIBO diagnostic through a quantitative assessment of the exhaled gases (value described in ppm, i.e. parts per million) and also through the evaluation of the presence or absence of symptoms reported by patients during the course of the respective tests. Such symptoms, properly described in the analyzed results of the tests were stratified as mild (only one reported symptom), moderate (two symptoms reported) and severe (three or more symptoms reported).

We evaluated the results of the breath tests from patients of both sexes without age limitation, referred to conduct such tests because of gastrointestinal complaints such as abdominal pain and/or discomfort, flatulence, bloating, diarrhea, intestinal constipation, among others. A convenience sample which included all the results of the exhaled H_2 and CH_4 test held since the implementation of this breath test at the clinic for a year and a half (100 patients) was used. Similarly, the same number of patient test results that only performed the exhaled H_2 test during that same period, selected consecutively and randomly (100 patients) was also evaluated. For greater uniformity in the interpretation of the results, we included only the results of patients who underwent the test using glucose as a substrate, excluding the results of those who took the test with lactulose as substrate.

The results were reviewed and analyzed by the interpretation of the figures obtained from the excretion of H_2 and/or H_2 and CH_4 during the 2 hours that the test was performed. The patient results of H_2 breath tests considered positive were those who had an increase in excretion of H_2 in at least 12 ppm relative to the baseline value, obtained before the substrate intake (glucose). Correspondingly, the patient results of H_2 and CH_4 breath tests considered positive were those who had increase in excretion of H_2 or CH_4 or both gases in at least 12 ppm relative to the baseline value. Furthermore, an analysis was done of the symptoms description reported by patients during the whole period in which the tests were performed, in addition to the proper stratification as previously mentioned.

The data is presented by absolute and relative frequencies. The association between the results of breath tests in relation to gender and the occurrence of symptoms was assessed by Fisher's exact test. Statistical analyzes were carried out in the free software R version 3.1.3 and *P* values less than 0.05 were considered significant (5% significance level).

RESULTS

The sample consisted of 200 consecutive results of breath tests of patients with clinical suspicion of SIBO. We selected 100 results of patient tests who only performed the exhaled H_2 test and 100 patient results who underwent the exhaled H_2 and CH_4 test in the same period.

We found that the H_2 and the H_2 and CH_4 exhalation tests were performed predominantly in women, accounting for 66% and 72% of samples, respectively.

It was observed that 56 results (56%) of exhaled H_2 breath tests were positive for SIBO. In addition, 63% of them had reported intestinal symptoms during the tests, with 16% classified as severe symptoms, 12% classified as moderate and 35% as mild symptoms. The time required for the test to became positive ranged from 15 to 120 minutes between the analyzed results. We also observed the difference of the baseline value and the maximum peak of exhaled H_2 which ranged between 12-85 ppm in these analyzed results.

The analysis of the test results from patients who underwent the exhaled H_2 and CH_4 test showed that 64 patients (64%) tested positive for SIBO. In addition, there were reports of intestinal symptoms in 48% of the results of the analyzed tests, 15% were classified as severe symptoms, 13% as moderate symptoms and 20% as mild symptoms. The time required for the test positivity ranged from 15 to 120 minutes for H_2 and 15 to 90 minutes for CH_4, between the analyzed results. There was also the difference from

the baseline and the maximum peak obtained from exhaled gases ranged between 12-144 ppm (H_2) and 14-50 ppm (CH_4).

Table 1 shows the gender distribution and the occurrence of symptoms resulting from the H_2 test. The occurrence of symptoms during the test was significantly associated with the test result (*P*-value 0.000), indicating that, among patients with a positive result, the proportion of those with symptoms (80.4%) was significantly higher than among patients with negative results (40.9%)

TABLE 1. Gender distribution and symptoms occurrence for test result of H_2 exhale test employing glucose as a substrate

Variables	Positive (n=56)		Negative (n=44)		*P*-value
	n	%	n	%	
Gender					1.000
Female	37	66.1	29	65.9	
Male	19	33.9	15	34.1	
Symptoms					0.000
Symptomatic	45	80.4	18	40.9	
Asymptomatic	11	19.6	26	59.1	

Table 2 shows the distribution of gender and occurrence of symptoms resulting from the H_2 and CH_4 test. There was a significant association between the affirmation of both gases (H_2 and CH_4) and gender (*P*-value 0.020), indicating that the proportion of female patients with positive results (100%) was significantly higher than among patients with negative results (61.1%). The isolated affirmation of exhaled H_2 was not significantly associated with gender (p-value 0.454) or with the occurrence of symptoms (*P*-value 0.814). Similarly, the isolated affirmation of methane was not significantly associated with gender (*P*-value 0.359) nor the occurrence of symptoms (*P*-value 1.000). In cases of simultaneous affirmation of H_2 and CH_4, there was also no significant association with the symptoms (*P*-value 0.735).

TABLE 2. Gender distribution and symptoms occurrence for test result of H_2 and CH_4 exhale test employing glucose as a substrate

Variables	Positive		Negative		*P*-value
	n	%	n	%	
Positive only for H_2	36		36		
Gender					0.454
Female	26	72.2	22	61.1	
Male	10	27.8	14	38.9	
Symptoms					0.814
Symptomatic	19	52.8	17	47.2	
Asymptomatic	17	47.2	19	52.8	
Positive only for CH_4	18		36		
Gender					0.359
Female	14	77.8	22	61.1	
Male	4	22.2	14	38.9	
Symptoms					1.000
Symptomatic	8	44.4	17	47.2	
Asymptomatic	10	55.6	19	52.8	
Positive H_2 and CH_4	10		36		
Gender					0.020
Female	10	100.0	22	61.1	
Male	0	0.0	14	38.9	
Symptoms					0.735
Symptomatic	4	40.0	17	47.2	
Asymptomatic	6	60.0	19	52.8	

DISCUSSION

There is a misconception that SIBO is a rare clinical condition that occurs only in a small number of patients with anatomic abnormalities of the proximal digestive tract or severe digestive motility disorders[18]. Recent studies show that an excessive number of bacteria is found in several gastrointestinal disorders, surviving especially in patients with celiac disease, inflammatory bowel disease, chronic liver diseases and gastrointestinal functional disorders, especially in the irritable bowel syndrome[4,14].

It is known that the prevalence of SIBO in healthy volunteers is lower when compared to symptomatic patients, especially those patients with inflammatory bowel disease, irritable bowel syndrome and abdominal[7,11] distension syndrome. The presence of overgrowth, as detected by the exhaled H_2 test has been described in 0-12.5% in the asymptomatic[20] volunteer group. However, it is important to note that the actual prevalence of the syndrome is not well known due to remaining difficulties for its diagnosis in daily practice[2].

In the present study, we observed a higher prevalence of females in patients with intestinal symptoms in the two analyzed tests. However, a study by Erdogan et al.[15] demonstrated that gender does not influence the presence of overgrowth, not corroborating with our results. What probably differs from our results is that most patients referred to such tests were suffering from gastrointestinal functional disorders, especially irritable bowel syndrome, known to be more prevalent in women, at a ratio of 4:1[10,11].

The study from Erdogan et al. also showed that the prevalence of bacterial overgrowth in patients with intestinal symptoms (diarrhea and flatulence, without changes in digestive endoscopy) was only 27.3% when using the H_2 and CH_4 test[6]. However, when the duodenal culture (gold standard for the diagnosis of SIBO) was also performed this prevalence increased to 44.6%. These authors concluded in the end that 65.5% of patients reporting gastrointestinal symptoms during the test had an overgrowth of microorganisms in the small intestine. In our study, we found a prevalence of 64% positivity overgrowth, a very similar rate to that acquired by North American researchers[18].

We observed in this study that the presence or absence of digestive symptoms while performing both tests shows a direct correlation with the result thereof. In patients who tested positive for SIBO, symptom reporting was significantly more frequent than in those with negative results.

Given that the H_2 and CH_4 are produced in the intestine by microorganisms that degrade carbohydrates, the breath test which detects these gases is carried out in order to evaluate the presence of bacterial overgrowth[18,22]. Thus, the use of the test that identifies the production of both gases is essential for greater accuracy in the diagnosis of the syndrome. The addition of methane to the exhaled H_2 test increases the sensitivity for the diagnosis of SIBO, allowing detection of the syndrome in a subgroup of individuals that do not produce H_2, but produce CH_4 as a major byproduct

of carbohydrate fermentation[16]. According to numerous studies that identified CH_4 exhaled in breath tests, it is estimated that 30%-62% of healthy individuals excreting this Gas[6,22]. As about 15% of humans do not produce H_2, devices capable of detecting both gases, greatly increase the sensitivity of the test, reducing false negative results.

In this study the data analyzed was from two breath tests using different samples. SIBO prevalence was 56% in the tests conducted in apparatus that detects only expired H_2 and 64% in those performed in another apparatus capable of detecting both exhaled H_2 and CH_4. In this last test, it was found that methane was solely responsible for positivity in 18% of patients, while the H_2 was solely responsible for 36% of this total. Together, these gasses were responsible for the positivity in 10% of the sample.

Our results show that approximately 20% of patients identified with SIBO only got their SIBO diagnosis by methane identification in the exhaled gas, which is consistent with the results found in the literature[6,16,20]. Thus, it is essential that the breath tests to diagnose SIBO be performed on devices capable of identifying the exhaled H_2 and CH_4, in order to increase sensitivity and diagnostic accuracy.

CONCLUSION

The data obtained in our study are consistent with those of the most recent literature on this topic. We proved the importance of utilizing the breath test that identifies the exhaled H_2 and CH_4 in order to increase the diagnostic accuracy of small intestine bacterial overgrowth. Our results confirm previous findings that showed that 15% to 20% of individuals do not produce hydrogen gas in their intestine.

It was also possible to observe a high prevalence of SIBO in patients with digestive symptoms during the two hours that the test is performed. Furthermore, we found that the presence and severity of symptoms during the making thereof is more frequent in patients with a positive result, correlating directly with the diagnosis of bacterial overgrowth.

Our study reinforces the need for routine introduction, in our midst, of devices able to detect both gases (H_2 and CH_4) for the most accurate diagnosis of SIBO, increasing thus the sensitivity and reliability of this test, with a significant reduction of false negative results.

Authors' contributions

Martins CP participated in the study design, data interpretation, manuscript writing, revised the final version of the manuscript to be published; participated in the protocol/project development, data collection and manuscript writing/editing; Chaves CHA participated in the protocol/project development, data collection and manuscript writing/editing; Castro MGB participated in the study design and performing the tests; Gomes IC participated in the statistical analysis; Passos MCF was a coordinator, participated in the study design, data interpretation, manuscript writing, and she revised the final version of the manuscript to be published. Study supervision: Passos MCF.

Martins CP, Chaves CHA, Castro MGB, Gomes IC, Passos MCF. Prevalência de supercrescimento bacteriano do intestino delgado em pacientes com sintomas intestinais funcionais. Arq Gastroenterol. 2017;54(2):91-5.

RESUMO – Contexto – O supercrescimento bacteriano do intestino delgado é uma síndrome heterogênea, caracterizada pelo aumento no número e/ou presença de uma microbiota atípica no intestino delgado. Os sintomas do supercrescimento bacteriano do intestino delgado são inespecíficos englobando quadro de dor/distensão abdominal, diarreia e flatulência. Devido ao maior custo e complexidade para a realização do aspirado jejunal, padrão ouro para o diagnóstico da síndrome, tem sido utilizado rotineiramente o teste do hidrogênio (H_2) expirado, utilizando glicose ou lactulose como substrato, que é capaz de determinar, no ar expirado, a concentração de H_2 produzida a partir do metabolismo bacteriano intestinal. Entretanto, em decorrência de uma parcela de indivíduos apresentar uma microbiota metanogênica, não produtora de H_2, justifica-se a realização do teste em aparelhos capazes de detectar, concomitantemente, a concentração de H_2 e metano (CH_4) expirados. **Objetivo** – O presente estudo teve como objetivo determinar a prevalência de supercrescimento bacteriano do intestino delgado em pacientes com sintomas digestivos, através de uma análise comparativa dos testes respiratórios empregando H_2 ou H_2 e CH_4 associados, utilizando a glicose como substrato. **Métodos** – Foram avaliados 200 pacientes de ambos os sexos, sem limitação de idade, encaminhados a um Laboratório de Teste Respiratório para realização do teste de H_2 (100 pacientes) e de H_2 e CH_4 expirados (100 pacientes) devido a queixas gastrointestinais, a maioria deles portadores de distúrbios funcionais gastrointestinais. **Resultados** – Os resultados obtidos indicaram uma significativa prevalência do supercrescimento bacteriano do intestino delgado no teste do H_2 e no teste do H_2 e CH_4 expirados (56% e 64%, respectivamente) em pacientes com sintomas gastrointestinais, além de maior predominância no sexo feminino. Constatou-se ainda, que o gás metano foi isoladamente responsável pela positividade em 18% do total de pacientes. **Conclusão** – Os dados encontrados no presente estudo demonstram condizentes com os achados da literatura atual e reforçam a necessidade da utilização de aparelhos capazes de captar os dois gases (H_2 e CH_4 expirados) para melhorar a sensibilidade e, consequentemente, a acurácia do diagnóstico de supercrescimento bacteriano do intestino delgado na prática médica diária.

DESCRITORES – Crescimento bacteriano. Intestino delgado. Testes respiratórios. Hidrogênio. Metano.

REFERENCES

1. Attaluri A, Jackson M, Valestin J, Rao SS. Methanogenic flora is associated with altered colonic transit but not stool characteristics in constipation without IBS. Am J Gastroenterol. 2010;105:1407-11.
2. Cole C, Ziegler T. Small Bowel bacterial overgrowth: a negative factor in gut adaptation in pediatric SBS. Curr Gastroenterol Rep. 2007;9:456-62.
3. Costa MBG, Azeredo Jr. IL, Marciano RD, Caldeira LM, Bafutto M. Evaluation of small intestine bacterial overgrowth in patients with functional dyspepsia through H2 breath test. Arq Gastroenterol. 2012;49:279-83.
4. Costello BPJ, Ledochowski M.; Ratcliffe NM. The importance of methane breath testing: a review. J Breath Res. 2013; 7:7-16.
5. Dukowicz AC, Lacy BE, Levine GM. Small Intestinal Bacterial Overgrowth: A Comprehensive Review. Gastroenterol Hepatol (N Y). 2007;3:112-22.
6. Erdogan A, Rao SSC, Gulley D, Jacobs C, Lee YY, Badger C. Small intestinal bacterial overgrowth: duodenal aspiration *vs* glucose breath test. Neurogastroenterol Motil. 2015;27:481-9.
7. Gasbarrini A, Corazza GR, Gasbarrini G, Montalto M, Di Stefano M, Basilisco G, et al. Methodology and indications of H2-breath testing in gastrointestinal diseases: the Rome Consensus Conference. Aliment Pharmacol Ther. 2009;29:1-49.
8. Khoshini R, Dai SC, Lezcano S, Pimentel M. A systematic review of diagnostic tests for small intestinal bacterial overgrowth. Dig Dis Sci. 2008;53:1443-54.
9. Lacy LB, Mearin F, Chang L, Chey WD, Lembo AJ, Simren M, et al. Rome IV: Bowel Disorders. Gastroenterology. 2016;150:1393-1407e5.
10. Lovell RM, Ford AC. Global prevalence of and risk factors for irritable bowel syndrome: a meta-analysis. Clin Gastroenterol Hepatol. 2012;10:712-21.e4.
11. Mitsui T, Kagami H, Kinomoto H, Ito A, Kondo T, Shimaoka K. Small bowel bacterial overgrowth and rice malabsorption in healthy and physically disabled older adults. J Hum Nutr Diet. 2003;16:119-22.
12. Passos MCF, Ramos AFP. Supercrescimento Bacteriano do Intestino Delgado. Rio de Janeiro: Editora Graffito; 2014, p.79-90.
13. Pimentel M. Review article: potential mechanisms of action of rifaximin in the management of irritable bowel syndrome with diarrhea. Aliment Pharmacol Ther. 2016;43:37-49.
14. Quera RP, Quigley EMM, Madrid AMS. Small intestinal bacterial overgrowth. An update. Rev Méd Chile. 2005;133:1361-70. Available from: http://www.scielo.cl/scielo.php?script=sci_arttext&pid=S0034-98872005001100013&lng=es
15. Quigley EM. Small intestinal bacterial overgrowth: what it is and what it is not. Curr Opin Gastroenterol. 2014;30:141-6.
16. Rezaie A, Pimentel M, Rao SS. How to Test and Treat Small Intestinal Bacterial Overgrowth: an Evidence-Based Approach. Curr Gastroenterol Rep. 2016;18:8.
17. Rivkin A, Rybalov S. Update on the Management of Diarrhea-Predominant Irritable Bowel Syndrome: Focus on Rifaximin and Eluxadoline. Pharmacotherapy. 2016;36:300-16.
18. Saad RJ, Chey WD. Breath testing for small intestinal bacterial overgrowth: maximizing test accuracy. Clin Gastroenterol Hepatol. 2014;12:1964-72.
19. Sachdev AH, Pimentel M. Gastrointestinal bacterial overgrowth: pathogenesis and clinical significance. Ther Adv Chronic Dis. 2013;4:223-31.
20. Shimura S, Ishimura N, Mikami H, Okimoto E, Uno G, Tamagawa Y, et al. Small Intestinal Bacterial Overgrowth in Patients with Refractory Functional Gastrointestinal Disorders. J Neurogastroenterol Motil. 2016;22:60-8.
21. Siddiqui I, Ahmed S, Abid S. Update on diagnostic value of breath test in gastrointestinal and liver diseases. World J Gastrointest Pathophysiol. 2016;7:256-65.
22. Triantafyllou K, Chang C, Pimentel M. Methanogens, methane and gastrointestinal motility. J Neurogastroenterol Motil. 2014;20:31-40.

ORIGINAL ARTICLE

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Inflammatory bowel disease: outpatient treatment profile

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ABSTRACT – Background – Crohn's disease and ulcerative colitis are the two major forms of inflammatory bowel disease. The incidence and prevalence of both conditions have increased and are progressively increasing. These diseases are frequently recurrent and clinically highly severe. In Brazil, the lack of epidemiological data related to such diseases has left these patients in a vulnerable state and contributed to increased morbidity. **Objective** – To describe the profiles of patients with inflammatory bowel disease treated in an outpatient service in Brazil. **Methods** – This descriptive, exploratory, and retrospective documentary study with a quantitative approach was performed in an outpatient treatment service for inflammatory bowel disease, at a university polyclinic located in Rio de Janeiro, Brazil, from May to July 2016. The study included 556 patients and was approved by the research ethics committee of the institution (CAAE no. 55179316.6.0000.5259/2016). **Results** – The data showed a high prevalence of inflammatory bowel disease in white female patients. Crohn's disease was diagnosed in more patients than was ulcerative colitis; the ileocolon was the most commonly affected location in patients with Crohn's disease. The stenotic phenotype was prevalent in patients with Crohn's disease. **Conclusion** – The prevalence of the stenotic phenotype in Crohn's disease in relation to others demonstrates the need for further investigations in this field of study in Brazil. In conclusion, the data showed that the epidemiologic profile of the study population is similar to that published in the national and international literature.

HEADINGS – Inflammatory bowel diseases. Crohn disease. Ulcerative colitis. Epidemiology.

INTRODUCTION

Crohn's disease (CD) and ulcerative colitis (UC) are the two major inflammatory bowel diseases (IBDs) that affect 1 in 200 people in developed countries, where they present an increasing incidence and prevalence. In European countries, at least a five-fold increase in the incidence of IBD in the last 30 years has been estimated[5,15].

IBD generally causes debilitating symptoms such as urgent diarrhea, rectal bleeding, vomiting, and anorexia, which often have an adverse effect on the social, professional, academic, family relationships, and sexual activity of patients[3,5,14,15].

UC, which involves only the rectum and colon, usually develops in the rectum (proctitis) and may extend proximally to the sigmoid and descending colon (left sided colitis) or the entire colon (pancolitis). The location and disease activity index determine the therapeutic approach[2].

CD may affect the gastrointestinal tract anywhere between the mouth and the anus; inflammation occurs intermittently, with spots caused by the disease in areas of healthy mucosa. CD may be classified as inflammatory, stenotic, or penetrating. The genetic component is more prevalent in CD than in UC. Other rheumatologic, dermatologic, ophthalmologic, hepatobiliary, and extraintestinal diseases may occur concomitantly[2].

Among the various classifications proposed for UC and CD, the Montreal classification is the most commonly used as it distinguishes clinical subphenotypes of CD according to location, behavior, and age at symptom onset, and those of ulcerative colitis according to the extent of the disease and age at symptom onset. Such a classification is desirable for correlating specific disease phenotypes with possible clinical outcomes and prognosis to select a better therapeutic approach and a more appropriate follow-up for each patient[4,5].

Reference centers responsible for treating patients with IBD are often required to justify their need for resources in order to provide appropriate treatments for patients. Studies have shown that the costs related to treating patients with IBD are nearly 2.2 billion dollars per year in the United States[15].

In Brazil, patients with CD and UC are not necessarily reported resulting in a lack of epidemiologic data with respect to these diseases. This situation places patients in a vulnerable state, as incipient studies and the poor publication records on this group of diseases contribute to their late diagnosis and increased morbidity[3,4,6,8,9,14].

It is difficult to determine the epidemiologic data of IBD in Brazil and all developing countries owing to deficiencies in data recording systems and the inability to access data other than those in the public health system. Given this reality, specialty pharmacies and reference centers for the treatment of IBD are favorable places for performing IBD-related studies[6,8,9,14].

The outpatient follow-up of patients with IBD aims to control symptoms and induce disease remission, adapt patients to their chronic condition and improve their quality of life, minimize toxicity and monitor the adverse effects of prescribed drugs, and postpone or reduce the incidence of relapse[1].

Therefore, this study aimed to describe the profile of patients with IBD in a university outpatient service in Rio de Janeiro.

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METHODS

This retrospective, documentary, and descriptive quantitative study was performed in an outpatient treatment service for IBD of a University Polyclinic located in Rio de Janeiro. The study was performed between May and July 2016, and included 556 patients. This investigation followed the ethical guidelines recommended by the Brazilian National Committee for Ethics in Research of the Brazilian National Health Council, and was approved by the Research Ethics Committee of the Institution (CAAE no. 55179316.6.0000.5259/2016).

The inclusion criteria were patients with IBD registered in the outpatient service for IBD treatment and undergoing outpatient treatment during the data collection period. Patients who had undergone treatment for <3 months were excluded from the study owing to the lack of necessary data for surveying their full profile.

The variables studied were sex, age, demographic data, diagnosis, disease location, intestinal CD phenotype, effective treatment, previous surgeries, and the occurrence of perianal disease.

The data were retrieved from medical records by using an appropriate data collection tool that enabled the collection of the socio-demographic, clinical, endoscopic, radiologic, and imaging profiles of each study participant.

Age was analyzed as a continuous variable, whereas the average and minimum and maximum values were retrieved and analyzed by using a line chart to find the prevalence peaks according to age.

The Montreal classification, which subdivides the disease according to three main phenotypic characteristics, i.e. age at diagnosis (A, "age"), topographic location (L, "location"), and clinical behavior (B, "behavior"), was used to categorize the CD location. The location is defined as the point of the maximum anatomical extent of the disease at any time. There were four possible classifications according to location: terminal ileal (L1, limited to the lower third of the small intestine, with or without cecal involvement), colonic (L2, anywhere between the cecum and the rectum without involvement of the upper digestive tract), ileocolonic (L3, terminal ileum and anywhere between the ascending colon and rectum), and isolated to the upper gastrointestinal tract (L4, anywhere above the terminal ileum, except the mouth). If there is a simultaneous proximal (L4) and distal (L1–L3) involvement, an L4 category should be added as a modifier; if no distal involvement was found, L4 was considered to be of the exclusive form. This resulted in seven possible classifications according to location: L1, L2, L3, L4, L1+L4, L2+L4, and L3+L4[4,10].

Concerning the clinical behavior of CD, three classifications were used: nonstenotic and nonpenetrating/inflammatory (B1), stenotic (B2), and penetrating/fistulizing (B3). Nonstenotic and nonpenetrating/inflammatory clinical behavior (B1) occurs in the presence of inflammation without any stenotic or fistulizing evidence. On the other hand, stenotic behavior (B2) is defined by a narrowing of the intestinal lumen. Penetrating behavior (B3) is defined when abdominal fistulae or inflammatory masses and/or abscess occur at any period during the progression of disease[4,10,12,15].

To ensure correct classification and differentiation of the clinical behavior of CD, data from enterography reports obtained from magnetic resonance imaging or computed tomography scans were used preferentially, as well as endoscopic reports of the study patients when appropriate.

The extent of UC was classified as follows: ulcerative proctitis (E1), involvement limited to the rectum; left colitis (E2), involvement extending to the splenic flexure; and extensive colitis (E3), involvement extending beyond the splenic flexure[3,5].

The data were processed, stored, and analyzed by using Microsoft Excel 2010 and the Statistical Package for Social Sciences version 20.0. For characterization of the sample and descriptive analysis of the variables, the data were summarized by using descriptive statistics in frequency distribution tables. The results were discussed and compared with the data of currently available relevant studies.

RESULTS

Among the 556 patients with IBD who participated in the study, 331 (59.3%) were women and 225 (40.7%) were men. The median age of the 432 participants was 49.7 years ,range, 15–86 years (Figure 1).

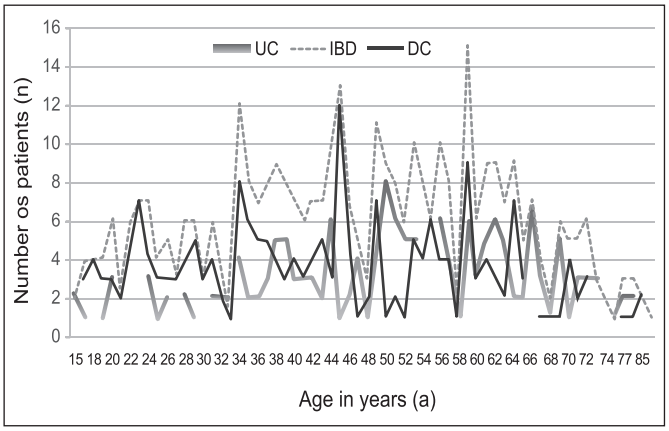

FIGURE 1. Distribution of inflammatory bowel disease (IBD), Crohn's disease (CD), and ulcerative colitis (UC) in patients according to age. Rio de Janeiro, Brazil, 2016.

The data relative to the places of residence of the participants showed that most of the patients treated at the outpatient service lived in the same municipality (53.70%; n=290), whereas those from the other 33 neighboring municipalities in the city of Rio de Janeiro were also identified. The patient with the farthest residence was living 344 km away from the reference center. Considering the regional distribution in the state, most of the treated patients were living in the metropolitan region (94.32%; n=382), followed by those who were living in the Coastal Lowlands region (2.72%; n=11), Northern Rio de Janeiro (1.23%; n=5), Costa Verde (0.74%; n=3), and Middle Paraíba and Central-South Rio de Janeiro (0.25%; n=1 each). The other patients (0.49%; n=2) were living in other states of the southeastern region of Brazil.

Concerning race/self-reported color, the data of 431 patients were surveyed: 47.80% (n=206) self-reported as white, 38.52% (n=166) as dark skinned, and 13.69% (n=59) as black.

The medical diagnoses included the data of 447 patients. CD was found in 56.38% (n=252) and UC was found in 38.26% (n=171), whereas 5.37% (n=24) of patients were lacking a diagnosis.

The most common types of UC according to inflammatory location were extensive colitis (E3) 35.67%, (n=61) and proctitis (E1) 30.99%, (n=53) (Figure 2).

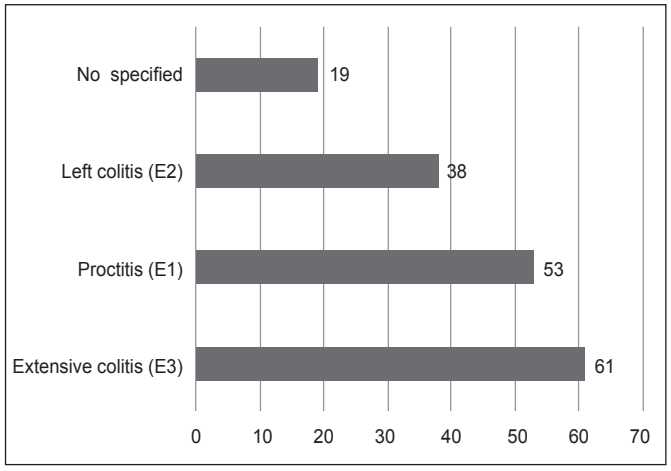

FIGURE 2. Number of patients with ulcerative colitis according to inflammatory location. Rio de Janeiro, Brazil, 2016.

The data from 88 patients with CD were surveyed according to the Montreal classification; the most prevalent type of CD according to location was ileocolonic (L3) (57.95%, n=51), followed by colonic (L2) (18.18%, n=16), ileal (L1) (11.63%, n=10), pancolonic (L2+L4) (10.23%, n=9), and jejunoileal (L1+L4) (2.33%, n=2).

According to the phenotypic classification of CD, the stenotic phenotype (B2) was the most prevalent (35.23%; n=31), followed by the nonstenotic and nonpenetrating/inflammatory (B1) (25%; n=22) and penetrating/fistulizing (B3) (23.86%; n=21). However, 15.91% (n=14) of patients were lacking a classification (Table 1).

TABLE 1. Number and proportion of patients with Crohn's disease according to location and phenotype. May to July 2016, Rio de Janeiro, Brazil

Variables		n	%
Localization	Intestinal phenotype		
Ileocolonic (L3)	Stenotic (B2)	19	21.59
	Inflammatory (B1)	7	7.95
	Penetrating (B3)	19	21.59
Colonic (L2)	Stenotic (B2)	5	5.68
	Inflammatory (B1)	7	7.95
	Penetrating (B3)	1	1.14
Ileal (L1)	Stenotic (B2)	5	5.68
	Inflammatory (B1)	3	3.41
	Penetrating (B3)	1	1.14
Pancolonic (L2+L4)	Stenotic (B2)	2	2.27
	Inflammatory (B1)	3	3.41
Jejunoileal (L1+L4)	Inflammatory (B1)	1	1.14
	Penetrating (B3)	1	1.14
	Not identified	14	15.91
Total		88	100%

Of the 252 patients with CD, 17.46% (n=44) had some type of perianal disease and 12.69% (n=32) had undergone a surgical procedure owing to disease complications (Figure 3).

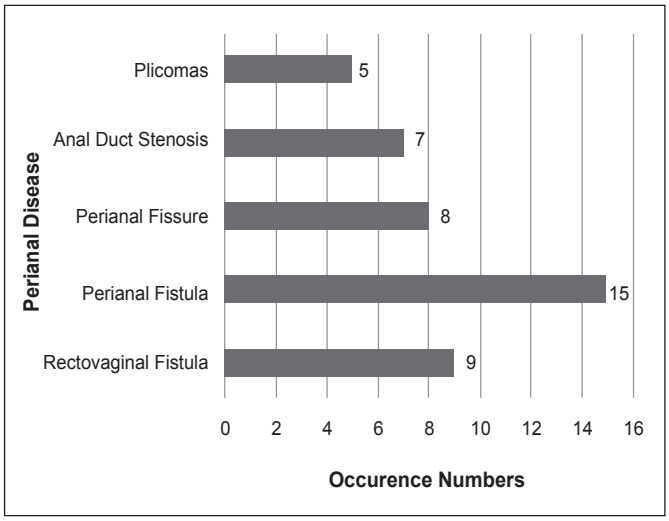

FIGURE 3. Distribution of occurrence numbers for perianal disease. Rio de Janeiro, Brazil, 2016.

DISCUSSION

The results of this study showed a predominance of female patients, thus corroborating other studies performed in Brazil. The data released in April 2016 by the department of pharmaceutical care and strategic inputs of the state of Rio de Janeiro are consistent with those obtained in the outpatient service. That publication indicated that 58.92% of patients with IBD were women and 41.08% were men[3,4,8,14]. In contrast, most European studies showed a higher prevalence of IBD among men; in European countries, women primarily develop CD and men develop UC[2,12].

Young adults are commonly diagnosed as having both CD and UC. Most new diagnoses were in the age group of between 15 and 40 years, whereas another peak of incidence was between 50 and 80 years[4,11,15]. Concerning the age group of the patients included in this study, most cases of IBD occurred at age between 30 and 45 years, whereas another peak of incidence occurred around age 60 years. The results found in this study contradict those found in the international literature. Most studies indicated a higher prevalence of IBD in white patients, followed by dark-skinned, black, and yellow patients, which was confirmed in this study population with a higher prevalence of IBD in white women[2,3,14,15].

The demographic profile of patients showed a similarity to other Brazilian reference centers that offer health care to patients living in neighboring regions. It is noteworthy that patients usually travel great distances to seek specialized medical services. This indicates the need for expanding the coverage of more complex services to ensure access and proper use for all patients who need such services. Although telehealth defined as health-care services provided to people separated by distance and time is available by using technologies such as telephones, computers, or interactive video streaming, these resources are underutilized in South America for improving patient assistance programs despite the widespread use of both landlines and cell phones by these populations[13].

Similar to the epidemiological studies on the profile of patients with IBD conducted by Kleinubing-Júnior et al. and Barros et al., the extent of involvement of UC in this study showed a prevalence of extensive colitis (E3) and rectitis/proctitis (E1)[3,8].

The Montreal classification, owing to its diagnostic and therapeutic implications, has been increasingly used in patients with CD. In most studies related to classification according to location, the ileocolonic type was the most frequent, followed by the ileal and colonic types[2–6,8–10,12,14]. In this study, patients with CD showed a higher prevalence of the ileocolonic type (L3), corroborating the results of recent multicenter studies that included countries in Europe, North America, and Oceania[2].

However, the data found in this study showed a greater prevalence of patients with CD in the colonic region (L3) than in the ileal region (L1). Multicenter and review studies have pointed out that the nonstenotic and nonpenetrating/inflammatory phenotype (B1) is the most frequent[2,5]. Considering the phenotypic classification of CD, the patients evaluated in this study were not predominantly found as having an inflammatory phenotype, as described in the literature. Most patients showed a penetrating phenotype, followed by the stenotic and inflammatory phenotype, respectively, with a slightly different percentage, which may be because of the low number of participants assessed with this classification.

The natural history and clinical progression of IBD are rather heterogeneous: up to 20% of patients with ulcerative colitis need to undergo a colectomy owing to the complications of the disease, whereas >50% of patients with CD require surgery within 10 years after the diagnosis. On the other hand, up to 50% of patients with ulcerative colitis and 30% with CD tend to show a relatively passive disease progression without the need for immunosuppression or surgery[13].

Therefore, the findings in the literature are in contrast to the results of this study, which showed that patients with CD needed more surgical procedures than those with IURC. Surgical approaches were performed in 12.69%, that is, <20% of patients with CD[5,13]. Perianal disease affects approximately 33% of patients with CD after 10 years of disease progression and presents a high morbidity, mainly due to pain and drainage in the area, which may lead to complications such as dermatitis and pruritus.

The most frequent types of injury are perianal fistula, abscess, fissure, plicoma, and stenosis, as confirmed by a Brazilian meta-analysis of 67 articles in 2012. Some studies found perianal involvement in approximately 26% of patients with CD[4,7,13].

This study found a lower prevalence of perianal disease than did other available studies. The most frequent type of injury was perianal fistula, followed by abscess, fissure, plicoma, and stenosis. In this manner, the occurrence of perianal disease and the percentage of surgical approaches described in this study did not exceed those described in domestic and international studies[4,5].

CONCLUSION

The data from the analysis of the epidemiologic profiles of patients with IBD in this reference center in Rio de Janeiro showed great similarity with the profiles found in national studies; however, in general, nationwide studies to verify possible regional variations in the epidemiologic profile have not been performed throughout the country.

The demographic data of patients demonstrated the need for expanding specialized medical services and the use of strategies supporting distance interventions that provide comprehensive health care for patients.

The phenotypic classification of patients with CD did not show a prevalence of the inflammatory phenotype, as described in the literature. The observed high prevalence of the stenotic phenotype indicates the need for further investigations in this field of study in Brazil.

In conclusion, the other results of this study agree with those in the international literature. This study is expected to be useful for other researchers and may serve as a basis for welfare policies for patients with CD and UC in Brazil, leading to a more effective and safe therapy for these patients.

Authors' contributions

Santos RM, Carvalho ATP, Silva KS, Sá SPC, Santos AH, Sandinha MR participated in the planning, investigation, preparation of the manuscript, approval, and final submission to *Arquivos de Gastroenterologia*.

Santos RM, Carvalho ATP, Silva KS, Sá SPC, Santos AH, Sandinha MR. Doença inflamatória intestinal: perfil do paciente em tratamento ambulatorial. Arq Gastroenterol. 2017;54(2):96-100.

RESUMO – Contexto – A Doença de Crohn e a retocolite ulcerativa idiopática são as duas principais formas de doença inflamatória intestinal e possuem crescente incidência e prevalência, tendem a ser progressivas, cursam com recidivas frequentes e assumem formas clínicas de alta gravidade. No Brasil a escassez de dados epidemiológicos relacionados a tais agravos deixa essas pessoas em estado de vulnerabilidade e contribui para o aumento da morbidade**. Objetivo –** Descrever o perfil dos pacientes portadores de doença inflamatória intestinal atendidos em um ambulatório de doenças inflamatórias intestinais do estado do Rio de Janeiro. **Métodos –** Trata-se de uma pesquisa documental retrospectiva, exploratória, descritiva em abordagem quantitativa, realizada de maio a julho de 2016, em uma policlínica universitária, localizada no Rio de Janeiro, em ambulatório de tratamento de doenças inflamatórias intestinais. O estudo foi aprovado pelo Comitê de Ética em Pesquisa da instituição CAAE: 55179316.6.0000.5259 /2016, e contou com 556 participantes. **Resultados** – Os dados revelam um predomínio das doenças inflamatórias intestinais, nos pacientes do sexo feminino, de cor branca. A doença de Crohn foi diagnosticada em um maior número de indivíduos em relação a retocolite ulcerativa idiopática. Os dados do perfil de localização intestinal mostram a região ileocolônica como mais afetada na doença de Crohn. Foi evidenciado predomínio do fenótipo estenosante na doença de Crohn. **Conclusão** – O predomínio do fenótipo estenosante na doença de Crohn, evidencia a necessidade de estudos aprofundados sobre a temática no Brasil. No mais, os dados obtidos demonstram um perfil epidemiológico da população semelhante ao divulgado em estudos nacionais e internacionais.

DESCRITORES – Doenças inflamatórias intestinais. Doença de Crohn. Colite Ulcerativa. Epidemiologia.

REFERENCES

1. Amo L, Gonzalez-Lama Y, Suarez C, Blazquez I, Matallana V, Calvo M, et al. Impact of the incorporation of a nurse in an inflammatory bowel disease unit. Gastroenterol Hepatol. 2016;39:318–23.
2. Bardhan KD, Simmonds N, Royston C, Dhar A, Edwards CM; on behalf of the Rotherham IBD Database Users Group. J Crohns Colitis. 2010;4:405–12.
3. Barros PAC, Silva AMR, Neto MAFL. The epidemiological profile of inflammatory bowel disease patients on biologic therapy at a public hospital in Alagoas. J Coloproctol (Rio J). 2014;34:131–5.
4. Bechara CDS, Lacerda Filho A, Ferrari MDLA, Andrade DAR, Luz MMPD, Silva RGd. Montreal classification of patient operated for Crohn's disease and identification of surgical recurrence predictors. Rev Col Bras Cirurg. 2015;42:97–104.
5. Cleynen I, Boucher G, Jostins L, Schumm LP, Zeissig S, Ahmad T, et al. Inherited determinants of Crohn's disease and ulcerative colitis phenotypes: a genetic association study. Lancet. 2016;387:156–67.
6. da Rosa JR, da Silva Júnior JF, da Rosa MI. Perfil epidemiológico de portadores de doença inflamatória intestinal Arq Catarin Med. 2014;43:53–8.
7. Eglinton TW, Barclay ML, Gearry RB, Frizelle FA. The spectrum of perianal Crohn's disease in a population-based cohort. Dis Colon Rectum. 2012;55:773–7.
8. Kleinubing-Júnior H, Pinho MSL, Ferreira LC, Bachtold GA, Merki A. Perfil dos pacientes ambulatoriais com doenças inflamatórias intestinais. ABCD, Arq Bras Cir Dig. 2011;24:200–3.
9. Oliveira FM, Emerick APC, Soares EG. Aspectos epidemiológicos das doenças intestinais inflamatórias na macrorregião de saúde leste do Estado de Minas Gerais. Ciênc Saúde Coletiva. 2010;15(Suppl 1).
10. Rebelo A, Rosa B, Moreira MJ, Cotter J. Da Classificação de Viena para a Nova Classificação de Montreal: Caracterização Fenotípica e Evolução Clínica da Doença de Crohn. J Portug Gastrenterol. 2011;18:99–100.
11. Ricci Jr JER. Segurança e riscos do tratamento da doença inflamatória intestinal durante gravidez e aleitamento. HU rev.2011;37(3),set.
12. Shirazi KM, Somi MH, Bafandeh Y, Saremi F, Mylanchy N, Rezaeifar P, et al. Epidemiological and clinical characteristics of inflammatory bowel disease in patients from northwestern Iran. Middle East J Dig Dis. 2013;5:86–92.
13. Solberg IC, Lygren I, Jahnsen J, Aadland E, Høie O, Cvancarova M, et al. Clinical course during the first 10 years of ulcerative colitis: results from a population-based inception cohort (IBSEN Study). Scand J Gastroenterol. 2009;44:431–40.
14. Souza MM, Belasco AGS, Aguilar-Nascimento JE. Perfil Epidemiológico dos Pacientes Portadores de Doença Inflamatória Intestinal do Estado de Mato Grosso. Rev Bras Coloproct. 2008;28:324–8.
15. Squires SI, Boal AJ, Naismith GD. The financial impact of a nurse-led telemedicine service for inflammatory bowel disease in a large district general hospital. Frontline Gastroenterol. 2016;7:216–21.

ORIGINAL ARTICLE

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Genotype association *GSTM1* null and gastric cancer: evidence-based meta-analysis

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ABSTRACT – Background – Gastric cancer is the fourth most common cancer in men and the sixth among women, except for non-melanoma skin tumors, in Brazil. Epidemiological evidences reveal the multifactorial etiology of this cancer, highlighting risk factors such as: infection by the bacterium *Helicobacter pylori*, advanced age, smoking, chronic alcohol abuse, eating habits and genetic polymorphisms. Considering the context of genetic polymorphisms, there is the absence of the *GSTM1* gene. The lack of *GSTM1* function to detoxify xenobiotics and promote defense against oxidative stress leads to increased DNA damage, promoting gastric carcinogenesis. This process is multifactorial and the development of gastric cancer results from a complex interaction of these variables. **Objective –** The aim of this study was to investigate the association of *GSTM1* null polymorphism in the pathogenesis of gastric cancer. **Methods** – A meta-analysis was conducted from 70 articles collected in SciELO and PubMed databases, between September 2015 and July 2016. In order to evaluate a possible association, we used the odds ratio (OR) and confidence interval of 95% (CI 95%). To assess the heterogeneity of the studies was used the chi-square test. Statistical analysis was performed using the BioEstat® 5.3. **Results** – This study included 70 studies of case-control, including 28,549 individuals, which were assessed for the null polymorphism of the *GSTM1* gene, and of which 11,208 (39.26%) were cases and 17,341 (60.74%) were controls. The final analysis showed that the presence of the *GSTM1* gene acts as a protective factor against the development of gastric cancer (OR=0.788; 95%CI 0.725-0.857; $P<0.0001$). Positive statistical association was found in Asia (OR=0.736; 95%CI 0.670-0.809; $P<0.0001$) and Eurasia (OR=0.671; 95%CI 0.456-0.988; $P=0.05$). However, statistically significant data was not obtained in Europe (OR=1.033; 95%CI 0.873-1.222; $P=0.705$) and America (OR=0.866; 95%CI 0.549-1.364; $P=0.534$). Therefore, the results can not be deduced around the world. **Conclusion –** This meta-analysis concluded that the presence of the *GSTM1* gene is a protector for the emergence of gastric cancer, especially in Asian countries, but this result was not found in Europe and America.

HEADINGS – Stomach neoplasms. Genetic polymorphism. Meta-analysis.

INTRODUCTION

Malignant tumors of the stomach are present predominantly in the form of three histologic types: adenocarcinoma accounts for 95% of the tumors; lymphoma, diagnosed in about 3% of cases; and leiomyosarcoma, initiated in tissues that give rise to the muscles and bones[74].

In Brazil, gastric cancer is the fourth most common cancer in men and the sixth among women, except for nonmelanoma skin tumors[74]. In the rest of the world, according to the International Agency for Research on Cancer (GLOBOCAN), gastric cancer is the fourth most common cancer in men and the fifth in women[17]. More than 70% of gastric cancer cases occur in developing countries. The incidence rate of this disease is two times higher in males than in females. The peak incidence occurs mostly in men, around 70 years old. About 65% of patients diagnosed with this cancer are over 50 years old[17,74].

The highest mortality rates are recorded in Latin America, especially Costa Rica, Chile and Colombia. However, the greatest number of cases occurs in Japan, where they found 780 patients per 100,000 inhabitants. Gastric cancer does not have a good prognosis, and mortality remains high throughout the world[74].

A study conducted by the National Cancer Institute José Alencar Gomes da Silva (INCA), demonstrated that the median survival of gastric cancer after surgical resection was 15 months (0-65 months), with higher survival rates for stage I and II and lower survival for stage III and IV[11]. European data shows that only 21% of patients survive more than five years after diagnosis[7]. And therefore, in Brazil and in the world, it is a major public health problem.

The process of carcinogenesis is multifactorial and not completely understood. In addition to nutritional and behavioral factors, genetic characteristics have shown to be increasingly important. The development of gastric cancer results from a complex interaction of these variables[68,86,89]. Genetic susceptibility may modify the effect of environmental exposure, thus explaining the variations in incidence of cancer around the world[23,34].

The individuality of genetic susceptibility is critical in the various factors that influence carcinogenesis, such as: protection of the gastric mucosa in the face of infection by *Helicobacter pylori*; inflammatory response to the infection; capacity of detoxification and antioxidant protective action; cell proliferation capacity; variability in the DNA repair process; apoptotic pathway[15,49,80,92].

In addition, part of the susceptibility is determined by indi-

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vidual differences in bioactivation of proto-oncogenes and detoxification of carcinogens due to metabolism and degree of penetrance of inherited polymorphisms[34,49].

Several genes with low penetrance were identified as potential carcinogens. Glutathione S-transferase (*GST*) is a superfamily of homo- and hetero-dimeric enzymes that catalyze the conjugation of potential carcinogens in glutathione, playing an important role in protecting cell structures, including DNA[89].

In humans, eight distinct *GST* genes were found, among them five are widely distributed: alpha (*GSTA*), sigma (*GSTs*), mi (*GSTM*), pi (*GSTP*) and theta (*GSTT*)[9,49]. Located on chromosome 1p13.3, the *GSTM1* plays an important role in detoxification of xenobiotics[89]. Among the isoforms in the *GST*, the *GSTM1* has a particular function, because the null polymorphism results from the total absence of the enzyme produced by it[80].

The most common *GSTM1* polymorphism gene is a homozygous deletion (null), which has been associated with reduced enzyme activity and increased vulnerability to cytogenetic damage[89]. The *GST* enzymes commonly act on environmental pollutants, such as benzopyrene and other polycyclic aromatic hydrocarbons, which are important carcinogens. Lack of *GSTM1* function to detoxify xenobiotics and promote defense against oxidative stress leads to increased DNA damage, promoting gastric carcinogenesis[61].

The association between *GSTM1* null genotype and gastric cancer was first described by Strange in 1991 in the British population. In recent years, several studies have attempted to demonstrate a positive association between *GSTM1* null and gastric cancer. However, the results have been conflicting regarding this association[80,89].

Considering both genders, gastric cancer is the fifth most frequent cancer and third leading cause of cancer death worldwide. Proper management of this disease is a major public health problem, both nationally and internationally. Knowledge of the interactions and molecular changes involving the carcinogenic process can lead to a way for the control of gastric cancer. In this perspective, the genetic role of polymorphisms has acquired a lot of relevance in the scientific community[80]. Given this universal scenario, the aim of this study is to investigate the association of *GSTM1* null polymorphism in the genesis of gastric cancer and compare the different effects of this association in Asia, America, Europe and Eurasia.

METHODS

The meta-analysis is a research technique that selects and extracts studies results through strict procedures. The results are then summarized by statistical analysis in order to reduce the subjectivity of the traditional methods of narrative review. Thus, combining results from different primary studies and their use in recent years, added significantly to the area of health by the high degree of recommendation associated with levels of evidence that it translates[63,67].

The main steps of a meta-analysis are: (1) the literature, (2) the transformation of the results of each study group on a common measure, (3) checking the homogeneity of the results, (4) modeling variation between studies, and finally (5) the sensitivity analysis[20].

Search and retrieval studies

The research of articles was conducted from databases of Scientific Electronic Library Online (SciELO) and PubMed National Center for Biotechnology Information, USA (NCBI), between September 2015 and July 2016, for the extraction of relevant studies that estimated the association between *GSTM1* polymorphism and the risk of gastric cancer. For this, the following keywords were selected: "GSTM1", "gastric cancer", "Glutathione S-transferase M1", and "Meta-analysis". During the research, articles in Portuguese, English and Spanish were selected. Limitations were not made regarding the size of the sample obtained by surveyed items.

Inclusion and exclusion criteria

In the context of meta-analysis, it is important to assess the heterogeneity, as it consists in the variability or differences between studies in relation to the estimating effects and therefore its identification is critical to assess the degree of confidence in the results. Overall, the authors divide the heterogeneity into three types: clinical, methodological or statistical[58,73]. In order to minimize these parameters, the inclusion criteria are defined: (1) Case-control studies; (2) histologic confirmation of adenocarcinoma; (3) gene by PCR Research *GSTM1*; (4) articles published from 1990 to 2015; (5) articles in languages: English, Portuguese and Spanish; (6) articles fully accessed by researchers.

Data extraction

The extraction of relevant data were made by two researchers independently, discrepancies in data collection were discussed and a consensus was reached among researchers. Data taken from the table were: year of publication of the article, lead author, country, continent, number of cases and controls and presence of polymorphisms of *GSTM1* in cases and controls.

Statistical analysis

Heterogeneity is defined as the diversity of the studies and can strongly affect the results. The diversity can then be evaluated for heterogeneity χ^2 test[58,73]. Thus, the genotypic frequencies of all articles were grouped into a single table and diversity was assessed with the use of heterogeneous χ^2 test in 2x2 contingency tables, to compare the different odds ratios (OR) with a confidence interval of 95%, determined in their studies.

If the heterogeneity of the χ^2 test reveals a $P>0.05$, the null hypothesis is confirmed, i.e., the studies are homogeneous. So it is recommended to use the fixed-effect tests that assume that all studies point in the same direction. In this context, the most used is the Mantel-Haenszel test. On the other hand, if the heterogeneity χ^2 test results in a $P<0.05$, it indicates diversity and heterogeneity between studies. Therefore, the use of random or random effect tests is recommended, such as the DerSimonian-Laird tests[4,6,26,91].

Global association tests were then used to assess the significance of the correlation between the polymorphism of gene *GSTM1* and gastric cancer for all studies combined. To estimate the effect of gene polymorphism in the development of gastric cancer, the amounts of each study were combined with fixed and random tests of effects utilizing the software BioEstat® 5.3[26]. Either for fixed-effect tests or for the random effects, odds ratios are calculated, its confidence intervals (95%) and the weights for each individual and combined study, generating an estimated combined effect. In addition, the tests elaborate a graphical *forest plot* type. The advantage of these charts is to summarize, in the same space, all the information on the effect and the contribution of each study for analysis[73].

As the grouping of all studies showed heterogeneity, we applied the random effects test DerSimonian-Laird for all genotypic

possibilities: the presence or absence of the *GSTM1*. This same grouping strategy was used to stratify studies by more frequent locations: Asia (50 studies), Europe (12 studies), America (5 studies) and Eurasia (3 studies). For groupings with the presence of gastric cancer realized in Asia, America and Europe, they utilized the DerSimonian-Laird test. On the other hand, for the variables of the locations in Eurasia, the fixed effect Mantel-Haenszel test was applied.

RESULTS

In this meta-analysis, after researching databases, we selected 174 articles on the polymorphism of the *GSTM1* gene, published between the years 1991 to 2015. Of these articles, 104 articles were discarded for not meeting the inclusion criteria (Figure 1). Thus, 70 articles were brought together that evaluated the association of *GSTM1* polymorphism and gastric cancer.

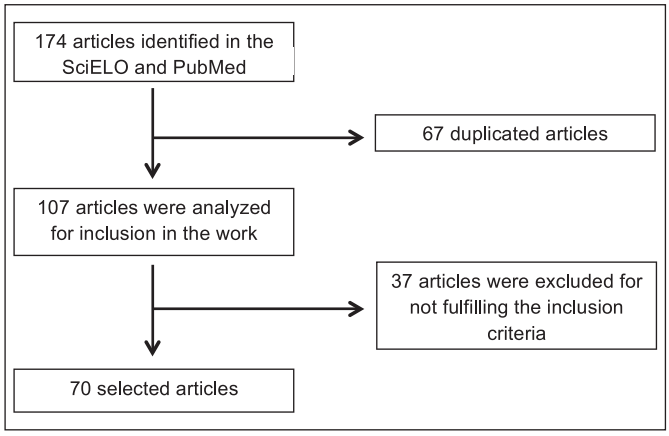

FIGURE 1. Identification of criteria, inclusion and exclusion, of the meta-analysis studies.

Among the articles selected, 50 were made in Asia, 12 in Europe, 5 in the Americas (North, Central and South) and 3 in Eurasia. This study included 28,549 individuals assessed for polymorphism of the *GSTM1* gene, of which 11,208 (39.26%) were part of the group of cases diagnosed with gastric cancer, and 17,341 (60.74%) were part of the control group. For comparison purposes, to group the data, the frequency of the presence of the *GSTM1* gene in cases and controls were, respectively, 46.0% and 50.4%.

The data of the *GSTM1* polymorphism of the gene were grouped into each article. Thus, the odds ratios (OR) were calculated for each study, their variations within the 95% confidence interval and significance probabilities (*P*).

For each cluster, we applied the DerSimonian-Laird test, except for the grouping in Eurasia, where we used the Mantel-Haenszel. The odds ratios were calculated with the grouping of all studies for: America (OR=0.866; 95%CI 0.549-1.364; *P*=0.534; Figure 2), Eurasia (OR=0.671; 95%CI 0.456-0.988; *P*=0.05; Figure 3), Europe (OR=1.033; 95%CI 0.873-1.222; *P*=0.705; Figure 4) and Asia (OR=0.736; 95%CI 0.670-0.809; *P*<0.0001).

The graphics generated in the meta-analysis are the *forest plot* type. This type of chart, each line represents one study, with the latter, in the shape of a rhombus, represents the combined results. The result of each study is described in graphical and numerical forms. In graphic form, the central squares represent the relative risk

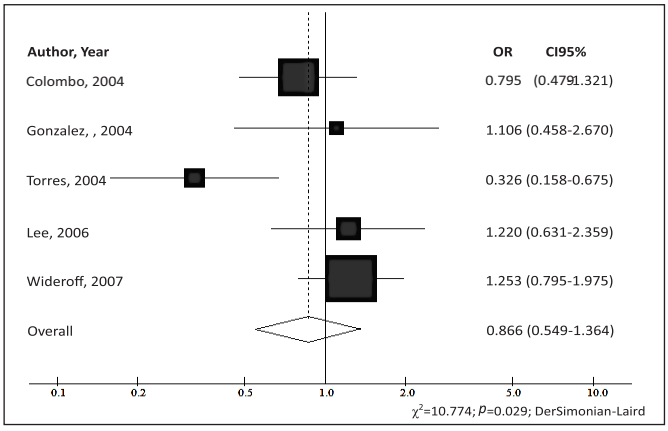

FIGURE 2. Odds ratios (OR) and confidence interval of 95% (95%CI) of the association between gastric cancer and the presence of *GSTM1* for the studies carried out in America with the Chi-square test of significant heterogeneity (DerSimonian-Laird test).

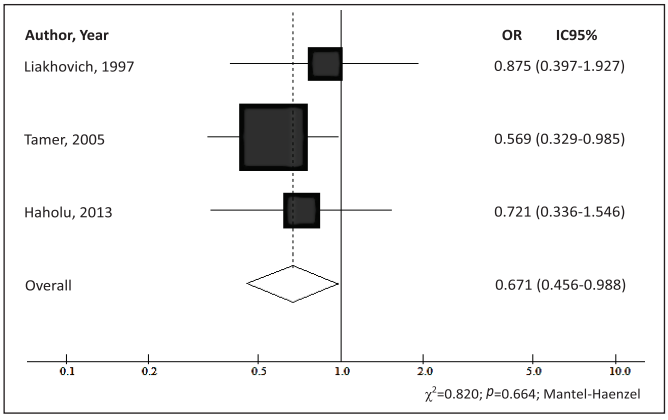

FIGURE 3. Odds ratios (OR) and confidence interval of 95% (95%CI) of the association between gastric cancer and the presence of *GSTM1* for the studies carried out in Eurásia with the Chi-square test of significant heterogeneity (Mantel-Haenzel test).

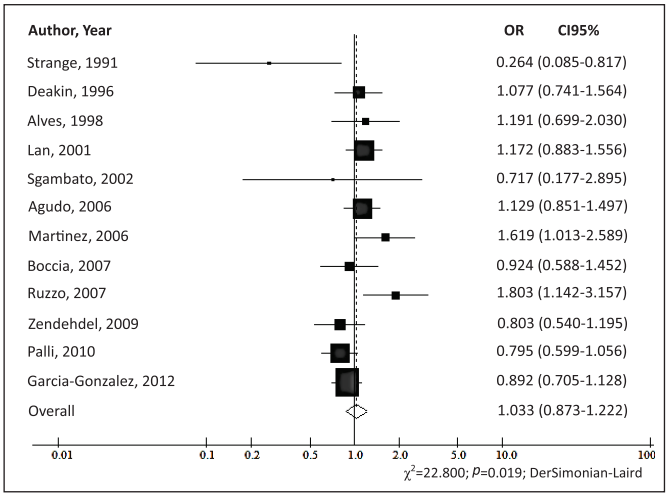

FIGURE 4. Odds ratios (OR) and confidence interval of 95% (95%CI) of the association between gastric cancer and the presence of *GSTM1* for the studies carried out in Europa with the Chi-square test of significant heterogeneity (DerSimonian-Laird test).

(RR) or odds ratio and the traits represent the confidence intervals (CI). When the CI does not exceed the null line (position 1.0 in the chart), it can be said that the study is statistically significant, both individually and for the combined value. The larger the sample group considered in the study, narrower will be the CIs and greater will be the area of the square, showing more accurate results and greater contribution to the meta-analysis.

Additionally, information on the polymorphism of gene *GSTM1* were grouped into each article, we calculated the OR, the variation of OR within the 95% confidence interval and the probability of significance (*P*) (Table 1). When applying DerSimonian-Laird test, the OR of all the combined work (OR=0.788; 95%CI 0.725-0.857; *P*<0.0001) showed that the presence of the gene is a protective factor for gastric cancer.

TABLE 1. Distribution of the polymorphism of the *GSTM1* gene in patients with gastric cancer, in the case and control groups in the published articles between 1991 and 2015 and the database of the current study

Author	Year	Case				Control				OR	CI (95%)		Weight
		GSTM1 (+)		*GSTM1* (-)		*GSTM1* (+)		*GSTM1* (-)			Inf.	Sup.	
		n	F (%)	n	F (%)	n	F (%)	n	F (%)				
Strange[75]	1991	5	26.3	14	73.7	29	59.2	20	40.8	0.264	0.085	0.817	2.999
Harada[25]	1992	5	26.3	14	73.7	44	52.4	40	47.6	0.175	0.057	0.531	3.105
Kato[31]	1996	34	53.1	30	46.9	59	49.2	61	50.8	1.169	0.639	2.128	10.544
Katoh[32]	1996	60	43.2	79	56.8	71	56.3	55	43.7	0.591	0.364	0.959	16.364
Deakin[14]	1996	64	47.1	72	52.9	261	45.2	316	54.8	1.077	0.741	1.564	27.563
Liakhovich[42]	1997	28	57.1	21	42.9	32	60.4	21	39.6	0.877	0.401	1.915	6.294
Ng[54]	1998	23	45.1	28	54.9	22	62.9	13	37.1	0.495	0.208	1.179	5.098
Wang[81]	1998	34	41.0	49	59.0	43	51.8	40	48.2	0.649	0.353	1.194	10.323
Alves[3]	1998	77	52.0	71	48.0	40	47.6	44	52.4	1.191	0.699	2.030	13.504
Oda[56]	1999	56	38.1	91	61.9	57	50.9	55	49.1	0.596	0.363	0.979	15.616
Jiang[29]	2000	17	41.5	24	58.5	27	65.9	14	34.1	0.377	0.156	0.911	4.919
Liu[43]	2000	36	36.4	63	63.6	178	48.9	186	51.1	0.601	0.381	0.941	18.481
Cai[8]	2001	35	36.8	60	63.2	51	54.3	43	45.7	0.496	0.278	0.884	11.481
Qian[60]	2001	34	38.2	55	61.8	50	53.2	44	46.8	0.548	0.305	0.984	11.201
Shen[70]	2001	67	55.4	54	44.6	80	66.1	41	33.9	0.638	0.381	1.071	14.351
Saadat[66]	2001	16	38.1	26	61.9	78	59.5	53	40.5	0.424	0.209	0.860	7.706
Setiawan[68]	2001	45	51.7	42	48.3	207	49.4	212	50.6	1.096	0.692	1.736	18.171
Lan[38]	2001	180	51.9	167	48.1	204	47.9	222	52.1	1.172	0.883	1.556	47.859
Wu[84]	2002	183	51.4	173	48.6	142	51.1	136	48.9	1.013	0.741	1.386	39.128
Gao[18]	2002	63	41.2	90	58.8	90	40.4	133	59.6	1.035	0.682	1.571	22.056
Gong[21]	2002	7	21.9	25	78.1	38	43.2	50	56.8	0.386	0.154	0.964	4.580
Zheng[94]	2002	28	30.4	64	69.6	44	47.8	48	52.2	0.482	0.264	0.877	10.674
Sgambato[69]	2002	3	37.5	5	62.5	47	47.0	53	53.0	0.717	0.177	2.895	1.971
Choi[10]	2003	34	42.5	46	57.5	82	46.3	95	53.7	0.859	0.506	1.459	13.683
Zhang[90]	2003	49	38.6	78	61.4	61	53.5	53	46.5	0.549	0.329	0.914	14.729
Zheng[93]	2003	168	53.7	145	46.3	106	55.2	86	44.8	0.941	0.656	1.348	29.623
Zhou[95]	2003	12	63.2	7	36.8	44	61.1	28	38.9	1.067	0.385	2.961	3.691
Colombo[12]	2004	53	53.0	47	47.0	88	58.7	62	41.3	0.795	0.479	1.321	14.916
Gonzalez[22]	2004	16	51.6	15	48.4	25	49.0	26	51.0	1.106	0.458	2.670	4.949
Torres[78]	2004	16	34.8	30	65.2	60	62.5	36	37.5	0.326	0.158	0.675	7.282
Roth[64]	2004	66	73.3	24	26.7	309	68.1	145	31.9	1.276	0.771	2.111	15.161
Suzuki[76]	2004	58	40.0	87	60.0	93	52.5	84	47.5	0.604	0.388	0.941	19.588
Shen[72]	2004	29	48.3	31	51.7	36	60.0	24	40.0	0.629	0.307	1.288	7.471
Shen[71]	2005	71	50.0	71	50.0	314	46.5	361	53.5	1.149	0.801	1.649	29.483
Lai[37]	2005	50	40.7	73	59.3	66	54.5	55	45.5	0.573	0.346	0.950	15.046
Li[41]	2005	33	33.0	67	67.0	36	58.1	26	41.9	0.360	0.188	0.690	9.108
Mu[51]	2005	69	35.2	127	64.8	158	40.2	235	59.8	0.810	0.568	1.155	30.500
Nan[52]	2005	149	37.3	251	62.8	254	41.4	360	58.6	0.842	0.650	1.090	57.576
Nan[53]	2005	34	31.8	73	68.2	90	40.9	130	59.1	0.677	0.417	1.100	16.312
Tamer[77]	2005	30	42.9	40	57.1	116	56.9	88	43.1	0.572	0.332	0.987	12.926
Lee[40]	2006	60	82.2	13	17.8	207	78.7	56	21.3	1.220	0.631	2.359	8.840
Hong[27]	2006	48	44.4	60	55.6	104	43.7	134	56.3	1.320	0.654	1.628	18.467
Zhou[96]	2006	33	33.0	67	67.0	36	58.1	26	41.9	0.360	0.188	0.690	9.108
Agudo[1]	2006	120	49.6	122	50.4	434	46.6	498	53.4	1.129	0.851	1.497	48.147
Martinez[47]	2006	65	66.3	33	33.7	180	54.7	149	45.3	1.619	1.013	2.589	17.438
Wideroff[83]	2007	55	47.4	61	52.6	87	41.8	121	58.2	1.253	0.795	1.975	18.540
Tripathi[79]	2007	45	59.2	31	40.8	61	61.0	39	39.0	0.928	0.507	1.699	10.493
Boccia[5]	2007	48	44.9	59	55.1	119	46.9	135	53.1	0.924	0.588	1.452	18.806
Ruzzo[65]	2007	91	72.2	35	27.8	83	57.6	61	42.4	1.898	1.142	3.157	14.851
Al-Moundhri[2]	2009	65	60.7	42	39.3	75	70.1	32	29.9	0.663	0.377	1.166	12.076
Masoudi[48]	2009	30	44.8	37	55.2	74	55.2	60	44.8	0.660	0.368	1.187	11.185
Malik[46]	2009	44	40.7	64	59.3	116	59.5	79	40.5	0.471	0.292	0.758	16.910
Moy[50]	2009	72	42.4	98	57.6	320	43.5	415	56.5	0.954	0.682	1.336	33.930
Piao[59]	2009	988	44.6	1225	55.4	776	45.7	923	54.3	0.959	0.845	1.089	238.192
Huang[28]	2009	55	45.5	66	54.5	84	60.9	54	39.1	0.538	0.329	0.881	15.813
Zendehdel[87]	2009	54	43.5	70	56.5	230	49.0	239	51.0	0.803	0.540	1.195	24.362
Nguyen[55]	2010	16	27.1	43	72.9	34	31.2	75	68.8	0.830	0.414	1.664	7.948
Yadav[85]	2010	84	63.2	49	36.8	150	55.6	120	44.4	1.367	0.894	2.090	21.286
Palli[57]	2010	130	43.9	166	56.1	271	49.6	275	50.4	0.795	0.599	1.056	47.660
Darazy[13]	2011	7	53.8	6	46.2	58	82.9	12	17.1	0.247	0.073	0.831	2.602
Luo[44]	2011	30	24.4	93	75.6	58	45.0	71	55.0	0.399	0.233	0.610	13.412
Zhang[88]	2011	89	45.9	105	54.1	218	52.9	194	47.1	0.755	0.534	1.063	32.927
Yadav[86]	2011	30	73.2	11	26.8	92	70.8	38	29.2	1.104	0.508	2.397	6.389
Jing[30]	2012	170	41.5	240	58.5	203	49.5	207	50.5	0.723	0.549	0.952	50.617
Malakar[45]	2012	45	44.1	57	55.9	107	52.5	97	47.5	0.718	0.446	1.155	16.970
Wang[82]	2012	90	69.8	39	30.2	112	81.2	26	18.8	0.540	0.307	0.949	12.050
Kim[33]	2012	41	40.2	61	59.8	76	38.0	124	62.0	1.098	0.676	1.785	16.271
Garcia-Gonzalez[19]	2012	274	49.2	283	50.8	290	52.1	267	47.9	0.892	0.705	1.128	69.682
Eom[16]	2013	214	44.9	263	55.1	217	45.6	259	54.4	0.971	0.753	1.253	59.142
Haholu[24]	2013	24	48.0	26	52.0	32	56.1	25	43.9	0.725	0.341	1.554	6.732
Total		5154	46.0	6054	54.0	**8736**	**50.4**	**8605**	**49.6**	**0.788**	**0.725**	**0.857**	***P*<0.0001**

The heterogeneity chi-square test was used in 2x2 tables of all the possibilities: Asia (χ^2=99,489; DF=49; *P*<0.0001); Europe (χ^2=22.800; DF=11, *P*=0.019); America (χ^2=10.774; DF=4; *P*=0.029); Eurasia (χ^2=0.820; DF=2, *P*=0.664); and finally, gathering all studies (χ^2=154,651; DF=69; *P*<0.0001). Thus, tests have revealed significant (*P*<0.05) with the American, Asian and European groupings, indicating that there is a difference between them only as a result of sampling error, i.e., the real effect is the same in each of the studies. However, in the Eurasia group, the test is found to be significant (*P*>0.05). However, data from all articles analyzed points to the same statistical direction (*P*<0.0001), then it is concluded that when all the samples are grouped, the articles are homogeneous and differences can be seen as resulting from random or common effects.

Table 2 shows an overview of all groups, with their heterogeneity χ^2 tests, indicating the *P*-value, which determines the type of test used in the meta-analysis. Verification tests of the correlation of the *GSTM1* gene polymorphism with gastric cancer, in various grouping situations, were sometimes random effects of DerSimonian-Laird, and at other times of fixed effect Mantel-Haenszel. It can be observed in Table 2 that the *p* values, in the meta-analysis, revealed no association between the variables studied (*P*>0.05) in Europe and America, and for the grouping of studies in Asia and Eurasia could be inferred that there is a positive association (Asia, *P*<0.0001 and Eurasia, *P*=0.05).

The data in Table 2 indicates that the meta-analysis of 70 case-control studies investigating the association between polymorphisms of *GSTM1* and the risk of developing gastric cancer was positively correlated, and therefore the presence of the gene a protective factor. Regarding the groupings performed, there is a positive correlation in the group of Asian and Eurasian studies, but no correlation was observed in studies conducted in Europe and America.

DISCUSSION

The pathogenesis of gastric cancer is not yet fully known. In recent years, it was understood that the knowledge that the process of developing this cancer is multifactorial, in which the environment and genetic susceptibility factors are decisive[35].

Among the biological factors, infection by the bacterium *Helicobacter pylori* plays an important correlation with gastric cancer due to their role in chronic atrophic gastritis, since gastric carcinoma is accompanied by hypochlorhydria in 85% to 90% of cases[39].

The *H. pylori* infection still remains the greatest risk factor for the development of gastric cancer, increasing the incidence of this cancer about six times. It is one of the most common infections of the population, with a worldwide prevalence estimated at between 50% and 90% in developing countries. However, it is important to mention that, in populations with a high prevalence of infection by *H. pylori*, a small fraction of infected develop gastric cancer[74].

The risk of intestinal metaplasia in the gastric antrum depends largely on the presence of a peptic ulcer and other factors, such as individual variability of the immune response, advanced age, smoking, chronic alcohol abuse, and eating habits. Therefore, additional factors alter the relationship of *H. pylori* to gastric carcinogenesis[61]. Chen and colleagues showed that risk factors such as smoking and *Helicobacter pylori* infection did not modify the association between *GSTM1* null and risk for gastric cancer, suggesting greater genetic influence in the etiology of this cancer[9].

Significant associations of the absence of the *GSTM1* gene and the incidence of gastric cancer were found in Asians, but it was not possible to demonstrate such an outcome in Caucasian and African populations, suggesting a possible influence of ethnic differences, genetic origins and the environment. The influence of *GSTM1* null allele can be masked by the presence of other causative genes, not yet identified, involved in the development of gastric cancer in Caucasians and Africans[34,61,97].

According to Rebbeck and colleagues, ethnic differences were found related to the prevalence of *GSTM1* deletion gene in different populations. In Japanese, this frequency varies between 48% to 51% of the population, in Chinese between 35% to 65%, in Indians 33% to 36%, 50% in Caucasians and Africans 22% to 35%[62].

Another risk factor that already has well-established relationship in the genesis of gastric cancer is smoking. It is estimated that smoking increases the risk of this cancer by 50%. A meta-analysis conducted in 2008 by Ladeiras-Lopes and colleagues estimated that smoking is the main modifiable risk factor related to gastric cancer, especially in men[36].

Tobacco is composed of more than 4,000 different compounds, 50 proven carcinogens. Pollutants such as benzopyrene and other polycyclic aromatic hydrocarbons are substrates of enzymes of the GST family and have serious carcinogenic activity. These compounds are primarily metabolized by Phase I enzymes, detoxified and converted into inactive metabolites by enzymes of Phase II, enzymes expressed by the gene *GSTM1*[39].

The null genotype refers to the complete absence of enzyme activity *GSTM1*, and can thus increase the risk of gastric cancer. The lack of function of this gene leads to the accumulation of toxic intermediate, resulting in greater damage to DNA, which facilitates carcinogenesis[25,36,61].

TABLE 2. Summary of the groupings, showing the chi-square test of heterogeneity and the type of the test realized in the meta-analysis.

Parameters	Number of studies	Heterogeneity			Meta-analysis				
		χ^2	gl	*P*-value	Test	OR	CI (95%)	CI (95%)	*P*-value
Geral									
GSTM1 (+) x *GSTM1* (-)	70	154.651	69	<0.0001	DSL	0.788	0.725	0.857	<0.0001
Region									
America	5	10.774	4	0.0292	DSL	0.866	0.549	1.364	0.534
Asia	50	99.489	49	<0.0001	DSL	0.736	0.670	0.809	<0.0001
Eurasia	3	0.820	2	0.6637	MH	0.671	0.456	0.988	0.050
Europe	12	22.800	11	0.0189	DSL	1.033	0.873	1.222	0.705

DSL: DerSimonian–Laird; MH: Mantel–Haenszel.

CONCLUSION

The meta-analysis included 70 articles published between 1991 and 2015. The group generated a simultaneous universal evaluation of the *GSTM1* null polymorphism in 28,549 individuals, 11,208 patients with gastric cancer and 17,341 controls.

The current study showed that there is a significant positive association between *GSTM1* null genotype and gastric cancer, with $P<0.0001$. The presence of *GSTM1* gene is protective in cases of gastric cancer (OR=0.788).

Prior studies of this association are quite controversial. The work carried out in Asia are those that showed strong correlation between variables ($P<0.001$); on the other hand, as reported in previous references, the data collected in Europe ($P=0.705$) and the Americas ($P=0.534$) did not find such an association. It is important to note the composition of this meta-analysis, the Asian articles contributed 20,257 individuals, 8,465 cases and 11,792 controls. The Americas articles contributed 1,134 individuals (366 cases and 768 controls) and European data with 6,675 individuals (2,208 cases and 4,467 controls).

The process of carcinogenesis is multifactorial and not fully understood. Genetic susceptibility may modify the effect of environmental exposure, thus explaining the variations in the incidence of gastric cancer around the world and the results found in this meta-analysis: positive relationship between *GSTM1* null and gastric cancer, especially when considering Asian countries.

Future studies with larger range of individuals are needed to better understand the association between *GSTM1* null and gastric cancer, especially in the continents in which such an association were not found, such as Europe and the Americas.

Authors' contributions

Ribeiro RX: performed research, drafted the research project, took data collection, wrote the preliminary versions of the article. Nascimento CILL: performed research, drafted the research project, took data collection, wrote the preliminary versions of the article. Silva AMTC: oriented study at all stages of execution, did the study statistics and edited the preliminary versions of the article, generating the final version.

Ribeiro RX, Nascimento CILL, Silva AMTC. Associação do genótipo nulo *GSTM1* e o câncer gástrico: evidências baseadas em meta-análise. Arq Gastroenterol. 2017;54(2):101-8.

RESUMO – Contexto – No Brasil, o câncer gástrico é o quarto mais comum em homens e o sexto entre as mulheres, excetuando-se os tumores de pele não melanoma. Aspectos epidemiológicos evidenciam a etiologia multifatorial desta neoplasia, destacando como fatores de risco: a infecção pela bactéria *Helicobacter pylori*, idade avançada, tabagismo, etilismo crônico, hábitos alimentares e polimorfismos genéticos. No contexto dos polimorfismos genéticos, tem-se a ausência do gene *GSTM1*. A falta da função de *GSTM1* em detoxificar xenobióticos e promover defesa contra o estresse oxidativo, leva ao maior dano do DNA, favorecendo a carcinogênese gástrica. Este processo é multifatorial e o desenvolvimento do câncer gástrico resulta de uma interação complexa dessas variáveis. **Objetivo** – O objetivo do presente estudo foi investigar a associação do polimorfismo nulo de *GSTM1* na gênese do câncer gástrico. **Métodos** – Foi conduzida uma meta-análise a partir de 70 artigos colhidos dos bancos de dados: SciELO e PubMed, entre setembro de 2015 e julho de 2016. Para avaliar uma possível associação, utilizou-se o *odds ratio* (OR) e intervalo de confiança de 95% (IC 95%). Para avaliar a heterogeneidade dos estudos, utilizou-se o teste do qui-quadrado. A análise estatística foi realizada utilizando-se o BioEstat® 5.3. **Resultados –** A presente pesquisa contou com 70 estudos do tipo caso-controle que incluíram 28.549 indivíduos avaliados para o polimorfismo nulo do gene *GSTM1*, dos quais 11.208 (39,26%) eram casos e 17.341 (60,74%) eram controles. A análise final mostra que a presença do gene *GSTM1* funciona como um fator de proteção contra o desenvolvimento de câncer gástrico (OR=0,788; IC95% 0,725-0,857; $P<0,0001$). Associação estatística positiva foi encontrada na Ásia (OR=0,736; IC95% 0,670-0,809; $P<0,0001$) e Eurásia (OR=0,671; IC95% 0,456-0,988; $P=0,05$). No entanto, não temos dados com significância estatística da Europa (OR=1,033; IC95% 0,873-1,222; $P=0,705$) e América (OR=0,866; IC95% 0,549-1,364; $P=0,534$) para inferir proteção ao câncer gástrico no mundo. **Conclusão** – Esta meta-análise, conclui que a presença do gene *GSTM1* é protetora para o surgimento do câncer gástrico, principalmente nos países asiáticos, porém tal resultado não foi encontrado se comparado isoladamente os estudos realizados na Europa e na América.

DESCRITORES – Neoplasias gástricas. Polimorfismo genético. Meta-análise.

REFERENCES

1. Agudo A, Sala N, Pera G, Capellá G, Berenguer A, García N, et al. No association between polymorphisms in CYP2E1, GSTM1, NAT1, NAT2 and the risk of gastric adenocarcinoma in the European prospective investigation into cancer and nutrition. Cancer Epidemiol Biomarkers Prev. 2006;15:1043-5.
2. Al-Moundhri MS, Alkindy M, Al-Nabhani M, Al-Bahrani B, Burney IA, Al-Habsi H, et al. Combined polymorphism analysis of glutathione S-transferase M1/G1 and interleukin-1B (IL-1B)/interleukin 1-receptor antagonist (IL-1RN) and gastric cancer risk in an Omani Arab Population. J Clin Gastroenterol. 2009;43:152-6.
3. Alves GM. Glutathione S transferase mu polymorphism and gastric cancer in the Portuguese population. Biomarkers. 1998;3:441-7.
4. Ayres M, Ayres Jr. M, Ayres DL, Santos AS. BioEstat: aplicações estatísticas nas áreas das ciências bio-médicas. Belém: Sociedade Civil Mamirauá; 2007. p. 132-214.
5. Boccia S, Sayed-Tabatabaei FA, Persiani R, Gianfagna F, Rausei S, Arzani, D, et al. Polymorphisms in metabolic genes, their combination and interaction with tobacco and alcohol consumption and risk of gastric cancer: a case-control study in an Italian population. BMC Cancer. 2007;7:206.
6. Böhning D, Malzahn U, Dietz E, Schlattmann P. Some general points in estimating heterogeneity variance with the DerSimonian–Laird estimator. Biostatistics. 2002;3:445-57.
7. Bustamante-Teixeira MT, et al. Sobrevida em pacientes com câncer gástrico em Campinas, São Paulo, Brasil. Cad. Saúde Pública. 2006;22:1611-8.
8. Cai L, Yu SZ, Zhang ZF. Glutathione S-transferases M1, T1 genotypes and the risk of gastric cancer: a case–control study. World J Gastroenterol. 2001;7:506-9.
9. Chen B, Zhou Y, Yang P, Wu X. Glutathione S-transferase M1 gene polymorphism and gastric cancer risk: an updated analysis. Arch Med Res. 2010;41:558-66.
10. Choi SC, Yun KJ, Kim TH, Kim HJ, Park SG, Oh GJ, et al. Prognostic potential of glutathione S-transferase M1 and T1 null genotypes for gastric cancer progression. Cancer Lett. 2003;195:169-75.
11. Cola CB, et al. Tratamento cirúrgico do câncer gástrico em pacientes jovens: experiência de 05 anos no INCA. Revista Brasileira de Cancerologia. 2005;51:135-41.
12. Colombo J, Rossit AR, Caetano A, Borim AA, Wornrath D, Silva AE. GSTT1, GSTM1 and CYP2E1 genetic polymorphisms in gastric cancer and chronic gastritis in a Brazilian population. World J Gastroenterol. 2004;10:1240-5.

13. Darazy M, Balbaa M, Mugharbil A, Saeed H, Sidani H, Abdel-Razzak Z. CYP1A1, CYP2E1, and GSTM1 gene polymorphisms and susceptibility to colorectal and gastric cancer among Lebanese. Genet Test Mol Biomarkers. 2011;15:423-9.
14. Deakin M, Elder J, Hendrickse C, Peckham D, Baldwin D, Pantin C, et al. Glutathione S-transferase GSTT1 genotypes and susceptibility to cancer: studies of interactions with GSTM1 in lung, oral, gastric and colorectal cancers. Carcinogenesis. 1996;17:881-4.
15. Domenico P, Saieva C, Gemma S, Masala G, Gomez-Miguel MJ, Luzzi I, et al. GSTT1 and GSTM1 gene polymorphisms and gastric cancer in a high-risk Italian population. Int. J. Cancer. 2005;115:284-9.
16. Eom SY, Yim DH, Zhang Y, Yun JK, Moon SI, Yun HY, et al. Dietary aflatoxin B1intake, genetic polymorphisms of CYP1A2, CYP2E1, EPHX1, GSTM1, and GSTT1, and gastric cancerrisk in Korean. Cancer Causes Control. 2013;24: 1963-72.
17. Ferlay J, Soerjomataram I, Ervik M, Dikshit R, Eser S, Mathers C, et al.Cancer Incidence and Mortality Worldwide: IARC Cancer Base No. 11. [Internet]. [Accessed 2015 november 6]. Available from: http://globocan.iarc.fr.
18. Gao CM, Takezaki T, Wu JZ, Li ZY, Liu YT, Li SP, et al. Glutathione-S-transferases M1 (GSTM1) and GSTT1 genotype, smoking, consumption of alcohol and tea and riskof esophageal andstomach cancers:a case–control study of a high-incidence area in Jiang su Province. China Cancer Lett. 2002;188:95-102.
19. Garcia-Gonzalez MA, Quintero E, Bujanda L, Nicolas D, Benito R, Strunk M, et al. Relevance of GSTM1, GSTT1, and GSTP1 gene polymorphisms to gastric cancer susceptibility and phenotype. Mutagenesis. 2012;27:771-7.
20. Giannotti JG, Packer IU, Mercadante MEZ. Meta-análise para estimativas de correlação genética entre pesos ao nascer e desmama de bovinos. Scientia Agricola. 2002;59:435-40.
21. Gong L, Sun HL, Xu YQ. The study of correlations between the deletion of GSTM1 gene and gastric cancer. Wan Nan Yi Xue Yuan Xue Bao. 2002;21:181-3.
22. Gonzalez A, Ramirez V, Cuenca P, Sierra R. Polymorphisms in detoxification genes CYP1A1, CYP2E1, GSTT1 and GSTM1 in gastric cancer susceptibility. Rev Biol Trop. 2004;52:591-600.
23. Gu J, et al. GSTM1 null genotype is associated with increased risk of gastric ycancer in both ever-smokers and non-smokers: a meta-analysis of casa-control studies. Tumour Biol. 2014;35:3439-45.
24. Haholu A, Berber U, Karagoz B, Tuncel T, Bilgi O, Demirel D. Is there any association of glutathione S-transferase T1 (GSTT1) and glutathione S-transferase M1 (GSTM1) gene polymorphism with gastric cancers? Pol J Pathol. 2013;64:247-52.
25. Harada S, Misawa S, Nakamura T, Tanaka N, Ueno E, Nozoe M. Detection of GST1 gene deletion by the polymerase chain reaction and its possible correlation with stomach cancer in Japanese. Hum Genet. 1992;90:62-4.
26. Higgins JPT, White IR, Wood AM. Imputation methods for missing outcome data in metaanalysis of clinical trials. Clinical Trials. 2008;5:225-39.
27. Hong SH, Kim JW, Kim HG, Park IK, Ryoo JW, Lee CH, et al. Glutathione S-transferases (GSTM1, GSTT1and GSTP1) and N-acetyltransferase 2 polymorphisms and the riskof gastriccancer. J Prev Med Public Health. 2006;39:135-140.
28. Huang X, Lu YF, Xie NC, Zhang HT, Ning Z, Tan ZR. A case-control study on the association between genetic polymorphism of GSTM1 and gastric cancer susceptibility in population from Guangxi province of China. Wei Chang Bing Xue He Gan Bing Xue Za Zhi. 2009;18:97-9.
29. Jiang YH, Ju ZY, Ren CS. Study on the relationship between the glutathiones-transferase gene deletion environmental factors and susceptibility to gastric carcinoma. Zhong Guo Gong Gong Wei Sheng. 2000;16:877-9.
30. Jing C, Huang ZJ, Duan YQ, Wang PH, Zhang R, Luo KS, Xiao XR. Glulathione-S-transferases gene polymorphism in prediction of gastric cancer risk by smoking and Helicobacter pylori infection status. Asian Pac J Cancer Prev. 2012;13:3325-8.
31. Kato S, Onda M, Matsukura N, Tokunaga A, Matsuda N, Yamashita K, Shields PG. Genetic polymorphisms of the cancer related gene and Helicobacter pylori infection in Japanese gastric cancer patients. An age and gender matched case–control study. Cancer. 1996;77(8 Suppl):1654-61.
32. Katoh T, Nagata N, Kuroda Y, Itoh H, Kawahara A, Kuroki N, et al. GlutathioneS-transferase M1 (GSTM1) and T1 (GSTT1) genetic polymorphism and susceptibilityto gastric and colorectal adenocarcinoma. Carcinogenesis. 1996;17:1855-9.
33. Kim H, Um J, Kim Y. Glutathione S-transferase gene polymorphism in Korean subjects with gastric and colorectal cancer. Oriental Pharmacy and Experimental Medicine. 2012;12:307-12.
34. La Torre G, Boccia S, Ricciardi G. Glutathione S-transferase M1 status and gastric cancer risk: a meta-analysis. Cancer Letters. 2005;217:53-60.
35. La Torre G, Chiaradia G, Gianfagna F, De Lauretis A, Boccia S, Mannocci A, Ricciardi W. Smoking status and gastric cancer risk: an updated meta-analysis of case-control studies published in the past ten years. Tumori. 2009;95:13-22.
36. Ladeiras-Lopes R, Pereira AK, Nogueira A, Pinheiro-Torres T, Pinto I, Santos-Pereira R, Lunet N. Smoking and gastric cancer: systematic review and metaanalysis of cohort studies. Cancer Causes Control. 2008;19:689-701.
37. Lai KC, Chen WC, Tsai FJ, Li SY, Chou MC, Jeng LB. GlutathioneS-transferase M1 gene null genotype and gastric cancer risk in Taiwan. Hepatogastroenterology. 2005;52:1916-9.
38. Lan Q, Chow WH, Lissowska J, Hein DW, Buetow K, Engel LS, Ji B, Zatonski W, Rothman N. Glutathione S-transferase genotypes and stomach cancer in a population-based case–control study in Warsaw. Poland Pharmacogenetics. 2001;11:655-61.
39. Lao X, Peng Q, Lu Y, Li S, Qin X, Chen Z, Chen J. Glutathione S-transferase gene GSTM1, gene-gene interaction, and gastric cancer susceptibility: evidence from an uptodate meta-analysis. Cancer Cell Int. 2014;14:127.
40. Lee K, Caceres D, Varela N, Csendes DA, Rios RH, Quinones SL. Allelic variants of cytochrome P4501A1 (CYP1A1), glutathione S transferase M1 (GSTM1) polymorphisms and their association with smoking and alcohol consumption as gastric cancer susceptibility biomarkers. Rev Med Chil. 2006;134:1107-15.
41. Li H, Chen XL, Li HQ. Polymorphism of CYPIA1 and GSTM1 genes associated with susceptibility of gastric cancer in Shandong Province of China. World J Gastroenterol. 2005;11:5757-62.
42. Liakhovich VV, Vavilin VA, Gutkina NI, Laktionova IP, Makarova SI, Mitrofanov DV, et al. Genes and enzymes of the xenobiotic-metabolizing system in cancer pathology. Vopr Med Khim. 1997;43:330-38.
43. Liu Y, Xu RT, Sun GF, Shang XL, Wang Q. The relationship of GSTM1 gene homozygous deletion polymorphism of gastric cancer. Zhong Guo Yi Ke Da Xue Xue Bao. 2000;29:287-9.
44. Luo YP, Chen HC, Khan MA, Chen FZ, Wan XX, Tan B, Ou-Yang FD, Zhang DZ. Genetic polymorphisms of metabolic enzymes-CYP1A1, CYP2D6, GSTM1, and GSTT1, and gastric carcinoma susceptibility. Tumour Biol. 2011;32:215-22.
45. Malakar M, Devi KR, Phukan RK, Kaur T, Deka M, Puia L, et al. Genetic polymorphism of glutathione S-transferases M1 and T1, tobacco habits and risk of stomach cancer in Mizoram. India Asian Pac J Cancer Prev. 2012;13:4725-32.
46. Malik MA, Upadhyay R, Mittal RD, Zargar SA, Modi DR, Mittal B. Role of xenobiotic-metabolizing enzyme gene polymorphisms and interactions with environmental factors in susceptibility to gastric cancer in Kashmir Valley. J Gastrointest Cancer. 2009;40:26-32.
47. Martinez C, et al. Glutathione S-transferases mu 1, theta 1, pi 1, alpha 1 and mu 3 genetic polymorphisms and the risk of colorectal and gastric cancers in humans. Pharmacogenomics. 2006;7:711-8.
48. Masoudi M, Saadat I, Omidvari S, Saadat M. Genetic polymorphisms of GSTO2, GSTM1, and GSTT1 and risk of gastric cancer. Mol Biol Rep. 2009;36:781-4.
49. Meng X, et al. Glutathione S-transferase M1 null genotype meta-analysis on gastric câncer risk. Diagnostic Patology. 2014;9:122.
50. Moy KA, Yuan JM, Chung FL, Wang XL, Van Den Berg D, Wang R, Gao YT, Yu MC. Isothiocyanates, glutathioneS-transferase M1 and T1 polymorphisms and gastric cancer risk: a prospective study of men in Shanghai. China Int J Cancer. 2009;125:2652-9.
51. Mu LN, Lu QY, Yu SZ, Jiang QW, Cao W, You NC, et al. Green tea drinking and multigenetic index on the risk of stomach cancer in a Chinese population. Int J Cancer. 2005;116:972-83.
52. Nan HM, Park JW, Song YJ, Yun HY, Park JS, Hyun T, et al. Kimchi and soybean pastes are risk factors of gastric cancer. World J Gastroenterol. 2005;11:3175-81.
53. Nan HM, Song YJ, Yun HY, Park JS, Kim H. Effects of dietary intake and genetic factors on hyper methylation of the hMLH1 gene promoter in gastric cancer. World J Gastroenterol. 2005;11:3834-41.
54. Ng EK, Sung JJ, Ling TK, Ip SM, Lau JY, Chan AC, et al. Helicobacter pylori and the null genotype of glutathione-S-transferasemu in patients with gastric adenocarcinoma. Cancer. 1998;82:268-73.
55. Nguyen TV, Janssen MJ, van Oijen MG, Bergevoet SM, te Morsche RH, van Asten H, Laheij RJ, et al. Genetic polymorphisms in GSTA1, GSTP1, GSTT1, and GSTM1 and gastric cancer risk in a Vietnamese population. Oncol Res. 2010;18:349-55.
56. Oda Y, Kobayashi M, Ooi A, et al. Genotypes of glutathione S-transferase M1 and N-acetyltransferase 2 in Japanese patients with gastric cancer. Gastric Cancer. 1999;2:158-64.
57. Palli D, Polidoro S, D'Errico M, Saieva C, Guarrera S, Calcagnile AS, et al. Polymorphic DNA repair and metabolic genes: a multigenic study on gastric cancer. Mutagenesis. 2010;25:569-75.
58. Pereira MG, Galvão TF. Heterogeneidade e viés de publicação em revisões sistemáticas. Epidemiol. Serv. Saúde. 2014;23:775-8.
59. Piao JM, Shin MH, Kweon SS, Kim HN, Choi JS, Bae WK, et al. Glutathione-S-transferase (GSTM1, GSTT1) and the risk of gastrointestinal cancer in a Korean population. World J Gastroenterol. 2009;15:5716-21.

60. Qian Y, Xu YC, Shen HB, et al. A molecular epidemiological study on the relationship between glutathione S-transferase M1, T1 genetic polymorphism and susceptibility to gastric cancer. Zhong Guo Gong Gong Wei Sheng. 2001;17:101-3.
61. Qiu LX, Wang K, Lv FF, et al. GSTM1 null allele is a risk factor for gastric cancer development in Asians. Cytokine. 2011;55:122-5.
62. Rebbeck TR. Molecular epidemiology of the human glutathione S-transferase genotypes GSTM1 and GSTT1 in cancer susceptibility. Cancer Epidemiol Biomarkers Prev. 1997;6:733-43.
63. Rodrigues CL, Ziegelmann PK. Metanálise: um guia prático. Rev HCPA. 2010;30:436-47.
64. Roth MJ, Abnet CC, Johnson LL, Mark SD, Dong ZW, Taylor PR, Dawsey SM, Qiao YL. Polymorphic variation of Cyp1A1 is associated with the risk of gastric cardia cancer: a prospective case-cohort study of cytochrome P-4501A1 and GST enzymes. Cancer Causes Control. 2004;15:1077-83.
65. Ruzzo A, Canestrari E, Maltese P, Pizzagalli F, Graziano F, Santini D, et al. Polymorphisms in genes involved in DNA repair and metabolism of xenobiotics in individual susceptibility to sporadic diffuse gastric cancer. Clin. Clin Chem Lab Med. 2007;45:822–8.
66. Saadat I, Saadat M. Glutathione S-transferase M1 and T1 null genotypes and the risk of gastric and colorectal cancers. Cancer Lett. 2001;169:21-6.
67. Santos EJF, Cunha M. Interpretação crítica dos resultados estatísticos de uma meta-análise: Estratégias metodológicas. Millenium. 2013;85-8.
68. Setiawan VW, Zhang ZF, Yu GP, Li YL, Lu ML, Tsai CJ, et al. GSTT1 and GSTM1 null genotypes and the risk of gastric cancer: a case–control study in a Chinese population. Cancer Epidemiol Biomarkers Prev. 2000;9:73-80.
69. Sgambato A, Campisi B, Zupa A, Bochicchio A, Romano G, Tartarone A, et al. Glutathione S-transferase (GST) polymorphisms as risk factors for cancer in a highly homogeneous population from southern Italy. Anticancer Res. 2002;22(6B):3647-52.
70. Shen J, Wang R, Xu X. Application of the interaction models between the polymorphism(s) of metabolic gene(s) and environmental exposure. Zhonghua Liu Xing Bing Xue Za Zhi. 2001;22:61–4.
71. Shen XB, Pu XP, Zhang J, Zhu LJ. Influence of GSTM1 and GSTT1 genotypes and smoking, alcohol exposure on the occurrence of gastric cancer: case-control study from Nanjing, China. Huan Jing Yu Zhi Ye Yi Xue. 2005;22:325-9,382.
72. Shen XB, Zhang J, Zhu LJ et al. Relationship between glutathione S-transferase M1, T1 genetic polymorphisms, smoking and alcohol consumption and susceptibility to stomach cancer. Huan Jing Yu Jian Kang Za Zhi. 2004;21:210-4.
73. Silva AMTC. O polimorfismo do gene p5372 (RP) no câncer de cabeça e pescoço: estudo de associação e meta-análise. Tese de doutorado em Biologia Molecular. Universidade Federal de Goiás. 2009.
74. Silva JAG. Coordenação de Prevenção e Vigilância. Estimativa 2014: Incidência de Câncer no Brasil. Rio de Janeiro: INCA, 2014. [Internet]. [Accessed 2015 november 6]. Available from: http://www.inca.gov.br/estimativa/2014/estimativa-24042014.pdf
75. Strange RC, Matharoo B, Faulder GC, Jones P, Cotton W, Elder JB, Deakin M. The human glutathione S-transferases: a case–control study of the incidence of the GST1 0 phenotype in patients with adenocarcinoma. Carcinogenesis. 1991;12:25-8.
76. Suzuki S, Muroishi Y, Nakanishi I, Oda Y. Relationship between genetic polymorphisms of drug-metabolizing enzymes (CYP1A1, CYP2E1, GSTM1, and NAT2), drinking habits, histological subtypes, and p53 gene point mutations in Japanese patients with gastric cancer. J Gastroenterol. 2004;39:220-230.
77. Tamer L, Ates NA, Ates C, Ercan B, Elipek T, Yildirim H, et al. Glutathione S-transferase M1, T1 and P1 genetic polymorphisms, cigarette smoking and gastric cancer risk. Cell Biochem Funct. 2005;23:267-72.
78. Torres MM, Acosta CP, Sicard DM. Groot de Restrepo H: Genetic susceptibility and risk of gastric cancer in a human population of Cauca. Colombia. Biomedica. 2004;24:153-62.
79. Tripathi S, Ghoshal U, Ghoshal UC, Mittal B, Krishnani N, Chourasia D, et al. Gastric carcinogenesis: possible role of polymorphisms of GSTM1, GSTT1, and GSTP1 genes. Scand J Gastroenterol. 2008;43:431-9.
80. WANG H, et al. Glutathione S-transferase M1 null genotype associated with gastric cancer among Asians. Dig Dis Sci. 2010;55:1824-30.
81. Wang KY, Lin JT, Chen CJ, et al. The relationship between the genotypesm of cancer susceptibility genes and the development of gastric cancer. Zhong Hua Gong Gong Wei Sheng Za Zhi. 1998;17:226-41.
82. Wang WW, Yang WM, Zhang YX, Zhao SY. Research on the relationship of the susceptibility to elderly gastric cancer and GSTM1 gene polymorphism in Hainan. Xian Dai Yu Fang Yi Xue. 2012;39:6273–4.
83. Wideroff L, Vaughan TL, Farin FM, Gammon MD, Risch H, Stanford JL, Chow WH. GST, NAT1, CYP1A1 polymorphisms and risk of esophageal and gastric adenocarcinomas. Cancer Detect Prev. 2007;31:233-6.
84. Wu MS, Chen CJ, Lin MT, Wang HP, Shun CT, Sheu JC, Lin JT. Genetic polymorphisms of cytochrome p450 2E1, glutathione S-transferase M1 and T1, and susceptibility to gastric carcinoma in Taiwan. Int J Colorectal Dis. 2002;17:338-43.
85. Yadav DS, Devi TR, Ihsan R, Mishra AK, Kaushal M, Chauhan PS, et al. Polymorphisms of glutathione-S-transferase genes and the risk of aero digestive tract cancers in the Northeast Indian population. Genet Test Mol Biomarkers. 2010;14:715-23.
86. Yadav D, et al. Glutathione-S-transferase M1 and T1 genes and gastric cancer: a case control study in North Indian population. Gene. 2011;487:166-9.
87. Zendehdel K, Bahmanyar S, McCarthy S, Nyren O, Andersson B, Ye W. Genetic polymorphisms of glutathione S-transferase genes GSTP1, GSTM1, and GSTT1 and risk of esophageal and gastric cardia cancers. Cancer Causes Control. 2009;20:2031-8.
88. Zhang AP, Liu BH, Wang L, Gao Y, Li F, Sun SX: Glutathione S-transferase gene polymorphisms and risk of gastric cancer in a Chinese population. Asian Pac J Cancer Prev. 2011;12:3421-5.
89. Zhang X-L, Cui Y-H. GSTM1 null genotype and gastric cancer risk in the Chinese population: an updated meta-analysis and review. OncoTargets and therapy. 2015;8:969-75.
90. Zhang YC, Deng CS, Zhou Y, Zhu YQ. Association of glutathione S-transferase M1 and T1 genetic polymorphisms with Helicobacter pylori infection and gastric adenocarcinoma. Shi Jie Hua Ren Xiao Hua Za Zhi. 2003;11:1306-9.
91. Zhanga Z, Fud G, Wanga M, Tongc N, Wangb S, Zhanga Z. P53 codon 72 polymorphism and ovarian cancer risk: a meta-analysis. Journal of Nanjing Medical University. 2008;22;279-85.
92. Zhao Y, Deng X, Song G, Qin S, Liu Z. The GSTM1 Null Genotype Increased Risk of Gastric Cancer: A Meta-Analysis Based on 46 Studies. PLoS One. 2013;8:e81403.
93. Zheng ZL. CYP2E1, GSTM1, GSTT1 polymorphisms and susceptibilities to cardia and non-cardia gastric cancer in high-risk area of China, The Master Thesis of Fujian Medical University. 2003.
94. Zheng TR, Zheng QH, Gong FS, Xie YQ, Wang XR. Gene deletion polymorphisms of GSTT1 and GSTM1 and susceptibility to stomach neoplasm. Shi Yong Zhong Liu Za Zhi. 2002;17:155-7.
95. Zhou Q, Zheng ZY, Wang LD, et al. Prevalence of genetic polymorphisms of GSTM1, GSTT1 and GSTP1 in subjects with gastric cardia adenocarcinoma at Linzhou, Henan. Zheng Zhou Da Xue Xue Bao (Yi Xue Ban). 2003;38:327-9.
96. Zhou T, Fan W, Han W, et al. The study of the association of GSTM1 gene polymorphism with susceptibility to gastric cancer. Chin J Curr Adv Gen Surg. 2006;9:355-8.
97. Zhu Y, He Q, Wang J, Pan HF. The association between GSTM1 polymorphism and gastric cancer risk: a meta-analysis. Mol Biol Rep. 2012;39:685-91.

ORIGINAL ARTICLE

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Body composition in patients with Crohn's disease and ulcerative colitis

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ABSTRACT – Background – The nutritional status of individuals with inflammatory bowel diseases is directly related to the severity of the disease and is associated with poor prognosis and the deterioration of immune competence. **Objective –** To assess the nutritional status and the body composition of outpatients with inflammatory bowel diseases. **Methods –** A cross-sectional study was conducted with clinical and nutritional assessment of patients with Crohn's disease and ulcerative colitis. Patients were classified according to the clinical activity through Crohn's Disease Activity Index and Mayo Score. Nutritional assessment consisted of anthropometric measurements of current weight, height, mid-arm circumference, triceps skinfold thickness and thickness of adductor policis muscle, with subsequent calculation of BMI, arm muscle circumference and the mid-arm muscle area (MAMA). The phase angle (PhA) and lean and fat mass were obtained with the use of electrical bioimpedance. Descriptive statistics, chi-square test or Fisher exact test, ANOVA and *t*-test. **Results** – We evaluated 141 patients of which 54 (38.29%) had Crohn's disease and 87 (61.70%) ulcerative colitis. The mean age was 43.98 (±15.68) years in Crohn's disease and 44.28 (±16.29) years for ulcerative colitis. Most of the patients were in clinical remission of the disease (Crohn's disease: 88.89%; ulcerative colitis: 87.36%). Regarding the nutritional classification using BMI, it was found that 48.15% of Crohn's disease patients were eutrophic and 40.74% were overweight or obese; among patients with ulcerative colitis, 52.87% were classified as overweight or obese. When considering the triceps skinfold, it was observed in both groups a high percentage of overweight and obesity (Crohn's disease: 75.93%; ulcerative colitis: 72.42%). Crohn's disease patients showed the most affected nutritional status according to the nutritional variables when compared to patients with ulcerative colitis (BMI: 24.88 kg/m² x BMI: 26.56 kg/m², P=0.054; MAMA: 35.11 mm x MAMA: 40.39 mm, P=0.040; PhA: 6.46° x PhA: 6.83°, P=0.006). **Conclusion** – Patients with inflammatory bowel diseases have a high prevalence of overweight and obesity. Crohn's disease patients had more impaired anthropometric and body composition indicators when compared to patients with ulcerative colitis.

HEADINGS – Inflammatory bowel diseases. Crohn´s disease. Ulcerative colitis. Body composition. Nutritional assessment.

INTRODUCTION

Inflammatory bowel disease (IBD), including Crohn's disease (CD) and ulcerative colitis (UC), consists in a chronic inflammatory disorder of the gut with intestinal and systemic manifestations. The principal symptoms are diarrhea, abdominal pain, gastrointestinal bleeding, weight loss, malnutrition and fatigue. According to United States estimates for CD, incidence varies between 6 and 8 per 100,000 inhabitants, with a prevalence of 100 to 200 per 100,000 inhabitants. In the case of UC, the incidence in that country ranges between 9 and 12, with a prevalence of 205 to 240 per 100,000 inhabitants[15,17,22]. Studies have shown higher rates of incidence of IBD in developing countries, including Brazil[8,34,38].

Inflammatory state can lead to impaired nutritional status of patients with IBD. Undernutrition is a major complication among these patients and it is strongly associated with worst prognostic and increased risk of clinical and surgical complications[26]. Its prevalence may vary from 23% in outpatient units to 85% in hospitalized subjects[8,29]. Factors associated with undernutrition in these patients are inadequate food intake, chronic inflammatory state with increased energy requirements and losses from the gastrointestinal tract[8, 26].

On the other hand, the prevalence of overweight and obesity is increasing among these patients, especially in the last decades[7]. Changes in dietary patterns is pointed out as one of the factors causing cardiovascular diseases and, therefore, this population needs careful nutritional follow-up in order to precociously detect patients that are under nutritional risk.

Because of the abovementioned reasons, nutritional assessment is a key point to the management of IBD patients. The aim of the present study was to evaluate the nutritional status and to evaluate body composition of IBD outpatients.

METHODS

Subjects

A cross-sectional study was performed on outpatients with either CD or UC from March through December 2012. The diagnosis was based on conventional clinical, endoscopic, radiologic and histological criteria[25].Criteria for inclusion were age above 18 years and confirmed diagnosis of IBD. Criteria for exclusion were pregnancy, use of nutritional supplements, chronic diseases such as hepatitis B and C, HIV, chronic kidney disease, heart failure and refusal to participate in the study. The study was approved by the Research Ethics Committee of Botucatu Medical School, Sao Paulo State University (protocol # 4178/2012), and a written informed consent was signed by all subjects before their inclusion.

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Clinical assessment

Patients were evaluated in accordance to the clinical course of the disease. The Crohn's Disease Activity Index (CDAI) was used to assess the disease activity of CD patients[3]. The Montreal Classification was used to classify the disease according to the age of appearance, disease location and presence of complications such as stricture, fistula or perianal disease[32].

Mayo score was used to assess the clinical activity of UC patients[30]. To determine the extent of intestinal damage (distal colitis, left-sided colitis or pancolitis), retrospective data from the first diagnostic colonoscopy were used.

Anthropometric measurements

Nutritional assessment was performed using anthropometric measurements and body composition analysis. All of them were conducted by the sane dietitian. Nutritional assessment included collection of the following information: weight, height, body mass index (BMI), Mid-arm Circumference (MAC), Triceps Skinfold Thickness (TSF), Mid-Arm Muscle Circumference (MAMC), Mid-arm Muscle Area (MAMA) and Adductor Policis Muscle (APM).

The BMI and MAMC were classified according to World Health Organization[39] Lipschitz[20] and Frisancho[10]. The APM was measured according to Lameu et al.[19].The percentages of adequation of MAC, TSF, and MAMC were classified as previously described Blackburn; Harvey[5], Frisancho[11], Harrison[13], Lameu et al.[19]. The anthropometric measurements were taken in the morning, after the patients' clinical evaluation.

Body composition analysis

Bioelectrical impedance analysis (BIA) was measured using a tetrapolar and single-frequency equipment (Biodynamic-450, 800 μA; 50 kHz) and applied to the skin using adhesive electrodes with the subject lying supine. Phase angle (PhA) derived from BIA was determined as previously discussed[18] and its values were calculated as follows: PhA = arc tangent reactance/resistance x (180°/π)[9].

Fat-free mass (FFM) and fat mass (FM) were recorded according to the parameters given by the device. The values obtained were compared with values obtained for the healthy population[31] according to percentile, sex and age. Evaluations of bioelectrical impedance analysis were standardized according to Mattar[23] and Heyward Stolarrczyk[16].

Laboratory tests

Blood samples were routinely drown in order to evaluate hematocrit values, hemoglobin, total protein and albumin, C-reactive protein and erythrocyte sedimentation rate. All determinations followed standardized laboratory techniques.

Statistical analysis

Descriptive statistics were performed. Frequencies and percentages were used for qualitative variables and means and respective standard deviations were calculated for quantitative variables. Chi-square (X^2) tests were applied to investigate the association between CD and UC and the demographic variables, outcomes, medication and nutritional parameters. Fisher's exact test was used when the expected values were lower than five, with a statistically significant association when $P \leq 0.05$. In order to compare DC and UC with nutritional parameters, ANOVAs were carried out. The *t*-test was used to compare the groups for the variables with normal distribution. *P* value <0.05 was considered statistically significant. Statistical analysis were performed using SAS for Windows (version 9.1).

RESULTS

One hundred and forty-one patients were evaluated from March to December 2012. Among these, 54 (38.29%) had CD and 87 (61.70%) had UC.

Sample description

With respect to Crohn's disease, the average age of patients was 43.98 (±15.68) years and 59.26% were female. The majority of patients were in clinical remission of the disease, according to the CDAI. According to the Montreal classification, most of patients had complications of the disease, such as stenosing behavior (48.15%), penetrating behavior (27.78%) or perianal disease (40.74%).

Regarding UC, the average age of the patients was 44.28 (±16.29) years and 55.17% were women. Among these, 83.92% were in clinical remission according to the Mayo score. Regarding the extent of the disease, 45.98% had pancolitis, 31.03% had distal colitis and 22.99% had left-sided colitis.

Nutritional status and body composition of patients with Crohn's disease in contrast with patients with ulcerative colitis

The comparative analysis of nutritional status and body composition of patients with CD and UC found that patients with CD have characteristics typical of more impaired nutritional status when compared to patients with UC, as well as increased inflammatory activity evaluated by means of laboratory tests such as CRP and ESR (Table 1). Lower BMI, MAMC, MAMA (*P*=0.04), APM, lean body mass index (*P*=0.03), phase angle (*P*=0.006) and laboratory tests such as hematocrit (*P*=0.01) were verified in patients with CD.

TABLE 1. Comparative analysis of nutritional and laboratory parameters of patients with Crohn's disease (CD) and ulcerative colitis (UC)

Variables (n=141)	CD (n=54) Mean (MD)	UC (n=87) Mean (MD)	*P*-value
Average age (years old)	43.981(± 15.68)	44.28 (± 16.29)	0.91
Anthropometry			
BMI (kg/m²)	24.88(± 4.70)	26.56 (± 5.16)	0.054
MAC (cm)	29.74 (± 4.29)	30.38 (± 4.28)	0.39
TSF (mm)	21.68 (± 10.14)	19.83 (± 8.77)	0.25
MAMC (%)	90.64 (± 13.43)	95.46 (± 14.71)	0.052
MAMA (cm²)	35.11 (± 11.99)	40.39 (± 16.20)	0.040
APM (%)	105.12 (± 46.32)	118 (± 55.93)	0.16
Body composition			
PhA (°)	6.46 (± 0.76)	6.83 (± 0.80)	0.006
LM (%)	71.80 (± 8.49)	71.01 (± 7.28)	0.55
FM (%)	28.19 (± 8.49)	29.98 (± 7.28)	0.55
LBMI (kg/m²)	17.61 (± 2.74)	18.65 (± 2.77)	0.03
FBMI (kg/m²)	7.23 (± 2.99)	7.93 (± 3.29)	0.20
Laboratory tests			
Hb (g/dL)	13.10 (± 2.04)	13.85 (± 2.10)	0.055
Ht (%)	39.68 (± 5.56)	42.09 (± 4.93)	0.014
TP (g/dL)	7.37 (± 0.71)	7.37 (± 1.10)	0.96
Alb (g/dL)	4.01 (± 0.58)	4.14 (± 0.63)	0.26
CRP (mg/dL)	3.28 (± 5.42)	2.02 (± 3.16)	0.12
ESR (mm/h)	26.45 (± 16.66)	18.05 (± 17.96)	0.012

BMI: body mass index; MAC: mid-arm circumference; cm: centimeters; TSF: triceps skinfold thickness; mm: millimeters; MAMC: mid-arm muscle circumference; MAMA: mid-arm muscle area; APM: thickness of the adductor pollicis muscle; PhA: phase angle; LM: lean mass; FM: fat mass; LBMI: lean body mass index; FBMI: fat body mass index; kg: kilogram; Hb: hemoglobin; Ht: hematocrit; TP: total protein; Alb: albumin; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate.

On the classification of nutritional status, the body mass index (BMI) analysis showed that 11.11% CD patients had low weight, 48.15% were eutrophic and 40.74% were overweight and obese. In the case of patients with UC, 6.90% were underweight, 40.23% were eutrophic and 52.87% were overweight and obese (Table 2). Regarding the classification of nutritional status according to the Mid-arm circumference (% MAC) and the Mid-Arm Muscle Circumference (% MAMC), it was observed that most patients in both groups were eutrophic (Table 2).

TABLE 2. Nutritional status classification by body mass index (BMI), mid-arm circumference (MAC), triceps skinfold thickness (TSF), mid--arm muscle circumference (MAMC), mid-arm muscle area (MAMA) and thickness of adductor policis muscle (APM) of patients with Crohn's disease and ulcerative colitis

Variables	Crohn's disease (n=54)	Ulcerative colitis (n=87)	*P*-value
BMI classification (%)			0.5304
Low weight, n (%)	6 (11.11)	6 (6.90)	
Eutrophic, n (%)	26 (48.15)	35 (40.23)	
Overweight, n (%)	15 (27.78)	32 (36.78)	
Obesity, n (%)	7 (12.96)	14 (16.09)	
MAC classification (%)			0.7949
Severe malnutrition, n (%)	1 (1.85)	1 (1.15)	
Moderate malnutrition, n (%)	2 (3.70)	5 (5.75)	
Mild malnutrition, n (%)	9 (16.67)	8 (9.20)	
Eutrophic, n (%)	30 (55.56)	52 (79.77)	
Overweight, n (%)	8 (14.81)	12 (13.79)	
Obese	4 (7.41)	9 (10.34)	
TSF classification (%)			0.8965
Severe malnutrition, n (%)	3 (5.56)	6 (6.90)	
Moderate malnutrition, n (%)	2 (3.70)	4 (4.60)	
Mild malnutrition, n (%)	7 (12.96)	9 (10.34)	
Eutrophic, n (%)	1 (1.85)	5 (5.75)	
Overweight, n (%)	3 (5.56)	4 (4.60)	
Obese, n (%)	38 (70.37)	59 (67.82)	
MAMC classification (%)			0.1561
Severe malnutrition, n (%)	5 (9.26)	2 (2.30)	
Moderate malnutrition, n (%)	7 (12.96)	9 (10.34)	
Mild malnutrition, n (%)	13 (24.07)	21 (24.14)	
Eutrophic, n (%)	29 (53.70)	50 (57.47)	
Obese, n (%)	0.00	5 (5.75)	
MAMA classification (%)			0.0261
Severe malnutrition	10 (18.52)	6 (6.98)	
Moderate/Mild malnutrition	13 (24.07)	13 (15.12)	
Eutrophic	31 (57.41)	67 (77.91)	
APM classification (%)			0.2516
No depletion, n (%)	23 (43.40)	49 (57.65)	
Depletion light, n (%)	9 (16.98)	7 (8.24)	
Moderate depletion, n (%)	9 (16.98)	15 (17.65)	
Severe depletion, n (%)	12 (22.64)	14 (16.47)	

BMI: body mass index; MAC: mid-arm circumference; TSF: triceps skinfold thickness; MAMC: mid-arm muscle circumference; MAMA: mid-arm muscle area; APM: thickness of the adductor pollicis muscle.

In relation to the nutritional status by the parameter Triceps Skinfold Thickness (TSF%), a high percentage of obesity was observed in both groups and a small portion was classified as malnourished (Table 2). It was also observed, according to the Mid-Arm Muscle Area, that 42.59% CD patients were under mild, moderate or severe malnutrition and 22.10% patients with UC were under mild, moderate or severe malnutrition. According to the classification of nutritional status by the Thickness of Adductor Policis Muscle (APM), 22.64% CD patients had severe muscle depletion, while 16.47% patients with UC had this condition (Table 2).

Nutritional status and body composition of patients with Crohn's disease in activity contrasted to patients in clinical remission

When comparing patients with active CD and patients in clinical remission (Table 3), it was found that patients with the active disease were younger than patients in remission (P=0.008). Patients in clinical activity were observed to have lower averages of anthropometric variables, as observed for BMI (P<0.001), MAC (P=0.01), MAMC (P=0.04) and APM (P=0.009).

TABLE3. Anthropometric analysis, body composition and laboratory parameters of patients with Crohn's disease in activity and in clinical remission

Variables	Crohn's disease		*P*-value
	Activity n=24	Remission n=30	
Age (years old)	37.83 (± 12.81)	48.90 (± 16.21)	0.008
Female gender, n (%)	16.00 (66.67)	16.00 (53.33)	0.3218
Anthropometry			
BMI (kg/m²)	22.66 (± 4.32)	26.66 (± 4.28)	0.001
MAC (cm)	28.12 (± 3.85)	31.03 (± 4.24)	0.01
TSF (mm)	19.83 (± 9.76)	23.16 (± 10.36)	0.23
MAMC (%)	86.55 (± 12.21)	93.92 (± 13.65)	0.043
MAMA (cm²)	31.84 (± 10.74)	37.74 (± 12.45)	0.07
APM (%)	86.54 (± 37.87)	119.36 (± 47.70)	0.009
Body composition			
PhA (°)	6.42 (± 0.73)	6.49 (± 0.79)	0.75
LM (%)	73.86 (± 9.26)	70.15 (± 7.57)	0.11
FM (%)	26.13 (± 9.26)	29.84 (± 7.57)	0.11
LBMI (kg/m²)	16.45 (± 2.21)	18.54 (± 2.80)	0.004
FBMI (kg/m²)	6.21 (± 2.82)	8.05 (± 2.90)	0.02
Laboratory tests			
Hb (g/dL)	12.36 (± 2.22)	13.76 (± 1.63)	0.01
Ht (%)	37.77 (± 6.12)	41.37 (± 4.50)	0.02
TP (g/dL)	7.48 (± 0.81)	7.28 (± 0.62)	0.36
Alb (g/dL)	3.93 (± 0.74)	4.08 (± 0.36)	0.34
CRP (mg/dL)	4.06 (± 6.27)	2.64 (± 4.63)	0.39
ESR (mm/h)	30.81 (± 19.14)	22.61 (± 13.36)	0.09

BMI: body mass index; MAC: mid-arm circumference; TSF: triceps skinfold thickness; MAMC: mid-arm muscle circumference; MAMA: mid-arm muscle area; APM: thickness of the adductor pollicis muscle; PhA: phase angle; LM: lean mass; FM: fat mass; LBMI: lean body mass index; FBMI: fat body mass index; Hb: hemoglobin; Ht: hematocrit; TP: total protein; Alb: albumin; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate.

Nutritional status and body composition of patients with ulcerative colitis in activity contrasted to patients in clinical remission

Patients with active disease were younger than patients in remission (P=0.01). Regarding the anthropometric variables, lower averages in patients in clinical activity were observed, as showed BMI (P=0.01), MAC (P=0.001), MAMC% (P=0.01) and MAMA (P=0.02). Regarding the evaluation of body composition, significant differences were found between patients in activity and patients in remission as the % LM (P=0.01), % FM (P=0.01) and FBMI (P=0.009) (Table 4).

TABLE 4. Anthropometric analysis. body composition and laboratory parameters of patients with ulcerative colitis in activity and in clinical remission

Variables	Ulcerative colitis		*P*-value
	Activity n=21	Remission n=66	
Age (years old)	36.42 (± 15.59)	46.78 (± 15.81)	0.01
Female gender, n (%)	11.00 (52.38)	37.00 (56.06)	0.7677
Anthropometry			
BMI (kg/m²)	24.28 (± 4.21)	27.28 (± 5.25)	0.01
MAC (cm)	27.86 (± 4.92)	31.18 (± 3.75)	0.001
TSF (mm)	17.28 (± 7.77)	20.64 (± 8.97)	0.12
MAMC (%)	88.67 (± 17.47)	97.62 (± 13.14)	0.01
MAMA (cm²)	33.41 (± 15.05)	42.61 (± 16.03)	0.02
APM (%)	108.04 (± 44.11)	121.06 (± 59.06)	0.36
Body composition			
PhA (°)	6.61 (± 0.82)	6.90 (± 0.78)	0.14
LM (%)	74.51 (± 7.38)	69.88 (± 6.94)	0.01
FM (%)	25.48 (± 7.38)	30.11 (± 6.94)	0.01
LBMI (kg/m²)	17.98 (± 2.80)	18.87 (± 2.75)	0.20
FBMI (kg/m²)	6.32 (± 2.50)	8.45 (± 3.36)	0.009
Laboratory tests			
Hb (g/dL)	12.82 (± 3.09)	14.21 (± 1.51)	0.01
Ht (%)	39.91 (± 6.66)	42.82 (± 4.02)	0.02
TP (g/dL)	7.23 (± 0.94)	7.42 (± 1.16)	0.59
Alb (g/dL)	4.01 (± 0.46)	4.19 (± 0.68)	0.38
CRP (mg/dL)	1.33 (± 0.85)	2.22 (± 3.54)	0.34
ESR (mm/h)	21.19 (± 14.37)	17.10 (± 18.93)	0.42

BMI: body mass index; MAC: mid-arm circumference; TSF: triceps skinfold thickness; MAMC: mid-arm muscle circumference; MAMA: mid-arm muscle area; APM: thickness of the adductor pollicis muscle; PhA: phase angle; LM: lean mass; FM: fat mass; LBMI: lean body mass index; FBMI: fat body mass index; Hb: hemoglobin; Ht: hematocrit; TP: total protein; Alb: albumin; CRP: C-reactive protein; ESR: erythrocyte sedimentation rate.

DISCUSSION

Nutritional assessment is an important point for success in the management of patients with IBD because it can help identifying shortcomings or even nutritional excesses[24]. The first studies on nutritional assessment in IBD identified malnutrition as the main characteristic in these individuals[21]. Malnutrition may be the result of increased losses or malabsorption due to the extent of the bowel portion affected by the disease, surgical resections, fistula or other factors that determine the functional or anatomical reduction of intestinal absorptive surface and/or acceleration of intestinal transit[8,26].

Low rates of malnutrition were found in the present study according to the BMI, and most of the patients were classified as eutrophic when they were evaluated by MAC, MAMC or MAMA. When evaluated by the APM, 43.40% of patients with CD and 57.65% of patients with UC were found without muscle depletion. This can be explained by the presence of a larger number of subjects in clinical remission and by the fact that the study was conducted with outpatients. However, we observed lower values of nutritional parameters such as BMI, MAC and MAMC in patients with active disease when compared with patients in clinical remission, for both CD and UC patients, probably due to the increased energy demand related to inflammation and lower food intake due to the symptoms of IBD.

We observed high rates of overweight and obesity among patients with CD and UC according to BMI, TSF and MAC. Obesity, once considered a rare condition in IBD, has become increasingly prevalent, mainly in the last two decades[2,7]. It is believed that this is related to increased number of comorbidities, such as diabetes, hypertension, thromboembolic disease, infections, cancer[7]. It is also associated with increased risk of complications such as advanced age at diagnosis, greater number of relapses of the disease, perianal disease incidence, more frequent hospitalizations[6] and postoperative complications, including wound infection and dehiscence of surgical anastomoses[35]. CD patients classified as overweight or obese (BMI >25 kg/m²) required surgical procedure for the treatment of the disease in earlier stage (24 months), as a result of complications, when compared to patients with low weight (BMI <18.5 kg/m²) (252 months)[14]. An observational study conducted in Scotland in 2009 found that 18% of study participants with IBD were considered obese (BMI >30 kg/m²) and there was a significantly higher number of obese patients with CD than with UC (P=0.05)[35]. A previous study[6] reported earlier rates of 3% of obesity in DC, 32.4% of this population showing a BMI ≥25 kg/m². Comparison of the data suggests a drastic change in BMI of patients from the studies, demonstrating an increase in the rate of obese patients, as it has been observed in the general population[35].

Obesity is also a risk factor for developing cardiovascular diseases. Studies indicate an increased risk of developing cardiovascular disease in IBD patients as compared to non-IBD patients[12,28], what is probably associated with chronic inflammation. A Danish cohort study comparing individuals with and without IBD found a significantly higher risk of ischemic heart disease in the first year after diagnosis of IBD in patients. Furthermore, higher risk was observed among women than among men (P=0.03). A meta-analysis of nine studies observed an increase of 18% in the risk of stroke and ischemic heart disease in patients with IBD. The increased risk of stroke and ischemic heart disease was more prominent in females when compared to males, probably because of the inherent differences and non-modifiable risk factors[33].

Recently, obesity itself has been studied as a potential risk factor for the development of CD[7]. In a case-control research that evaluated patients with CD, UC and healthy controls, the authors found a significant association between the diagnosis of CD and obesity, even after adjusting for other known risk factors for the development of CD, such as age, smoking history, family history of the disease and history of appendectomy[36].

Nutritional assessment consists of evaluating the nutritional status and body composition. Body composition assessment done

through anthropometric measurements and bioelectrical impedance analysis (BIA) are valuable tools to identify nutritional status due to its low cost and easy applicability[1,4,27].It is worth noting that the gold standard method for assessing body composition is the Dual-Energy X-Ray Absorptiometry (DEXA), which allows direct and non-invasive measurement of bone mass, fat-free mass and fat mass. However, DEXA requires skilled personnel, has low affordability, high radiation exposure and it is considered a costly examination. In the present study, we used the BIA to assess body composition. Studies show good correlation between the parameters of body composition assessed by BIA and by DEXA[37]. The BIA is an inexpensive examination, noninvasive, easy to apply and does not use ionizing radiation. Furthermore, it provides the calculation of the phase angle, which is the parameter used with prognostic purposes in some chronic diseases.

We must emphasize the study participants should not be considered representative of the total population with IBD, once they come from a tertiary hospital with peculiar characteristics. Other limiting factors should be mentioned, as the sample size and the kind of design adopted (cross-sectional). Longitudinal studies with more patients are needed so that the real significance of the results is confirmed. Despite this need, nutritional guidance programs can already be adopted and encouraged with the aim of preventing or correcting nutritional deficits for the population under study.

CONCLUSION

In relation to nutritional status and body composition, it was found that patients with IBD showed high prevalence of overweight and obesity. The majority of the patients were in clinical remission. As for comparing the nutritional status among patients with CD and UC, it was found that patients with CD had anthropometric and body composition parameters more impaired than UC patients.

In spite of the limitations, the results of this study increase the knowledge about the importance of assessing the nutritional status of patients with Crohn's disease or ulcerative colitis and underscore the importance of the multidisciplinary team performance in the treatment centers.

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Authors' contributions

Back IR: collected data and design of the manuscript. Marcon SS: critical revision. Gaino NM: collected data. Vulcano DSB: design of the manuscript. Dorna MS: design of the manuscript. Sassaki LY: conception and design of the manuscript.

Back IR, Marcon SS, Gaino NM, Vulcano DSB, Dorna MS, Sassaki LY. Composição corporal de pacientes com doença de Crohn e colite ulcerativa. Arq Gastroenterol. 2017;54(2):109-14.

RESUMO – Contexto – O estado nutricional de indivíduos com doença inflamatória intestinal está diretamente relacionado à gravidade da doença e associado a mau prognóstico e deterioração da competência imune. **Objetivo –** Avaliar o status e a composição corporal de pacientes ambulatoriais com doença inflamatória intestinal. **Metódos –** Foi conduzido um estudo transversal com avaliação clínica e nutricional de pacientes com doença de Crohn e colite ulcerativa. Pacientes foram classificados de acordo com o índice de atividade clínica *Crohn's Disease Activity Index* e escore de *Mayo*. Avaliação nutricional foi composta peso atual, estatura, circunferência do braço, dobra cutânea tricipital e espessura do músculo adutor do polegar. Posteriormente, foram calculados índice de massa corporal, circunferência muscular do braço e área muscular do braço corrigida. O ângulo de fase e massa magra e massa gorda foram derivadas da bioimpedância elétrica. Foram realizados análise descritiva, teste de qui-quadrado ou exato de Fisher, teste *t* e ANOVA. **Resultados** – Foram avaliados 141 pacientes, sendo 54 (38,29%) com doença de Crohn e 87 (61,70%) com colite ulcerativa. A idade média foi de 43,98 (±15,68) anos em pacientes com doença de Crohn e 44,28 (±16,29) anos em pacientes com colite ulcerativa. A maioria dos pacientes estava em remissão clínica da doença (doença de Crohn: 88,89%; colite ulcerativa: 87,36%). O estado nutricional de acordo com o IMC foi 48,15% eutrófico e 40,74% sobrepeso/obesidade para doença de Crohn; entre os indivíduos com colite ulcerativa, 52,87% foram classificados como sobrepeso/obesidade. Ao se considerar dobra cutânea do tríceps, observou-se obesidade em ambos os grupos (doença de Crohn 75,93%; colite ulcerativa: 72,42%). Pacientes com doença de Crohn apresentam maiores variações de composição corporal quando comparados com pacientes com colite ulcerativa (IMC: 24,88 kg/m² x IMC: 26,56 kg/m², *P*=0,054; área do músculo do braço: 35,11mm x área do músculo do braço: 40,39 mm, *P*=0,040; ângulo de fase: 6,46° x ângulo de fase: 6,83°, *P*=0,006). **Conclusão** – Pacientes com doença inflamatória intestinal apresentaram alta prevalência de sobrepeso e obesidade. Indivíduos com doença de Crohn apresentaram parâmetros de composição corporal e de antropometria mais comprometidos, quando comparados com indivíduos com colite ulcerativa.

DESCRITORES – Doenças inflamatórias intestinais. Doença de Crohn. Colite ulcerativa. Composição corporal. Avaliação nutricional.

REFERENCES

1. Acuña K, Cruz T. Avaliação do Estado Nutricional de Adultos e Idosos e Situação Nutricional da População Brasileira. Arq Bras Endocrinol Metab. 2004;48:345-61.
2. Andrade MIS, Maio R, Dourado KF, Macêdo PFC Barreto Neto AC. Excessive weight – muscle depletion paradox and cardiovascular risk factors in outpatients with inflammatory bowel disease. Arq Gastroenterol. 2015;52:37-45.
3. Best WR, Becktel JM, Singleton JW, Kern F Jr. Development of a Crohn's disease activity index. National Cooperative Crohn's Disease Study. Gastroenterology.1976;70:439-44.
4. Bin CM. Comparação entre Método Funcional com Avaliação Subjetiva Global, Antropometria, Inquérito Alimentar e Análise Bioquímica na Estimativa do Estado Nutricional de Pacientes com Doença de Crohn em Remissão Clínica. [Dissertation]. Universidade Federal do Rio Grande do Sul, Faculdade de Medicina, Porto Alegre, 2007.
5. Blackburn GL, Harvey KB. Nutritional assessment as a routine in clinical medicine. Postgraduate Medicine. 1982;71:46-63.
6. Blain A, Cattan S, Beaugerie L, Carbonnel F, Gendre JP, Cosnes J. Crohn's disease clinical course and severity in obese patients. Clin Nutr. 2002;21:51–7.

7. Boutros M, Maron D. Inflammatory Bowel Disease in the Obese Patient. Clinics in Colonand Rectal Surgery. 2011;24:244-52.
8. Elia PP, Fogaça HS, Barros RG, Zaltman C, Elia CSC. Descriptive analysis of the social, clinical, laboratorial and anthropometric profiles of inflammatory bowel disease inwards patients from the "Clementino Fraga Filho" University Hospital, Rio de Janeiro, RJ, Brazil. Arq Gastroenterol. 2007;44:332-9.
9. Flora APL, Dichi I. Current aspects of nutritional therapy in inflammatory bowel disease. Rev Bras Nutr Clin. 2006;21:131-7.
10. Frisancho AR. Anthropometric standards for the assessment of growth andnutritional status. Michigan: University of Michigan;1990.
11. Frisancho AR. New norms of upper limb fat and muscle areas for assessment of nutritional status. Am J Clin Nutr. 1981;34:2540-5.
12. Gandhi S, Narula N, Marshall JK, Farkouh M. Are Patients with Inflammatory Bowel Disease at Increased Risk of Coronary Artery Disease? Am J Med. 2012;125:956-62.
13. Harrison GG, Buskirk ER, Lindsay Carter JE, Johnston FE, Lohman TG, Pollock ML. Skinfold thicknesses and measurement technique. In: Lohman TG, Roche AF, Martorell R. Anthropometric standardization reference manual. Champaign: Human Kinetics.1988; p.55-70.
14. Hass DJ, Brensinger CM, Lewis JD, Lichtenstein GR. The impact of increased body mass index on the clinical course of Crohn's disease. Clin Gastroenterol Hepatol. 2006;4:482–8.
15. Herrington LJ, Liu L, Lewis JD, Griffin PM, Allison J. Incidence and prevalence of inflammatory bowel disease in a Northern California managed care organization, 1996–2002. Am J Gastroenterol. 2008;103:1998-2006.
16. Heyward VV, Stolarczyk LM. Métodos de dobras cutâneas. In: Heyward VV, Stolarczyk LM. Avaliação da Composição Corporal aplicada: São Paulo: Manole; 2000; 23-45.
17. Kappelman MD, Rifas-Shiman SL, Kleinman K, Ollendorf D, Bousvaros A, Grand RJ, Finkelstein JA. The prevalence and geographic distribution of Crohn's disease and ulcerative colitis in the United States. Clin Gastroenterol Hepatol. 2007;5:1424-9.
18. Kyle UG, Soundar EP, Genton L, Pichard C. Can phase angle determined by bioelectrical impedance analysis assess nutritional risk? A comparison between healthy and hospitalized subjects. Clin Nutr. 2012;31: 875-81.
19. Lameu EB, Gerude MF, Corrêa RC, Lima KA. Adductor policis muscle: a new anthropometric parameter. 2004;39:57-62.
20. Lipschitz DA. Screening for nutritional status in the elderly. Prim Care. 1994;21:55-67.
21. Lochs, H. Basics in Clinical Nutrition: Nutritional support in inflammatory bowel disease. e-SPEN, the European e-Journal of Clinical Nutrition and Metabolism.2010; 8. p. 100-3.
22. Loftus CG, Lofus EV, Harmsen WS, Zinsmeister AR, Tremaine WJ, Melton LJ 3rd, Sandborn WJ. Update on the incidence and prevalence of Crohn' s disease and ulcerative colitis in Olmsted County, Minnesota, 1940-2000. Inflamm Bowel Dis. 2007;13:254-61.
23. Mattar R. Avaliação da Composição Corporal por bioimpedância: uma nova perspectiva. Rev Âmbito Méd Desp. 1997.
24. Mijac DD, Janković GL, Jorga J, Krstić MN. Nutritional status in patients with active inflammatory bowel disease: Prevalence of malnutrition and methods for routine nutritional assessment. Eur J Intern Med. 2010;21:315-9.
25. O'Morain C, Tobin A, Leen E, Suzuki Y, O' Riordan T. Criteria of case definition in Crohn's disease and ulcerative colitis. Scand J Gastroenterol. 1989;170:7-11.
26. Rocha R, Santana GO, Almeida N, Lyra AC. Analysls of fat and muscle mass in patients with inflammatory bowel disease during remission and active phase. J Nutr. 2008;101:676-9.
27. Royall D, Greenberg GR, Allard JP, Baker JP, Harrison JE, Jeejeebhoy KN. Critical assessment of body-composition measurements in malnourished subjects with Crohn's disease: the role of bioelectric impedance analysis. J Clin Nutr. 1994;59:325-30.
28. Rungoe C, Basit S, Ranthe MF, Wohlfahrt J, Langholz E, Jess T. Risk of ischaemic heart disease in patients with inflammatory bowel disease: a nationwide Danish cohort study. Gut. 2013;62:689-94.
29. Salviano FN, Burgos MGPA, Santos EC. Perfil socioeconômico e nutricional de pacientes com doença inflamatória intestinal internados em um hospital universitário. Arq. Gastroenterol. 2007;44:99-106.
30. Schroeder KW, Tremaine WJ, Ilstrup DM. Coated oral 5-aminosalicylicacid therapy for mildly to moderately active ulcerative colitis. A randomized study. N Engl J Med. 1987;317:1625-9.
31. Schutz Y, Kyle UUG, Pichard C. Fat-free mass index and fat mass index percentiles in Caucasians aged 18-98y. Int J Obes Relat Metab Disord. 2002; 26:953-60.
32. Silverberg MS, Satsangi J, Ahmad T, Arnott ID, Bernstein CN, Brant SR, et al. Toward an integrated clinical, molecular and serological classification of inflammatory bowel disease: Report of a Working Party of the 2005 Montreal World Congress of Gastroenterology. Can J Gastroenterol. 2005;Suppl A:5A-36A.
33. Singh S, Singh H, Loftus EV Jr, Pardi DS. Risk of Cerebrovascular Accidents and Ischemic Heart Disease in Patients with Inflammatory Bowel Disease: A Systematic Review and Meta-analysis. Clin Gastroenterol Hepatol. 2014;12:382-93.
34. Souza MH, Troncon LE, Rodrigues CM, Viana CF, Onofre PH, Monteiro RA, et al. Trends in the occurrence (1980-1999) and clinical features of Crohn's disease and ulcerative colitis in a university hospital in southeastern Brazil. Arq Gastroenterol. 2002;39:98-105.
35. Steed H, Walsh S, Reynolds N. A brief report of the epidemiology of obesity in the inflammatory bowel disease population of Tayside, Scotland. Obes Facts. 2009;2:370-2.
36. Suibhne TN, Raftery TC, McMahon O, Walsh C, O'Morain C, O'Sullivan M. High prevalence of overweight and obesity in adults with Crohn´s disease: Associations with disease and lifestyle factors. J Crohns Colitis. 2013;7:241-8.
37. Thibault R, Genton L, Pichard C. Body composition: Why, when and for who? Clin Nutr. 2012;31:435-47.
38. Victoria CR, Sassaki LY, Nunes HRC. Incidence and prevalence rates of inflammatory bowel diseases, in Midwestern of São Paulo state, Brazil. Arq Gastroenterol. 2009;46:20-5.
39. World Health Organization. Obesity: preventing and managing the global epidemic. Geneva: WHO, 1998 (Reports of WHO).

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SIM Brasil study – Women's Gastrointestinal Health: gastrointestinal symptoms and impact on the Brazilian women quality of life

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ABSTRACT – **Background** – Gastrointestinal symptoms seem to affect more women, due to hormonal and emotional issues, impacting the quality of life. The emotional state can affect the bowel functioning through a bidirectional communication system between the gut and the brain involving the neuroendocrine system. Altered bowel functioning and gastrointestinal symptoms can alter quality of life. **Objective** – The SIM study aimed to describe, characterize and quantify gastrointestinal symptoms reported by Brazilian women, their causes, feelings and impact. **Methods –** A structured electronic questionnaire was developed following qualitative phase for semantic formatting, and was administered to volunteer women in ten Brazilian cities. Descriptive and Bayesian statistics analyses were used. **Results –** From the 3029 respondent, 66% reported gastrointestinal symptoms. The most prevalent symptoms were gases (46%), abdominal distention and constipation (43%). The main causes were lifestyle and eating habits. Gastrointestinal symptoms affected quality of life in most women (62%), especially constipation (mood (89%), concentration (88%) and sexual life (79%)). Most common solutions were drinking water, teas, eating foods rich in fiber and probiotics. **Conclusion –** Gastrointestinal symptoms are highly prevalent in Brazilian women and negatively impact different aspects of quality of life (mood, concentration and sexuality). The bowel is an important emotional catalyst that can modulate the psychologic behavior. Better understanding of the interaction between the gut and the brain should help in the management of gastrointestinal symptoms to improve women's quality of life.

HEADINGS – Gastrointestinal tract. Women's health. Quality of life. Surveys and questionnaires.

INTRODUCTION

Gastrointestinal (GI) symptoms are quite frequent in the population, and some of the symptoms are common, such as constipation, diarrhea, bloating, abdominal pain and distension, although their prevalence are little known, mainly in Latin countries[4,10,18,23,33,36,38,40]. Gastrointestinal symptoms encompass different digestive symptoms that are widely observed in the general population[38,40]. The type, frequency and intensity of these symptoms vary between subjects[23], and may impact their daily life[38]. Abdominal pain/discomfort and bloating are often reported as the more troublesome and frequent symptoms, especially in patients with irritable bowel syndrome (IBS)[4,36]. Due to hormonal issues, it is assumed that women are most affected by them, especially with regard to constipation[32].

When these symptoms, generally associated to multiple factors, cannot be explained by any abnormality in the structure of the organs or biochemical markers, the GI symptoms is defined as a functional bowel disorder, such as irritable bowel syndrome (IBS) and functional constipation[6,10,12,21]. When the etiology of the problem is related to the structural or biochemical function, the problem is defined as organic[10]. According to Costa et al.[10], differentiate a functional bowel disorder from an organic disease is essential to alleviate the impact on the lives of patients and the public health service.

The installation of functional bowel disorders is directly related to lifestyle, and insufficient physical activity and poor diet are factors that can determine this scenario[16,32]. The emotional and stress state of the subject, factors comprising the lifestyle, are also closely related to GI symptoms[17,20,30]. Women seem to be more affected emotionally and have their quality of life (QoL) more affected by them than men[1].

The perception of the bowel may be affected by the emotional state of the individual. Currently, the study of the relationship between the bowel and brain is being widely spread. In the gut-brain axis, there is clear reciprocal relationship between these two major organs, where the operation of one influences the other. The corticotropin-releasing hormone - serotonin - is an important mediator of stress responses, influencing the brain and bowel[17,20,21,30]. The neuroendocrine system, and in particular, serotonin, are currently considered relevant factors in the context of GI symptoms because they relate to the functioning of the bowel[10,14,22,24]. The literature has shown that the composition of the intestinal microbiota also interferes with brain activity in humans, especially in the regions that control the sensations and emotions[39].

Understanding how the QoL of healthy subjects can be affected by GI symptoms is an expanding area of research[18], with experimental designs drawn by the fascinating organic relationship of the gut-brain axis. In this context, this study is justified by the scarce scientific literature on intestinal health of Brazilian women,

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especially in relation to the prevalence of GI symptoms and their impact on the QoL of the female population. The purposes of this study is describe, characterize and quantify the main GI symptoms reported by Brazilian women, their causes, the sensations related and the impact of these GI symptoms on the Brazilian women QoL, as well as the solutions adopted by women against the GI symptoms.

METHODS

Observational study on a national scale, conducted in two phases, as follows.

Qualitative phase

This stage aimed to characterize the main profiles of women who reported GI symptoms and identify how these women expressed such problems, through sensations and images. Individual interviews with psychologists (lasting an hour and a half) and individual journaling for 15 days were conducted with 21 female volunteers (aged from 25 to 70 years, in the city of São Paulo). At this stage, through the journal, the women were able to report in depth the feelings in the very moment they were experiencing them, also expressing their feelings through drawings and images. In the interviews, it was possible to access and discuss the individual experiences of each one of them.

Quantitative phase

Based on the results obtained in the qualitative phase, we structured a questionnaire entitled "Tell us the story of your latest GI problem" with 49 questions. At the beginning, through a list of symptoms, the women were asked to indicate the different GI symptoms that might concern them in general and to report whether they were "often", "occasionally" or "not at all" concerned by GI symptoms. After that, the questionnaire focused on the most recent GI symptom and asked to the women identified it from a list of usual symptoms previously described, associating this GI symptom with a series of pictures and images representing their sensations during the episode[7,8].

The questionnaire was very didactical including general instructions at the beginning and at the sub sections in order to facilitate the understanding and the questions were focused on time, place of occurrence, severity, supposed trigger and consequences (social, emotional and physical) of the last GI symptom experienced, including the main strategies adopted to management the situation. The language was friendly and the structure was multiple choice. Here is an example of a question and the possible answers showed in Figure 1.

Today/yesterday		In the morning	
One week ago		In the afternoon	
2 weeks ago		In the evening	
1 month ago		In my sleep (night time or nap)	
More than one month ago			
In the week		I was at home	
At the weekend		I was outside home	

FIGURE 1. Example of possible answers, where only one choice was possible, to the questionnaire question: "Describe your circumstances when your latest GI problem you are telling us about started"

The questionnaire was made available electronically (web survey), self-administered, for women to access and fill in their homes, what facilitated the ability to complete the survey in the privacy of their homes and anonymously which encourages more truthful answers and the ability to complete the survey at the respondent's convenience.

In this data collection way (web survey), the respondents were not influenced by the interviewer, and the information could be collected in a variety of locations from a large number of respondents (sample size) with increased power of the statistical analysis. The sample consisted of 3.029 women who were selected voluntarily, to achieve a number of 2.000 women who reported some GI symptom. Pregnant women and those who had very serious digestive complaints were excluded. The selected women were from ten Brazilian capitals (São Paulo, Rio de Janeiro, Belo Horizonte, Curitiba, Porto Alegre, Salvador, Recife, Fortaleza, Manaus and Federal District-Brasilia), aged from 18 to 60 years, of classes A, B and C according to the economic classification criteria in Brazil, of the Brazilian Association of Research Companies (ABEP)[11].

Statistical tests

With the descriptive statistical analysis, we obtained the frequency of GI symptoms, when they occurred, the reported causes, symptoms and sensations attributed by women, commonly used solutions and impact on QoL.

By grouping the type of symptoms, with Bayesian statistical analysis, we defined sets (clusters) of GI symptoms and their consequences related to age group, social class and regions of the country.

RESULTS

Qualitative phase

Four women profiles were identified based on their behavior in relation to the way they related to the GI symptoms they reported. These profiles have been defined from the way of involvement women had with their GI symptoms, which moved in a shaft that went from emotional to rational; and how women dealt with the problem, what was its management measures, which transitioned from functional to emotional. Figure 2 shows the main charac-

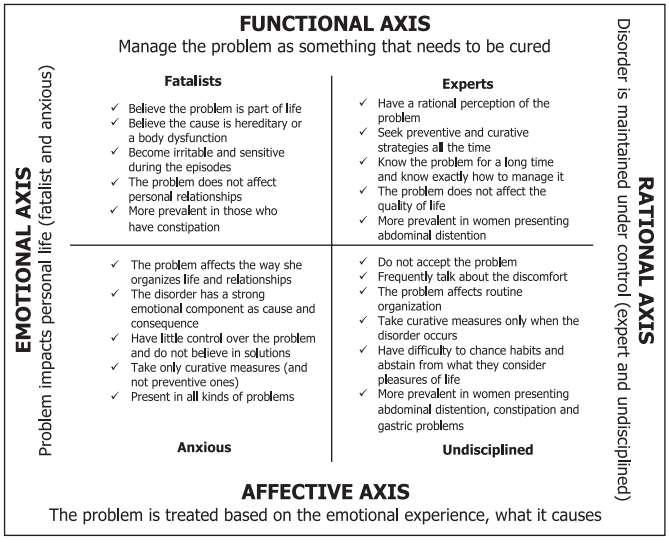


FIGURE 2. Women profiles identified in the qualitative phase

teristics of the four profiles found: fatalistic, experts, anxious and undisciplined.

Based on the reports of these women, four categories of GI disorders were also identified (constipation, diarrhea, gastric heaviness and abdominal distention), as well as how women expressed such problems through sensations reported and images associated with these problems (Figure 3).

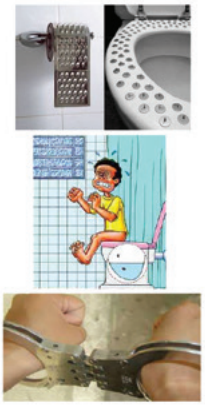
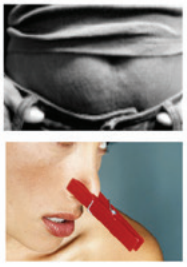
Categories	Sensations	Images
Constipation	anguish, suffering, sadness, difficulty, pollution, imprisonment, obstruction, immobility, weight, stress, bad mood	
Gastric Heaviness	weight, explosion, burning, aggression	
Abdominal Distention	pressure, discomfort, malaise, depression, decay, ugliness, shame	
Diarrhea	urgency, pain, sadness, confusion, disorganization, disruption, lack of control, isolation	

FIGURE 3. Main sensations and images associated with the categories of gastrointestinalconditions

Important findings were reported by the women during this study phase: i) to go to the bathroom outside home is an obstacle to meet their physiological needs; ii) they feel fear and shame, when other people could notice what they were doing in the bathroom.

Quantitative phase

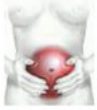
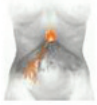
Of the 3029 women who responded to the questionnaire, 2000 reported GI symptoms, that is, two out of every three women. Of these, 46% reported having the problem weekly, 8% monthly and 12% every two months or less. Most women (72%) stated that the problems occurred during the week and almost half (42%) reported having the last GI symptom less than a week before.

The respondent study sample who reported GI symptoms showed the following distribution according to age group: 48% between 18 and 29 years, 28% from 30 to 39 years, 15% between 40 and 49 years, and 9% between 50 and 60 years. According to the classification by the Brazil criteria[11], 17% of women were from the social class defined as A, 51% belonged to class B and 32% to class C (considering the respondent study sample). The geographical distribution of the respondent sample was 3% in the North, 10% in Northeast, 7% in the Midwest, 63% in the Southeast and 17% in the South regions.

Among the causes for GI symptoms, those related to lifestyle that were reported most frequently were stress (61%) and insufficient physical activity (52%). Among the causes related to eating habits, 77% of women reported the consumption of fatty foods as the main cause of GI symptoms, and 46% of them said it was the sparkling water or soft drinks. The hasty lifestyle that includes the work routine and lack of time was mentioned as a major impediment for women to adopt a healthier lifestyle, controlling stress, practicing more physical activities and having a more balanced diet. Women mentioned some specific situations as aggravating to the problem, as eating in a hurry, staying seated for long periods, anxiety and stress.

Sensations reported in this stage of the research were quantified as shown in Table 1, which also displays the images associated with the sensations as they were presented in the questionnaire.

TABLE 1. Frequency of sensations reported by women

Sensations	Frequency (%)	Questionnaire images
Abdominal Distention / Weight	29	
Burning	21	
Gripes	17	
Lazy Intestine	14	
Bubbling	8	
None	11	

According to the typology of the symptoms, the Bayesian statistical analyzes defined 13 sets (clusters) as shown in Table 2. The most prevalent symptoms were gases in 46%, and abdominal distention and constipation, both in 43%. The sets (clusters) most prevalent in the age group from 18 to 29 years were the clusters 2, 6 and 11. The cluster 1 was the most prevalent for the age groups from 30 to 39 years and from 40 to 49 years. For the age group from 50 and 60 years, the most prevalent was cluster 7. There was no statistical difference between them for social class and geographic region.

TABLE 2. Definition and occurrence of clusters based on the type of symptoms

Cluster	Occurrence	Symptoms
1	9.8%	Constipation + abdominal distention + weight + gas + spasm + bubbling + plentiful stool + painful evacuation + body pain
2	10.3%	Constipation + gases + bubbling + plentiful stool + painful evacuation
3	7.9%	Abdominal distention + weight + gases + plentiful stool + painful evacuation + body pain + constipation
4	5.3%	Full + bloated stomach
5	7.4%	Constipation + painful evacuation + gases
6	7.8%	Constipation + painful evacuation
7	5.9%	Diarrhea + weight + abdominal distention + gases + bubbling + spasms + plentiful stool + acid reflux + body pain
8	5.1%	Bubbling + spasms + gases + diarrhea
9	7.4%	Nausea + bloated stomach
10	7.1%	Weight + pressure + bloated stomach + acid reflux + body pain
11	7.5%	Diarrhea + cramps
12	7.4%	Plentiful stool
13	11.0%	Bubbling

In an effort to "solve" the disorders, 73% of women reported drinking water, 40% reported taking herbal tea, 38% reported eating foods rich in fiber, 36% reported consuming probiotic yogurt, 34% reported eating papaya and 33% reported eating yogurt was the strategy adopted. None of the women who answered the questionnaire sought medical help to solve GI symptoms. They justified this behavior with the reasons described in Table 3.

TABLE 3. Frequency of the reasons why women who reported gastrointestinal conditions did not seek medical attention

Reason	Frequency (%)
I do not consider it a disease	25.5
I expect the problem to be solved on its own	20
I take medications that do not require a prescription	18.5
Doctors do not treat this problem seriously enough	17
I know that the cause is my poor eating habits, so I take medicine	5
Other	14

Most women (62%) reported that GI symptoms affected their QoL in general. Almost 70% of them said that the problems interfered in the mood, 66% said they affect the concentration and 57% state they affected their sex life (Figure 4). Assessing specifically women who reported any problem related to the slow intestinal transit time, it was found that the impact on their QoL was even more significant: for 89% there was an impact on mood, for 88% there was an impact on concentration and for 79% on sexual life.

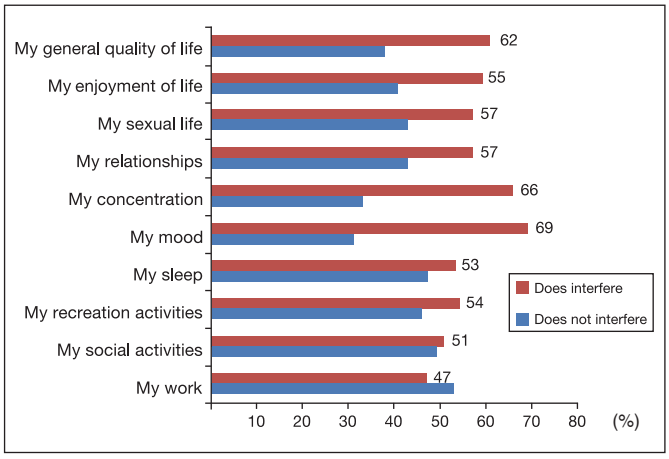


FIGURE 4. Gastrointestinal conditions impact on Brazilian women quality of life

DISCUSSION

The SIM (Women's Gastrointestinal Health) study allowed for the first time a representative and comprehensive evaluation of the intestinal health of Brazilian women, once that this study is the first one done in country and also in Latin America on this topic. According SIM study, in Brazilian female population, the GI symptoms are related by two-thirds of women, affecting their QoL, showing that this is an important topic to be discussed and addressed in medical community. The main GI symptoms reported were functional and the main causes attributed by women were those related to lifestyle and to eating habits, affecting their QoL in general (mainly mood, concentration and sexual life).

According Longstreth et al.[23], the IBS symptoms are predominant in female population occurring in 10% to 20% of adults and adolescents around the world, and these symptoms come and go over time impacting QoL. The main symptoms are bloating (present in 96% of IBS patients, more prevalent in women); constipation (present in around 30% of people throughout the world and also more common in women); and functional diarrhea.

In Brazil, in a recent cross-sectional study performed in Florianópolis with 605 women, with 40 years old (median age), visiting a public health service for reasons that were not related with GI symptoms, the constipation was self-reported by 24.5%[32]. Around the world, in a cross-sectional study in the USA, through telephone survey, 2510 subjects (men and women) answered questions about their GI symptoms in the month previous to the research, focusing on the frequency, duration, intensity and impact of symptoms, and 40.5% of respondents reported having had one or more symptoms in the previous month[33]. A study conduct in Netherlands, shows that 26% of the population reported GI symptoms, with a higher prevalence among female population, and flatulence (71%),

bloating (63%) and borborygmi (60%) were the most frequently symptoms[38]. In a survey performed by Silva et al.[35], it is estimated that constipation affects from 2% to 28% of the population in Western countries, and it is difficult to estimate the true prevalence of this problem because there are different classifications for diagnosis. These studies reinforce the importance of findings of this SIM study, where 66% of the Brazilian women had some type of GI symptoms with significant impact on their QoL.

The causes of GI symptoms considered functional are related to multiple factors, and lifestyle seem to be play an important role in this process[2,21,34,35]. It is known that diet composition is extremely important to bowel function[16] and the stress also exerts great influence on the functioning of the GI tract[17,20]. Also, the lifestyle is a factor responsible for the composition of the intestinal microbiota[3,25] and stress can directly affect its composition[5,30]. According to Bailey et al.[5], exposure to stress affects the stability of the microbiota and may result in bacterial translocation. The diet also changes the composition of the microbiota[19], which in turn can determine bowel function. According to Annalisa et al.[3], intestinal microbiota is influenced by several factors such as age, diet habit and lifestyle.

The human body is populated by a multitude of microorganisms called human microbiota. They inhabit surfaces of the human body[5], with a genetic diversity superior to men, since they make up to 100 trillion cells, which is ten times more than the number of human cells[27]. Many of these microorganisms are part of the intestinal microbiota, with over 500 species of bacteria, which due to such expressive characteristics have an ability to interact with their host, impacting their health and well-being[5,6,9,15,18,25,27]. The composition of the intestinal microbiota may be related to GI symptoms, mainly related to abdominal pain and abdominal distention. According to Jalanka-Tuovine et al.[18], subjects who had abdominal pain showed five times less *Bifidobacterium* than those without pain. Also, may be related with well-being. In a study with rats, Bercik et al.[6] concluded that the composition of the intestinal microbiota influences behavior and brain chemistry, and GI symptoms and intestinal dysbiosis can contribute to psychiatric disorders. Tillisch et al.[39] conducted a study with women to investigate the relationship between brain function and probiotic yogurt consumption, which acts in the modulation of the intestinal microbiota. The authors concluded that the consumption of fermented yogurt with probiotics (for 4 weeks, 250 grams per day) interferes with brain activity, with greater emphasis on regions of emotions and sensations[39].

It is important to note that the way the slow intestinal transit is seen in society directly affects the occurrence of GI symptoms and may even be their cause. This fact is seen as a taboo especially among women and has a psychological impact, since in this SIM study (during the first phase) they reported that the fact of having to go to the bathroom outside home is an obstacle to meet their physiological needs. Thus slowing the defecation reflex, an essential factor that causes constipation. The anal sphincters are a complex system between the inner smooth muscle, the striated muscle and the external puborectalis muscle, which are organized to execute fecal continence mechanism in conjunction with a network of nerves and the central nervous system[28,29,31]. According to Rajasekaran et al.[29], the internal smooth muscle is primarily responsible for the pressure of the anal canal on rest and the external striated muscle has greater responsibility on the increase in anal canal pressure, with voluntary tightening of the sphincter. The behavior of inhibiting the reflection of the anal sphincter, slowing it, can contribute to constipation. According to Oliveira et al.[26], constipation is more prevalent in women, and among the factors that could explain this scenario, in addition to psychological and hormonal issues, are births and gynecological surgeries that can damage the pelvic muscles and their innervation. In postmenopausal women, genital prolapses are more frequent and also help to explain constipation[26].

The emotional aspect related to the functioning of the bowel is often overlooked, but imprints an important relationship with it[17]. Serotonin, a hormone related to wellbeing sensation, is closely related with the bowel[10], since most of this hormone is synthesized, stored and released in the intestine[24]. The enterochromaffin cells are neuroendocrine cells in the mucosa of the GI tract and are responsible for the serotonin synthesis[14,22,24]. According to Mawe and Hoffman[24], serotonin is altered in patients with GI symptoms, especially with the slow intestinal transit, a factor that impacts the individual's wellbeing sensation. The corticotropin-releasing hormone acts on the gut-brain axis, mediating stress responses and influencing both brain and intestine, and the perception of the latter is affected by the emotional state of the subject[17,21,30].

The age range is related to gastrointestinal symptoms and their occurrences. In the present study, it was observed that the symptoms of clusters 1 and 7 were the most frequent in the age groups that includes the menopause and postmenopausal period (ranging in this study from 40 up to 60 years). These sets (clusters) are the ones that concentrate the largest combination and variety of symptoms, which might be related to the tangle of feelings that affect women in these age groups due to the hormonal decline that is characteristic of menopause.

Empirically, women relate dietary factors as an adjunct in the solution of GI symptoms. In addition to the increased consumption of fiber and water[34], probiotic yoghurt is an important factor in the improvement of GI symptoms, since the probiotic microorganisms are able to modulate the intestine microbiota and determine bowel function. In a review study conducted by Waitzberg et al.[41], the authors evaluated two clinical trials on the action of a fermented milk product with *Bifidobacterium animalis lactis* CNCM I-2494 and four other probiotics on the health of the host, especially with regard to digestive health and the functions of the GI tract. It was found that consumption of the product for four weeks had health benefits for healthy women, with a significant decrease in GI symptoms and increased digestive comfort[41]. Probiotics, by imprinting a relationship with the intestinal functions, contribute to the health and wellbeing of the subject[9,13]. This fact was also observed in the SIM study, where some women were using probiotics to solve their GI problems, especially those with slow intestinal transit.

Negative impact on QoL in healthy subjects can be one of the disorders generated by GI symptoms[18]. In a Brazilian study, performed in Florianópolis, with 605 adult women, visiting a public health service for reasons that were not related with GI symptoms, 25.1% had the diagnosis for constipation and about 80% of them stated that constipation had medium or great interference in their lives[32]. The SIM study showed similar results, where GI symptoms affected the QoL in general of the most of the evaluated women.

Around the world, other studies showed similar data. In a study conducted in France with 253 subjects with IBS, through telephone survey, Amouretti et al.[1] found that those subjects with IBS had lower QoL scores, statistically significant when compared with the French general population. Low scores in HRQoL (Health-Related Quality of Life) were correlated with greater intensity of abdominal

pain and discomfort. The same results were found in Netherlands, comparing the population with and without GI symptoms: the impact on the QoL was significantly more negative in the population with GI symptoms ($P<0.01$)[38]. Another study of Sandler et al.[33] showed that 65% of respondents reported that the symptoms had moderate to severe intensity and that these symptoms affected daily activities, impacting significantly the day-to-day life of the American population studied. Daily activities such as work, are affected by GI symptoms, which impacts on the economy. In a study by Sun et al.[37], with data from the National Health and Wellness Survey (NHWS), where 1.430 subjects had chronic constipation, the authors found a statistically significant impact ($P<0.01$) of this health condition with the loss of productivity at work, with more absenteeism in those with chronic constipation compared with the control group (9.08% vs 5.20%).

GI symptoms are seen as harrowing and stressful by most women. According to Amouretti et al.[1], women's QoL is most affected by GI symptoms. Once the GI problem happens, many women deal with it with distress and anxiety, which acts against the improvement of symptoms and so on in a vicious cycle, a behavior complicating the other, confusing cause and consequence of the problem. As previously mentioned in results section, most of women said that their problems occurred during the week, i.e. on working days, which may be related to the period of increased stress and haste of women. Anxiety and depression are related to mood swings, which have great influence in the GI tract, contributing to the occurrence of functional bowel disorders[17].

Besides this psychological scenario of stress and anxiety, feelings of shame are assigned to the act of defecating. Some women in the SIM study said (in the first phase) that the mere fact that other people could notice what they were doing in the bathroom, make them feel fear and shame. Such event can be explained precisely by the association of evacuation to the feeling of rejection, threatening the human search for belonging and acceptance. For Sandler et al.[33], women were more likely to report their problems than men when they were not related to the stool itself, but for diarrhea or sift stool, the numbers were similar between women and men (27.1% vs 26.7%)[33]. This relationship between the stool and the emotional commitment was also reported by Amouretti et al.[1], who observed in his study in the French population that subjects with diarrheal IBS had a worse emotional domain score in HRQoL when compared to the scores of individuals with IBS with constipation predominance.

Functional bowel disorders should not be seen as less important neither by the population that suffers from them or health professionals. The education of professionals on the matter, with the correct and transparent approach to the subject with their patients can ease the intensity, frequency and duration of them. It is known that the adoption of a healthier lifestyle, effectively contributes to the treatment of functional bowel disorders[2,21,34,35]. It is the role of health professionals to advise and recommend to their patients the adoption of a healthier lifestyle, an attitude that would cause them to have an adequate motility and consequently decrease the consumption of laxative medications. The exceptions would be for those with chronic constipation, where laxatives are indicated, under medical supervision, as initial part of the treatment[34]. The misuse and abuse of laxatives may cause unnecessary side effects and major impacts on the health of subjects, since these substances can cause side effects and change motor, secretory and absorptive functions of the GI tract[2,34].

Is important to reinforce that the SIM study is the first one done in Brazil and Latin America, allowing a representative and comprehensive evaluation of the intestinal health of Brazilian women for the first time. Its data allow some considerations: i) educational measures are necessary for clarification of the population that bowel function is a normal physiological function, which acts directly to the proper functioning of the organism as a whole, including the mental and emotional aspects; ii) besides the general population, health professionals should be better instructed to have a more transparent approach to GI symptoms with their patients, which certainly ease the psychological impact of talking about this topic, as well as physiologically abbreviate intensity, frequency and duration of GI symptoms; and iii) its demystifying is critical, as it is still seen as a taboo by society, especially in the female population.

One of the main limitations of the second phase of the study, related to the way of questionnaire administration (web survey), was the possible lack of access to the Internet. Also, language, cultural differences and regional expressions that exist in the different parts of the country, and the potential for respondents to misunderstand questions or the terms being used. In this survey, the use of items that were easy to understand and images to facilitate communication, minimized this latter risk.

CONCLUSION

This observational study on national scale shows that GI symptoms are present in the day-to-day life of two-thirds of Brazilian women without social class distinction and in all regions of the country, affecting more significantly their QoL. The main GI symptoms reported were functional and the causes attributed by women were those related to lifestyle and eating habits.

Most of the evaluated women reported that GI symptoms affected their QoL in general, affecting mainly mood, concentration and sexual life. Such evidence allows to conclude that the bowel, because of its tenuous connection with the brain, becomes an important emotional catalyst, and may modulate negatively the psychological behavior of women, giving rise to a psycho-physiological vicious cycle that begins with negative feelings such as low self-esteem, stress, distress, anger and anxiety, triggering intestinal discomfort, which through their physical symptoms, reports the internal imbalance in the female body. Thus, uncomfortable with the GI reactions, the QoL of these women is directly affected, which invariably aggravate and make chronic the emotions that stimulate the initial cycle.

Social standards and lifestyle directly affect the female perspective on the intestine as an organ. The confrontation of the taboo of evacuating as a natural need is critical, requiring public awareness that the intestine is part of the body, and that with all its complexity of operation, it has immense relevance.

Authors' contributions

Del'Arco APWT: research implementation, data analysis, text editing. Magalhães P: data analysis, text revision. Quilici FA: research coordination, data analysis, text revision.

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RESUMO – **Contexto** – Sintomas gastrointestinais parecem afetar mais as mulheres, devido a problemas hormonais e emocionais, afetando a qualidade de vida. O estado emocional pode afetar o funcionamento do intestino por meio de um sistema de comunicação bidirecional entre o intestino e o cérebro que envolve o sistema neuroendócrino. Alterações da função intestinal e sintomas gastrointestinais podem afetar a qualidade de vida. **Objetivo** – O estudo SIM teve como objetivo descrever, caracterizar e quantificar os sintomas gastrointestinais relatados por mulheres brasileiras, suas causas, sentimentos e impacto. **Métodos** – Questionário eletrônico estruturado foi desenvolvido após a fase qualitativa para formatação semântica, e foi administrado a mulheres voluntárias em 10 cidades brasileiras. Foram realizadas análises estatísticas descritivas e Bayesiana. **Resultados** – A partir dos 3029 respondentes, 66% relataram sintomas gastrointestinais. Os sintomas mais prevalentes foram gases (46%), distensão abdominal e constipação (43%). As principais causas relatadas foram estilo de vida e hábitos alimentares. Sintomas gastrointestinais afetaram a qualidade de vida da maioria das mulheres (62%), especialmente a constipação (humor (89%), concentração (88%) e vida sexual (79%)). As soluções mais comuns adotadas foram beber água, chás, comer alimentos ricos em fibras e probióticos. **Conclusão** – Sintomas gastrointestinais são altamente prevalentes nas mulheres brasileiras e impactam negativamente diferentes aspectos da qualidade de vida (humor, concentração e sexualidade). O intestino é um catalisador emocional importante que pode modular o comportamento psicológico. Melhor compreensão da interação entre o intestino e o cérebro pode ajudar na gestão dos sintomas gastrointestinais para melhorar a qualidade de vida das mulheres.

DESCRITORES – Trato gastrointestinal. Saúde da mulher. Qualidade de vida. Inquéritos e questionários.

REFERENCES

1. Amouretti M, Le Pen C, Gaudin AF, Bommelaer G, Frexinos J, Ruszniewski P, et al. Impact of irritable bowel syndrome (IBS) on health-related quality of life (HRQoL). Gastroenterol Clin Biolog. 2006;30:241-6.
2. Andre SB, Rodriguez TN, Moraes Filho JPP. Constipação Intestinal. Rev Bras Med. 2000;57:15-7.
3. Annalisa N, Alessio T, Claudette TD, Erald V, Antonino L, Nicola D. Gut microbioma population: an indicator really sensible to any change in age, diet, metabolic syndrome, and life-style. Mediators Inflamm. 2014;2014:901308
4. Azpiroz F, Malagelada JR. The pathogenesis of bloating and visible distension in irritable bowel syndrome. Gastroenterol Clin North Am. 2005;34:257-69.
5. Bailey MT, Dowd SE, Galley JD, Hufnagle AR, Allen RG, Lyte M. Exposure to a social stressor alters the structure of the intestinal microbiota: Implications for stressor-induced immunomodulation. Brain Behavior Immun. 2011;25: 397-407.
6. Bercik P, Denou E, Collins J, Jackson W, Lu J, Jury J, et al. The intestinal microbiota affects central levels of brain-derived neurotropic factor and behavior in mice. Gastroenterol. 2011;141:599-609.
7. Carruthers HR, Miller V, Morris J, Evans R, Tarrier N, Whorwell PJ. Using art to help understand the imagery of irritable bowel syndrome and its response to hypnotherapy. Int J Clin Exp Hypn. 2009;57:162-73.
8. Carruthers HR, Morris J, Tarrier N, Whorwell PJ. Reactivity to images in health and irritable bowel syndrome. Aliment Pharmacol Ther. 2010;31:131-42.
9. Ciorba MA. A gastroenterologist's guide to probiotics. Clin Gastroenterol Hepatol. 2012;10:960-8.
10. Costa F, Mumolo MG, Marchi S, Bellini M. Differential diagnosis between functional and organic intestinal disorders: Is there a role for non-invasive tests? World J Gastroenterol. 2007;13:219-23.
11. Critério de Classificação Econômica Brasil. Associação Brasileira de Empresas de Pesquisa (ABEP). Available from: http://www.abep.org/criterio-brasil
12. Francesconi CF. Algoritmos diagnósticos da fundação Roma para sintomas gastrointestinais normais: apresentação. Arq Gastroenterol. 2012;49(s.1):6-8.
13. Gerritsen J, Smidt H, Rijkers GT, Vos WM. Intestinal microbiota in human health and disease: the impact of probiotics. Genes Nutr. 2011;6:209-40.
14. Gershon MD, Tack J. The serotonin signaling system: from basic understanding to drug development for functional GI disorders. Gastroenterol. 2007;132:397-414.
15. Holmes E, Li JV, Athanasiou T, Ashrafian H, Nicholson JK. Understanding the role of gut microbiome-host metabolic signal disruption in health and disease. Trends in Microbiol. 2011;19:349-59.
16. Howarth L, Petrisko Y, Furchner-Evanson A, Nemoseck T, Kern M. Snack selection influences nutrient intake, triglycerides, and bowel habits of adult women: a pilot study. J Amer Diet Assoc. 2010;110:1322-27.
17. Huerta-Franco MR, Vargas-Luna M, Tienda P, Delgadillo-Holtfort I, Balleza-Ordaz M, Flores-Hernandez C. Effects of occupational stress on the gastrointestinal tract. World J Gastrointest Pathophysiol. 2013;4:108-18.
18. Jalanka-Tuovinen J, Salonen A, Nikkila J, Immonen O, Kekkonen R, Lahti L, et al. Intestinal microbiota in healthy adults: Temporal analysis reveals individual and common core and relation to intestinal symptoms. PlosOne. 2011;6:e23035.
19. Kashyap PC, Marcobal A, Ursell LK, Larauche M, Duboc H, Earle KA, et al. Complex interactions among diet, gastrointestinal transit, and gut microbiota in humanized mice. Gastroenterol. 2013;144:967-77.
20. Konturek PC, Brzozowski T, Konturek SJ. Stress and the gut: pathophysiology, clinical consequences, diagnostic approach and treatment options. J Physiol Pharmacol. 2011;62:591-99.
21. Lee YJ, Park KS. Irritable bowel syndrome: Emerging paradigm in pathophysiology. World J Gastroenterol. 2014;20:2456-69.
22. Li HJ, Johnston B, Aiello D, Caffrey DR, Giel-Moloney M, Rindi G, et al. Distinct cellular origins for serotonin-expressing and enterochromaffin-like cells in the gastric corpus. Gastroenterol. 2014;146:754-64.
23. Longstreth GF, Thompson WG, Chey WD, Houghton LA, Mearin F, Spiller RC. Functional bowel disorders. Gastroenterol. 2006;130:1480-91.
24. Mawe GM, Hoffman JM. Serotonin signaling in the gastrointestinal tract: functions, dysfunctions, and therapeutic targets. Nat Rev Gastroenterol Hepatol. 2013;10:473-86.
25. Nicholson JK, Holmes E, Kinross J, Burcelin R, Gibson G, Jia W, et al. Host-gut microbiota metabolic interactions. Science. 2012;336:1262-67.
26. Oliveira SCM, Pinto-Neto AM, Góes JRN, Conde DM, Santos-Sá D, Costa-Paiva L. Prevalência e fatores associados à constipação intestinal em mulheres na pós-menopausa. Arquiv Gastroenterol. 2005;42:24-9.
27. Qin J, Li R, Raes J, Arumugam M, Burgdorf KS, Manichanh C. et al. A human gut microbial gene catalogue established by metagenomic sequencing. Nature. 2010;464:59-65.
28. Raizada V, Bhargava V, Karsten A, Mittal RK. Functional morphology of anal sphincter complex unveiled by high definition manometery & 3-dimensional ultrasound imaging. J Neurogastroenterol Motility. 2011;23:1013-28.
29. Rajasekaran MR, Jiang Y, Bhargava V, Littlefield R, Lee A, Lieber RL, et al. Length-tension relationship of the external anal sphincter muscle: implications for the anal canal function. American J Physiol Gastrointestinal and Liver Physiol. 2008;295:367-73.
30. Rodino-Janeiro BK, Alonso-Cotoner C, Pigrau M, Lobo B, Vicario M, Santos J. Role of corticotropin-releasing factor in gastrointestinal permeability. J Neurogastroenterol and Motility. 2015;21:33-50.
31. Rodriguez L, Siddiqui A, Nurko S. Internal anal sphincter relaxation associated with bisacodyl-induced colonic high amplitude propagating contractions in children with constipation: a colo- anal reflex? J Neurogastroenterol Motility. 2012;24:1023-39.
32. Sacomori C, Silveira C, Sperandio FF, Cardoso FL. Prevalence, repercussion and factors associated with intestinal constipation in women in Florianopolis. J Coloproctol. 2014;34:254-9.

33. Sandler RS, Stewart WF, Liberman JN, Ricci JA, Zorich NL. Abdominal pain, bloating, and diarrhea in the United States: Prevalence and impact. Digestive diseases and sciences. 2000;45:1166-71.
34. Santos Junior JCM. Laxantes e purgativos: O paciente e a constipação intestinal. Rev Brasil Coloproctol. 2003;23:130-4.
35. Silva AM, Lins, DC, Azevedo SL, Dias BF, Paiva PB. Diagnóstico de enfermagem: Constipação intestinal funcional associado ao estilo de vida. 2010. 10º SINADEn. Available from: http://www.abeneventos.com.br/10sinaden/anais/files/0091.pdf
36. Spiegel B, Bolus R, Harris LA, Lucak S, Naliboff B, Esrailian E, et al. Measuring IBS patient reported outcomes with an abdominal pain numeric rating scale: results from the proof cohort. Aliment Pharmacol Ther. 2009;3:1159-70.
37. Sun SX, Dibonaventura M, Purayidathil FW, Wagner JS, Dabbus O, Mody R. Impact of chronic constipation on health-related quality of life, work productivity, and healthcare resource use: an analysis of the National Health and Wellness Survey. Dig Dis Sci. 2011;56:2688-95.
38. Tielemans MM, Focks JJ, Rossum LGM, Eikendal T, Jansen JBMJ, Laheij RJF, et al. Gastrointestinal symptoms are still prevalent and negatively impact health-related quality of life: a large cross-sectional population based study in The Netherlands. PlosOne. 2013;8:e69876.
39. Tillisch K, Labus J, Kilpatrick L, Jiang Z, Stains J, Ebrat B, et al. Consumption of fermented milk product with probiotic modulate brain activity. Gastroenterol. 2013;144:1394-401.
40. Van Kerkhoven LA, Eikendal T, Laheij RJ, Van Oijen MG, Jansen JB. Gastrointestinal symptoms are still common in a general Western population. Neth J Med. 2008;66:18-22.
41. Waitzberg DL, Quilici FA, Michzputen S, Passos MCF. The effect of probiotic fermented milk that includes Bifidobacterium lactis CNCM I-2494 on the reduction of gastrointestinal discomfort and symptoms in adults: a narrative review. Nutricion Hospitalaria. 2015;32:501-9.

ORIGINAL ARTICLE

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Action of vitamin E on experimental severe acute liver failure

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ABSTRACT – **Background** – Severe Acute Liver Failure (ALF) is a life-threatening clinical syndrome characterized by hepatocyte necrosis, loss of hepatic architecture, and impairment of liver functions. One of the main causes of ALF is hepatotoxicity from chemical agents, which damage hepatocytes and result in increase of reactive oxygen species. The vitamin E isoform is the one with the strongest biological antioxidant activity. **Objective** – To evaluate the antioxidant effect of vitamin E in this ALF model. **Methods** – We used 56 rats (mean weight of 300 g) divided into eight groups, four groups assessed at 24 hours and 4 assessed at 48 hours after induction: control group (CO); Vitamin E (Vit. E); Thioacetamide (TAA) and Thioacetamide + Vitamina E (TAA+Vit.E). Rats were submitted to injections of thioacetamide (400 mg/kg i.p.) at baseline and 8 hours later. Vitamin E (100 mg/kg ip) was administered 30 minutes after the second dose of thioacetamide. The 48-hour group rats received two additional doses of vitamin E (24h and 36h). At 24h or 48 hours after the administration of the first dose of TAA, rats were weighed and anesthetized and their blood sampled for evaluation of liver integrity through enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT). Liver tissue was sampled for assessment of lipid peroxidation (LPO) by the technique TBARS, antioxidant enzymes SOD, CAT, GPx and GST activity, levels of the NO_2/NO_3 and histology by H&E in two times. The results were expressed as mean ± standard deviation and statistically analyzed by ANOVA followed by Student-Newman-Keuls, with $P<0.05$ considered as significant. **Results** – After treatment with vitamin E, we observed a reduction in liver enzymes AST (U/L) (101.32±19.45 in 24 hours and 97.85±29.65 in 48 hours) related to the TAA group (469.56± 0.69 in 24 hours and 598.23±55.45 in 48 hours) and ALT (U/L) (76.59±8.56 in 24 hours and 68.47±6.49 in 48 hours) compared to the TAA group (312.21±10.23 in 24 hours and 359.15±17.58 in 48 hours). There was a reduction of LPO (nmol/mg Prot) in the TAA+Vit.E group (0.77±0.07 in 24 hours and 0.95±0.08 in 48 hours) compared to the TAA group (1.50±0.07 in 24 hours e 1.65±0.16 in 48 hours). SOD decreased in the TAA+Vit.E group (49.48±9.47 in 24 hours and 62.45±18, 47 in 48 hours), related to the TAA group (98.46±15.48 in 24 hours and 154.13±21.46 in 48 hours), as well as GST (nmol/min/mg Prot) in the TAA+Vit.E group (350.57±36.93 in 24 hours and 453.29±13.84 in 48 hours) compared to the TAA group (561.57±64.56 in 24 hours and 673.43±38.13 in 48 hours). There was an increase in CAT (pmol/min/mg Prot) in the TAA+Vit.E group (3.40±0.44 in 24 hours and 3.0±0.35 in 48 hours) compared to the TAA group (1.65±0.21 in 24 hours and 1.86±0.42 in 48 hours). The GPx (nmol/min/mg Prot) increased in 24 hours in the TAA+Vit.E group (1.01±0.16) compared to the TAA group (0.41±0.04) and decreased in 48 hours (1.19±0.17) compared to the TAA group (1.76±0.21). There was a reduction in NO2/NO3 (mmol/L) levels in the TAA+Vit.E group (31.47±4.26 in 24 hours and 38.93±5.20 in 48 hours) compared to the TAA group (49.37±5.12 in 24 hours and 53.53±5.97 in 48 hours). The histopathological evaluation showed a decrease in liver injury (necrosis and inflammation) in both studied times. **Conclusion** – These results suggest that vitamin E was able to protect the liver from lesions caused by thioacetamide.

HEADINGS – Acute liver failure. Thioacetamide. Oxidative stress. Antioxidants.

INTRODUCTION

Severe Acute Liver Failure (SALF) is a syndrome with high morbidity and mortality rates and low prevalence. It is characterized by sudden onset in patients with previously normal liver with rapid progress, leading to hepatocellular insufficiency, which translates into extensive metabolic disturbances, particular susceptibility to bacterial or fungal infections, collapse of multiple organs, coagulopathy, and central nervous system disorders, with mortality reaching 80%[6,24,27]. The severe acute attack on the hepatic parenchyma can have different etiologies, such as drugs, xenobiotics and viruses. At present, the treatment of excellence in most cases is liver transplantation. The availability of organs is limited, however, precluding the use of such therapy in all necessary cases. Furthermore, few are the hospitals that have competent surgical teams to effectively perform liver transplantation. The acknowledged therapeutic effectiveness of N-acetylcysteine in cases of SALF triggered by intake of large doses of paracetamol gives us an indication of the possible therapeutic application of other compounds that can act in such situations. So, attentions have been focused on the possibility of restoring liver mass and function through various treatments, in an attempt to delay or arrest the progress of the disease[18,24,28,29,31].

Research on experimental models of ALT play an extremely important role for the study of its pathogenesis and the many stages

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of its course. Thioacetamide (TAA) is a xenobiotic known as a potent hepatotoxic, carcinogenic and cirrhosis-inducing agent in rats[5,12]. Its administration causes the death of hepatic cells by both centrilobular necrosis and apoptosis[10,14,20]. This process involves reactive oxygen species (ROS), which leads to oxidative stress (OS), with increased damage to DNA, proteins and lipids from the excessive generation of free radicals (FR)[15,29].

Both ROS and reactive nitrogen species (RNS) as well as other free radicals are critical intermediaries in the physiopathogenesis and physiopathology of hepatocyte lesion[9]. Bioactive products resulting from lipoperoxidation are highly implicated as being key abnormalities responsible for the hepatic injury[11].

The organism relies on an antioxidant defense system against ROS and RNS, which is divided in two main types: enzymatic, such as enzymes superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT) and glutathione S-Transferase (GST); and non-enzymatic, such as glutathione (GSH), ascorbic acid (vitamin C), flavonoids, vitamin E, among others[26].

Vitamin E is a component of vegetable oils that is found in nature in four different forms: α, β, γ, and δ-tocopherol. Vitamin E is the main antioxidant vitamin transported in the blood flow by the lipid phase of plasma lipoprotein particles[16].

The α-tocopherol isoform is the one with the strongest biological antioxidant activity and is widely distributed in tissues and plasma. In the non-hydrophobic portion of a-tocopherol there is the hydroxyl radical (HO), whose atom of hydrogen is easily removable. So, when peroxyl and/or alcoxyl radicals are generated during lipid peroxidation, they are likely to combine with fatty acids of the tail of vitamin E, thus stopping to withdraw electrons from membrane fatty acids. Therefore, vitamin E, owing to its structural characteristics, acts as chain breaker, i.e. a scavenger of free radicals, thus precluding lipoperoxidation (LPO)[30].

Given the physiopathogeny of severe SALF involving the formation of ROS and RNS, the hepatotoxic ability of TAA and the antioxidant effects of Vitamin E, this work was designed to investigate the action of this vitamin on SALF in rats.

METHODS

Ethical considerations

Animal handling complied with the ethical principles established by Federal Law No. 11.794, which regulates the scientific use of experimental animals in Brazil. This project was approved by the Ethical Research Committee of *Universidade Luterana do Brasil* (ULBRA) for Animal Use (CEUA- Protocol 2012 – 43P).

Animals and research design

Fifty-six male Wistar rats with mean weight of 300 g were used, divided in two experiments according to time of interest, 24h and 48h (28 animals per experiment). Each experiment comprised four groups: control (CO) group, Vitamin E (Vit. E) group, Tioacetamide (TAA) group and Tioacetamide + Vitamin E (TAA+Vit. E). Each experimental group was composed of 7 animals (n=7 based on sampling calculation) obtained from the animal facility of ULBRA. Along the study period the animals were kept in plastic boxes lined with wood shavings on a 12h light/dark cycle and room temperature between 20 and 25ºC. They had free access to food and water.

Thioacetamide (Sigma Chemical Co., St. Luis, MO, USA) was diluted in 1 mL of 0.9% NaCl vehicle and administered with intraperitoneal injection (i.p.). Vitamin E (α–tocopherol), supplied in gelatinous capsules with oil by Importadora Química DELAWERE®, was administered at a dose of 125 mg/kg (i.p.)

Experimental protocol

The CO-24h group received three doses of 0.9% NaCl vehicle, with the second dose given 8 hours after the first and the third and last dose 30 minutes after the second. Thioacetamide was administered at two doses of 400mg/Kg (i.p.) each with an interval of 8 hours, while vitamin E was given at a dose of 125 mg/kg (i.p.) 30 minutes after the second dose of TAA. In the 48-hour experiment, two additional doses of vitamin E were administered, with the second dose given 24 hours after the start of the experiment and the third, 36 hours after it. Doses of vehicle at the same dilution (0.9% NaCl, 1 mL) were administered to the groups in both experiments in order to expose the animals to the same number of administrations. At the end of each experiment, animals were weighed and anesthetized with ketamine 95 mg/kg and xylazine hydrochloride 8 mg/kg (i.p.). Blood samples were collected from the retro-orbital plexus for hepatic integrity assays and livers were dissected out for posterior analyses. At the end of each experiment (24h and 48h), animals were killed by exsanguination under deep anesthesia.

Plasma analyses

Liver integrity was determined by evaluation of enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in plasma using the commercial kit Boehriner Mannheim (Germany). AST (340 nm) and ALT (340 nm) activities were obtained by kinetic assay using the commercial liquiform kit Labtest®.

Liver homogenates

Nine mL of phosphate buffer solution (1.15% KCL) per gram of tissue (liver) and phenylmethylsulfonyl fluoride (PMSF) at a concentration of 100 mM in isopropanol (10 µL/mL of KCl) were used. The tissue was homogenized in ULTRA-TURRAX for 40 seconds at 0-2ºC and subsequently centrifuged for 10 min at 3000 rpm in refrigerated centrifuge. The precipitate was discarded and the supernatant removed and frozen at -80ºC for posterior biochemical analyses[19].

Protein

The Bradford method (1976) was used to quantify proteins, with bovine albumin as standard (SIGMA). The samples were spectrophotometrically measured at 595 nm, and the concentrations expressed in mg/mL and used to calculate thiobarbituric acid reactive substances (TBARS) levels and antioxidant enzyme activity.

Lipoperoxidation

The amount of malondialdehyde generated by lipoperoxidation was measured by TBARS, a technique that measures the quantity of substances reacting with thiobarbituric acid. Tissue samples were placed in test tubes with a mixture of 10% thrichloroacetic acid (TCA) and 0.67% thiobarbituric acid (TBA). They were subsequently warmed in bath for 30 min and chilled in ice for about 5 min. After chilling the samples, 1.5 mL of n-butyl alcohol was added to extract the pigment formed. After this procedure they were placed in stirrer for 45 sec and centrifuged for 10 min at 3000 rpm. Finally, the stained product, present in the upper fraction, was read spectrophotometrically at a wavelength of 535 nm. The obtained TBARS concentration was expressed as nmol per milligram of protein[4].

Analyses of antioxidant enzymes

Glutathione S-transferase (GST) is based on an enzyme that catalyzes the formation of 1 mmol of DNP-SG per minute at 30°C using 1mM of the concentration of (reduced) GSH and CDNB, detected spectrophotometrically at 340 nm, values expressed in mmol/min/mgprot[21]. The analysis of superoxide dismutase (SOD) activity is defined as its ability to inhibit a detection system that reacts with O^{-}_{2}. The technique of measuring SOD is based on the inhibition of this reaction with adrenalin, detected spectrophotometrically at 480 nm. The data were expressed as units of SOD per milligram of protein (USOD/ mg prot.)[25]. The analysis of catalase activity (CAT) is defined by the breakdown of hydrogen peroxide in water and oxygen, being directly proportional to its enzymatic activity, detected spectrophotometrically at 240 nm. The results were expressed in µmoles per milligram of protein (mmoles of H_2O_2)[3]. Glutathione peroxidase (GPX) can be studied by measuring the rate of consumption of nicotinamide adenine dinucleotide (NADPH) in the reduction of glutathione oxidase, detected spectrophotometrically at 340 nm and its activity expressed in nmoles per minute per milligram of protein (nmol/min/mg prot)[13].

Evaluation of nitric oxide metabolites – nitrites e nitrates

Nitric oxide production was measured indirectly through a colorimetric quantitative test by the Griess reaction. It is based on the enzymatic reduction of nitrates to nitrites in the presence of nitrate reductase and NADPH, and posterior reaction of the formed nitrites (or initially present in the samples) with Griess reagent (mixture of sulfanilamide and naphthyl ethylenediamine, specific for NO_2^-). However, as the excess of NADPH used inhibits the Griess reaction, it is necessary to oxidize all of the NADPH not used in the reduction of nitrates. This is achieved by adding nitrate reductase. The reading was performed in a microplate reader at 540nm and the results expressed in mmol of NO_2/NO_3.

Histological analysis

Histological analyses were performed on liver samples preserved in 10% formaldehyde solution for 24h, which were then embedded in paraffin and cut in 3 mm slices using a rotating microtome. Histological examinations were performed using hematoxylin-eosin staining. A single pathologist, blinded to experimental protocol, analyzed all livers under a binocular Labophot NIKON microscope, at 100X magnification.

Statistical analysis

The results were expressed as mean ± standard error for each experimental group. The software GrapPad Instat, version 3.0 was used for the statistical analysis. For symmetrical data, simple ANOVA was used to compare the differences found in each studied parameter. The complementary Student-Newman-Keuls test was used as well for multiple comparisons. The level of significance for each comparison was at least 5% ($P<0.05$).

RESULTS

Liver integrity

Table 1 shows the results of AST and ALT evaluation at 24h and 48h. The TAA groups showed a significant increase ($P<0.001$) as compared to the CO group at both times, while the TAA groups receiving Vit. E (125 mg/kg) reduced these enzymes significantly ($P<0.001$) at both these times, decreasing and protecting from the damage triggered by TAA. It was thus demonstrated that Vit. E doses contributed to a protective effect on the liver tissue.

TABLE 1. Evaluation of enzymes AST and ALT (U/L) in the different experimental groups at the two studied times (24h and 48h)

Grupos	CO	VIT. E	TAA	TAA + Vit. E
24 h				
AST	39.05±6.55	39.99±5.23	469.56±0.69[a]	101.32±19.45[b]
ALT	22.36±3.45	21.56±2.64	312.21±10.23[a]	76.59±8.56[b]
48 h				
AST	43.12±5.63	41.56±3.45	598.23±55.45[a]	97.85±29.65[b]
ALT	29.48±3.12	32.45±3.05	359.15±17.58[a]	68.47±6.49[b]

CO: control group, Vit. E: Vitamin E group; TAA: Thiocetamide group; TAA+Vit.E: Thiocetamide with Vit. E group). Data are expressed as mean ± standard error of mean. n=7 per group, where a= significant increase ($P<0.01$) of TAA vs Controls, and b= indicates significant decrease ($P<0.001$) of TAA+Vit.E vs TAA.

Lipoperoxidation measurement

LPO results for the 24h and 48h groups can be seen in Figure 1, where a significant increase ($P<0.001$) of LPO was found in TAA groups as compared to the others. As Vit. E is administered at 24 h, LPO is reduced as compared to the TAA group ($P<0.001$), and the same was found at 48h. This is clear evidence that Vit. E administration in the TAA group reduces LPO at both studied times.

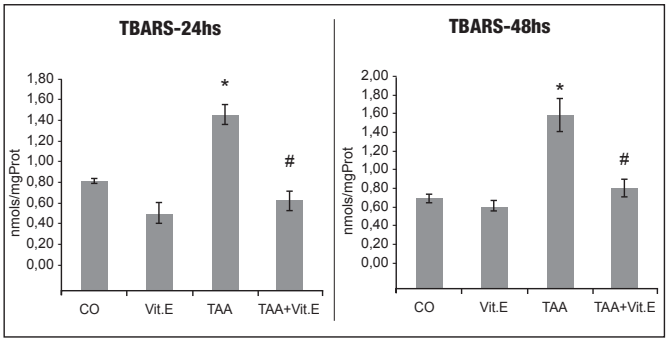

FIGURE 1. Evaluation of lipoperoxidation through TBARS (nmol/mg prot) in the different experimental groups assessed at 24h and 48h. CO: control group, Vit. E: Vitamin E group; TAA: Thiocetamide group; TAA+Vit.E: Thiocetamide with Vit. E group. Data are expressed as mean ± standard error of mean. n=7 per group. *Significant increase ($P<0.05$) of TAA vs Controls and #significant decrease ($P<0.05$) of TAA+Vit.E vs TAA.

Antioxidant enzymes

SOD, CAT, GPx and GST activities were assessed at 24h and 48h (Table 2). GST and SOD activities were significantly increased in the TAA groups as compared to control groups at 24h and 48h, and decreased in the TAA+Vit.E groups as compared to the TAA groups at both times. Cat activity was significantly reduced in the TAA groups as compared to controls at 24h and 48 h, and increased in the TAA+Vit.E groups as compared to TAA at both studied times. GPx showed a different pattern across the studied times. At 24h GPx activity was reduced in the TAA as compared to controls and the TAA+Vit.E group had increased GPx activity as compared to the TAA group. At 48h, GPx activity was increased in the TAA group as compared to controls and was reduced in the TAA+Vit.E group as compared to TAA.

TABLE 2. Evaluation of antioxidant enzymes SOD, CAT, GPX and GST activities in the different studied groups and two studied times: 24h and 48h

Groups	SOD (USOD/min/mg Prot)	CAT (pmol/min/mg Prot)	GPX (nmol/min/mg Prot)	GST (nmol/min/mg Prot)
24 h				
CO	36. 47 ± 7.49	3.43 ± 0.68	0.76 ± 0.09	246 ± 11.41
Vit. E	28.46 ± 5.13	4.60 ± 0.21	0.73 ± 0.08	262.57 ± 8.68
TAA	98.46 ± 15.48[a]	1.65 ± 0.21[c]	0.41 ± 0.04[c]	561.57 ± 64.56[a]
TAA + Vit. E	49.48 ± 9.47	3.40 ± 0.44[d]	1.01 ± 016[b]	350.57 ± 36.93[b]
48 h				
CO	31.54 ± 7.68	3.299 ± 0.23	0.68 ± 0.08	318.71 ± 18.94
Vit. E	12.96 ± 6.48	3.45 ± 0.24	0.71 ± 0.07	299.57 ± 11.81
TAA	154.13 ± 21.46[a]	1.86 ± 0.42[a]	1.76 ± 0.21[a]	673.43 ± 38.13[a]
TAA + Vit. E	62.45 ± 18.47[b]	3.015 ± 0.35[b]	1.19 ± 0.17[b]	453.29 ± 13.84[b]

CO: control group, Vit. E: Vitamin E group; TAA: Thiocetamide group; TAA+Vit.E: Thiocetamide with Vit. E group. Data are expressed as mean ± standard error of mean. n=7 per group. **a**= significant increase (P<0.001) TAA vs Controls; **b**= significant decrease (P<0.001) of TAA+Vit.E vs TAA; **c**= significant increase (P<0.05) of TAA vs Control groups, and **d**= significant decrease (P<0.01) TAA+Vit.E vs TAA.

Nitrites e Nitrates

Figure 2 shows that nitric oxide metabolites increased significantly (P<0.001) in the TAA groups at both times and were significantly reduced in the Vitamin E-supplemented groups (P<0.001) at both studied times.

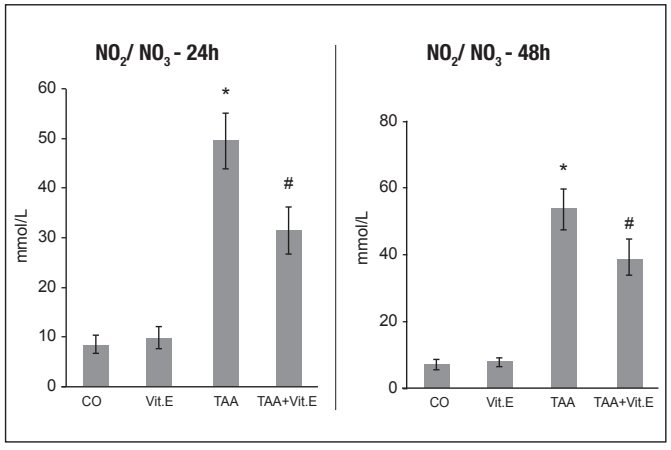

FIGURE 2. Evaluation of nitric oxide metabolites - nitrites and nitrates (mmol/L) in the different experimental groups at 24h and 48h. CO: control group, Vit. E: Vitamin E group; TAA: Thiocetamide group; TAA+Vit.E: Thiocetamide with Vit. E group. Data are expressed as mean ± standard error of mean. n=7 per group. a = significant increase (P<0.001) TAA vs Controls; b = significant decrease (P<0.001) of TAA+Vit.E vs TAA.

Histology

Histological evaluation of liver tissue was performed by hematoxylin & eosin (HE) staining at 200x magnification. As can be seen in Figures 3A and B (24h) and 4-A and B (48h), the CO and Vit.E groups showed normal hepatic parenchyma, with clear-cut hepatocyte cordons with well-preserved cytoplasms and nuclei. In the histology of the TAA group, shown in Figures 3C (24h) and 4C (48h), there is evidence of hepatocyte cordon disorganization, inflammatory infiltrate, and necrosis. In the TAA+Vit.E group, seen in Figures 3D (24h) and 4D (48h), note the preservation of hepatocyte cordons and decreased incidence of necrosis and inflammatory infiltrate in response to Vitamin E.

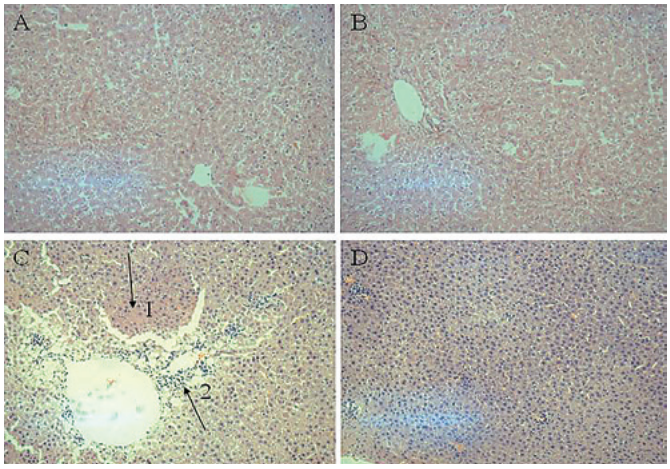

FIGURE 3. Photomicrograph of hepatic tissue by hematoxylin & eosin (HE) stain in the different experimental groups at 24h. Magnification: 200X. A = CO group, B = Vit. E group, C = TAA group and D = TAA+Vit.E group. Arrow 1: Necrosis. Arrow 2: Inflammatory infiltrate.

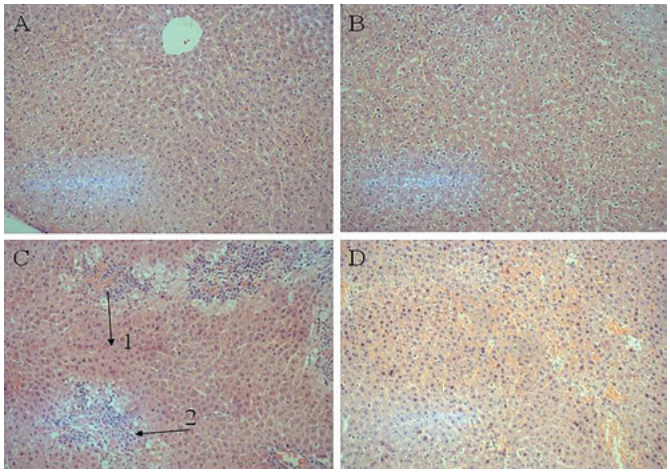

FIGURE 4. Photomicrograph of hepatic tissue by hematoxylin & eosin (HE) stain in the different experimental groups at 48h. Magnification: 200X. A = CO group, B = Vit. E group, C = TAA group and D = TAA+Vit.E group. Arrow 1: Necrosis. Arrow 2: Inflammatory infiltrate.

DISCUSSION

Severe SALF is an acute hepatic disorder with varied, poorly known physiopathogeny and established physiopathology with variants concerning etiology. Knowledge of these mechanisms is important to determine the pathways of the lesion and any potential effects of external intervention. The utilization of experimental models and antioxidant drugs comes to contribute to its understanding. Drugs that can delay the progress of the disease or reorganize the hepatic parenchyma are potential therapeutic agents for the disease.

In the present study, vitamin E was found to attenuate oxidative stress and inflammation in TAA-induced SALF. Enzymes AST and ALT were increased by about 1200% in the groups receiving TAA as compared to controls at 24h and 48h. Other authors also obtained similar results by inducing toxicity in rat livers through administration of AA doses (350 mg/kg). In Vitamin E-treated groups, there was a significant decrease of about 75% in AST and ALT as compared to TAA groups at 24h and 48h[7,28]. In another study also observed a reduction of these parameters in an experimental model of toxicity, treating with flavonoid quercitin[8].

In LPO evaluation, TBARS presented an increase of 59.5% and 94.1% at 24h and 48h, respectively, in the TAA group. In the Vit. E group, however, values were similar to those of the control group and significantly reduced as compared to TAA groups, with reductions of 51.3% and 42.4% at 24h and 48h, respectively. Such reduction demonstrates decreased LPO in the hepatic tissue due to the antioxidant action of vitamin E administered to these animals, which can be evidenced by histology, where one notes reorganization of the hepatic tissue and reduction of the inflammatory infiltrate and necrosis[2,17,28].

Administration of Vitamin E significantly reduced SOD activity by 49.7% at 24h and 59.48% at 48h, suggesting decreased production of $O_2^{\cdot}$, and consequent decreased LPO, as demonstrated by histology and TBARS levels. In a study investigating the preventive effect of quercetin in animals with TAA-induced SALF, de Oliveira et al., showed significantly increased SOD activity from the administration of the flavonoid. Other works have reported decreased SOD activity from the administration of other antioxidants[8,22,23].

CAT activity was significantly reduced in TAA groups as compared to CO groups by 51.9% and 43.6% at 24h and 48 h, respectively. TAA acts directly on the formation of $O_2^{\cdot-}$, which act on membrane lipids and form hydroperoxide lipids, thus accounting for reduced CAT activity. The use of Vit. E significantly restored CAT activity at 24h and 48h, with increases of 106% and 61.8%, respectively, making it similar to CO groups and reinforcing the antioxidant effect of Vitamin E. Another study found significant reduction of CAT activity in rats, evaluating the damage caused by oxidative stress induced by high-carbohydrate diet in a model of hepatic encephalopathy[1].

The behavior of GPx activity varied across the studied times. While in the TAA group there was a reduction of 46% in GPx activity in relation to the CO group at 24h, there was an increase of it (158.8%) at 48h, possibly explained by the reestablishment of the hepatic tissue. As compared to the TAA group, GPx activity in the TAA+Vit.E group presented an increase of 146.3% at 24h and a decrease of 32.3% at 48h, which can be explained by the decreased tissue aggression due to the protective effect of Vit. E, evidenced by lower TBARS levels and histology. The increase in GPx appears to accompany the accumulation of organic peroxides formed by LPO caused by TAA. Similar results were reported in a work using antioxidant quercetin and glutamine[7,31].

The high toxicity of TAA in hepatocytes triggered an increase in enzyme GST, through its detoxifying protective action, with increases of 128.2% and 111% at 24h and 48h, respectively. In the Vitamin E-treated group, however, the reduction was of 37.5% and 32.6% at 24h e 48h, respectively, owing to the preservation of hepatocytes by the exogenous antioxidant, as evidenced again by decreased TBARS levels and liver histology. Our findings corroborate those of other authors who evaluated GST activity in experimental model, using TAA as hepatotoxic inducer[31].

On histological evaluation of hepatic tissue using hematoxylin & eosin (HE) staining at a magnification of 200x, CO and Vit. E groups showed hepatic cells arranged in hepatocyte cordons around capillaries with aspects of normal distribution, and visible nuclei without inflammatory infiltrates or necrosis. On the other hand, the hepatic tissue in the TAA group displayed disorganization of hepatocytes, inflammatory infiltrates, and necrosis, which accounts for increased TBARS levels at both times. Histological evaluation of the Vit.E-treated group showed a reorganization of hepatocyte cordons and smaller incidence of inflammatory infiltrate or necrosis. These evidences coincide with significant reduction of TBARS and lower lipoperoxidation in the hepatic parenchyma at both studied times, promoted by Vitamin E antioxidant action. Another author also demonstrated Vitamin E antioxidant role when administered at 200 mg/Kg/day for 3 days[28].

The rise of metabolites NO_2 and NO_3 indicates an increase in NO production, which participates closely in the inflammatory and destructive process of the hepatic tissue[9]. Animals in TAA groups presented increase of NO metabolites that associate with the superoxide anion to form peroxinitrite, which is extremely damaging to hepatocytes. This increase was of 476% (24h) and 646% (48h) in relation to control groups, while Vitamin E-supplemented groups showed a reduction of 36.2% (24h) and 29.8% (48h) as compared to groups receiving TAA, which in a way can be explained by the improvement of the hepatic parenchyma. Similar findings were reported by other authors who assessed nitric oxide levels[7, 22].

From the findings it is possible to see that the Vitamin E group at 48h, despite having received two additional 100 mg/Kg doses of Vitamin E, presents values that are close to those obtained at 24h, suggesting that the dose of 100 mg/Kg administered intraperitoneally thirty minutes after TAA administration was already sufficient to protect the liver against the oxidative stress triggered by the drug.

CONCLUSION

Oxidative stress plays a key role in the aggravation of liver injury and structural and/or functional disorders of the liver. The use of antioxidants, such as vitamin E, seems promising as an attempt to prevent the complications resulting from oxidative stress and in the continuation of the disease. In the present study, TAA administration induced liver damage in rats at both 24h and 48h and administration of Vitamin E was effective in protecting the tissue at both these times. We suggest that Vitamin E can be an effective tool in the treatment of SALF and its resulting complications. However, studies with new approaches taking in

consideration apoptosis markers, inflammatory route, and DNA damage caused by oxidative stress will be helpful in elucidating these mechanisms.

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Authors' contributions

Miguel FM and Schemitt EG performed all of the research work; Colares JR and Hartmann RM were in charge of the experimental model; Morgan-Martins MI and Marroni NP designed the research work and the article review.

Miguel FM, Schemitt EG, Colares JR, Hartmann RM, Morgan-Martins MI, Marroni NP. Ação da vitamina E sobre a insuficiência hepática aguda grave experimental. Arq Gastroenterol. 2017;54(2):123-9.

RESUMO – **Contexto** – A Insuficiência Hepática Aguda Grave (IHAG) é uma síndrome clínica potencialmente fatal, na qual ocorre necrose dos hepatócitos, perda da arquitetura hepática e deterioração de suas funções. Dentre as principais causas da IHAG está a hepatotoxicidade decorrente de agentes químicos, que lesam os hepatócitos e acarretam aumento das espécies reativas de oxigênio. A vitamina E tem alta atividade antioxidante biológica e é amplamente distribuída nos tecidos. **Objetivo** – Avaliar o efeito antioxidante da Vitamina E no modelo de IHAG. **Métodos** – Foram utilizados 56 ratos, com peso médio de 300 g, divididos em oito grupos, quatro grupos avaliados em 24 horas e quatro em 48 horas após a indução: grupo controle (CO); Vitamina E (Vit.E); Tioacetamida (TAA) e Tioacetamida + Vitamina E (TAA+Vit.E). Os ratos foram submetidos a injeções de tioacetamida, na dose de 400 mg/Kg de peso i.p., no início do experimento e, posteriormente, após 8 horas. A vit E (100 mg//Kg i.p.) foi administrada 30 minutos após a segunda dose de tioacetamida. Os animais do tempo 48 horas receberam mais duas doses de vit. E (24h e 36h). Transcorridas 24 ou 48 horas após a administração da primeira dose de TAA, os animais foram pesados, anestesiados e o sangue retirado para a avaliação da integridade hepática através das enzimas Aspartatoaminotransferase (AST) e Alanina aminotransferase (ALT). O tecido hepático foi retirado para avaliação da lipoperoxidação através da técnica de TBARS, atividade das enzimas antioxidantes SOD, CAT, GPx, e GST, avaliação de NO_2/NO_3 e avaliação histológica pela coloração de hematoxilina e eosina nos dois tempos. Os resultados foram expressos como média ± erro padrão e a análise estatística utilizada foi ANOVA, seguido de teste de Student-Newman-Keuls, considerado significativo $P<0,05$. **Resultados** – Após o tratamento com a vit. E, observamos uma redução nas enzimas de integridade hepática AST (U/L) (101,32±19,45 em 24h e 97,85±29,65 em 48h) relacionado ao grupo TAA (469,56±20,69 em 24h e 598,23±55,45 em 48h) e ALT (U/L) (76,59±8,56 em 24h e 68,47±6,49 em 48h) comparado ao grupo TAA (312,21±10,23 em 24h e 359,15±17,58 em 48h). Houve uma redução da LPO (nmol/mg Prot), no grupo TAA+Vit.E (0,77±0,07 em 24h e 0,95±0,08 em 48h) comparado ao grupo TAA (1,50±0,07 em 24h e 1,65±0,16 em 48h). A SOD (USOD/min/mg Prot) diminuiu no grupo TAA+Vit.E (49,48±9,47 em 24h e 62,45±18,47 em 48h) relacionado ao grupo TAA (98,46±15,48 em 24h e 154,13±21,46 em 48h), assim como a GST (nmol/min/mg Prot) no grupo TAA+Vit.E (350,57±36,93 em 24h e 453,29±13,84 em 48h) comparado ao grupo TAA (561,57±64,56 em 24h e 673,43±38,13 em 48h). Houve aumento da CAT (pmol/min/mg Prot) no grupo TAA+Vit.E (3,40±0,44 em 24h e 3,01±0,35 em 48h) em relação ao grupo TAA (1,65±0,21 em 24h e 1,86±0,42 em 48h). A GPx (nmol/min/mg Prot) aumentou em 24h no grupo TAA+Vit.E (1,01±0,16) comparado ao grupo TAA (0,41±0,04) e diminuiu em 48h (1,19±0,17) em relação ao grupo TAA (1,76±0,21). Verificou-se redução nos níveis de NO_2/NO_3 (mmol/L) no grupo TAA+Vit.E (31,47±4,26 em 24h e 38,93±5,20 em 48h) em relação ao grupo TAA (49,37±5,12 em 24h e 53,53±5,97 em 48h). A avaliação histopatológica mostrou diminuição da lesão hepática (necrose e inflamação) em ambas os tempos estudados. **Conclusão** – Estes resultados sugerem que a vitamina E foi capaz de proteger o fígado de lesões causadas por tioacetamida.

DESCRITORES – Falência hepática aguda. Tioacetamida. Estresse oxidativo. Antioxidantes.

REFERENCES

1. Alzoubi KH, Khabour O F, Salah H A, Hasan Z, et al. Vitamin E prevents high-fat high –carbohidrates diet-induced memory impairment: The role of oxidative stress. Physiol Behav. 2013;119:72-8.
2. Bhatti FR, Mehmood A, Wajid N, Rauf M, Khan S, Riazuddin S, et al. Vitamin E protects chondrocytes against hydrogen peroxide-induced oxidative stress in vitro. Inflamm Res. 2013;62:781-9.
3. Boveris A, Chance B. The mitochondrial generation of hydrogen peroxide. General properties and effect of hyperbaric oxygen. Biochem J. 1973;134:707-16.
4. Buege JA, Aust SD. Microsomal lipid peroxidation. Methods Enzymol. 1978;52:302-9.
5. Chieli E, Malvadi G. Role of Cyt P-450 dependent and FAA containing mono oxygenases in the bioactivation of thioacetamide, thiobezamide and their sulophoxides. Biochem Pharmacol. 1985; 34:395-1.
6. Chu CJ, Hsiao CC, Wang TF, Chan CY, Lee FY, Chang FY, et al. Prostacyclin inhibition by indomethacin aggravates hepatic damage and encephalopathy in rats with thioacetamide-induced fulminant hepatic failure. World J Gastroenterol. 2005;11:232-6.
7. David C, Rodrigues G, Bona S, Meurer L, González-Gallego J, Tuñón M, et al. Role of quercetin in preventing thioacetamide-induced liver injury in rats. Toxicol Pathol. 2011;39:949-57.
8. De Oliveira CR, Ceolin J, De Oliveira RR, Schemitt EG, Colares JR, Bauermann LF, et al. Effects of quercetin on polychlorinated biphenyls-induced liver injury in rats. Nutr Hosp. 2014; 1141-8.
9. Diesen DL, Kuo PC. Nitric oxide and redox regulation in the liver: Part I. General considerations and redox biology in hepatitis. J Surg Res. 2010;162:95-109.
10. Diez-Fernandez C, Sanz N, Cascales M. Changes in glucose-6-phosphate dehydrogenase and malic enzyme gene expression in acute hepatic injury induced by thioacetamide. Biochem Pharmacol. 1996;51:1159-63.
11. Fedstein AE. Novel insights into the pathophysiology of nonalcoholic fatty liver disease. Semin Liver Dis. 2010;30:391-401.
12. Fitzhugh OG, Nelson AA. Liver tumours in rats fed thiourea or thioacetamide. Science, 1948; 108: 626–28.
13. Flohé L, Gunzler WA. Assays of glutathione peroxidase. Methods Enzymol. 1984;105:114-21.

14. Fontana L, Moreira E, Torres MI, Fernández MI, Ríos A, De Medina FS, et al. Serum amino acid changes in rats with thioacetamide-induced liver cirrhosis. Toxicology.1996;106:197-206.
15. Halliwell B, Gutteridge J. Free Radicals in Biology and Medicine. 4th edn. New York: Oxford University Press Inc; 2007.
16. Herrera E, Barbas C. Vitamin E: action, metabolism and perspectives. J Physiol Biochem. 2001; 57:43-56.
17. Jeon YH. Vitamin E, an antioxidant, as a possible therapeutic agent for treating pain. Korean J Pain. 2013;3:314-5.
18. Lee, WM. Acute liver failure in the United States. Semin. Liver Dis. 2003;23:217-26.
19. Llesuy SF, Milei J, Molina H, Boveris A, Milei S. Comparison of lipid peroxidation and myocardial damage induced by adriamycin and 4'- epiadriamycin in mice. Tumori. 1985,71:241-9.
20. Mangipudy RS, Chanda S, Mehendale HM. Tissue repair response as a function of dose in thioacetamide hepatotoxicity. Environ Health Perspect. 1995;103:260-7.
21. Mannervick BGC, Guthenberg C. Glutationa Transferase (human placenta). Methods Enzymol. 1981;77:231-5.
22. Marques C, Licks F, Zattoni I, Borges B, De Souza LER, Marroni, CA, et al. Antioxidant properties of glutamine and its role in VEGF-Akt pathways in portal hypertension gastropathy. World J Gastroenterol. 2013;19:4464-74.
23. Marques C, Mauriz JL, Simonetto D, Marroni CA, Tuñon MJ, González-Gallego J, Marrón NP. Glutamine Prevents gastric oxidative stress in an animal model of portal hypertension gastropathy. Ann Hepatol. 2011;4:531-39.
24. Mattos AA, Dantas-Corrêa EB. Tratado de Hepatologia. 4 Ed. Rubio: São Paulo, 2010. 1.024 p.
25. Misra HP, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem. 1972;247:3170-5.
26. Morgan-Martins MI. Estresse Oxidativo e Antioxidantes. In Marroni, NP. Radicais Livres no processo saúde-doença: da bancada à clínica/ Norma Possa Marroni, Maria Isabel Morgan-Martins, Marilene Porawski (organizadoras) – Curitiba, PR: CRV, 2012.
27. Muñoz RS, Maroja JL, Vasconcelos FR, Melo J. Avaliação cognitiva breve para detecção de encefalopatia em pacientes com doença hepática crônica. GE Port J Gastroenterol. 2013;20:255-60.
28. Mustafa HN, Awdan AS, Hegazy GA. Protective role of antioxidants on thioacetamide-induced acute hepatic encephalopathy: Biochemical and Ultrastructural study. Tissue and Cell. 2013;45:350-62.
29. Neal RA. Halpert, J. Toxicology of thionosulfer compounds. Annu Rev Pharmacol Toxicol. 1982;22:321-9.
30. Niki E. Role of vitamin E as a lipid-soluble peroxil radical scavenger: in vitro and in vivo edidence. Free Radical Biol Med. 2014;66:3-12.
31. Schemitt EG, Colares JR, Hartmann RM, Morgan-Martins MI, Marroni CA, Tuñón MJ, Marroni NP. Effect of glutamine on oxidative stress and inflammation in a rat model of fulminant hepatic failure. Nutr Hosp. 2016;33:92.

ORIGINAL ARTICLE

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Ex vivo model of rabbit intestinal epithelium applied to the study of colonization by enteroaggregative *Escherichia coli*

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ABSTRACT – Background – The diarrheal syndrome is considered a serious public health problem all over the world and is considered a major cause of morbidity and mortality in developing countries. The high incidence of enteroaggregative *Escherichia coli* in diarrheal syndromes classified as an emerging pathogen of gastrointestinal infections. After decades of study, your pathogenesis remains uncertain and has been investigated mainly using *in vitro* models of adhesion in cellular lines. **Objective** – The present study investigated the interaction of enteroaggregative *Escherichia coli* strains isolated from childhood diarrhea with rabbit ileal and colonic mucosa *ex vivo*, using the *in vitro* organ culture model. **Methods** – The *in vitro* adhesion assays using cultured tissue were performed with the strains co-incubated with intestinal fragments of ileum and colon over a period of 6 hours. Each strain was tested with three intestinal fragments for each region. The fragments were analysed by scanning electron microscopy. **Results** – Through scanning electron microscopy we observed that all strains adhered to rabbit ileal and colonic mucosa, with the typical aggregative adherence pattern of "stacked bricks" on the epithelium. However, the highest degree of adherence was observed on colonic mucosa. Threadlike structures were found in greater numbers in the ileum compared to the colon. **Conclusion** – These data showed that enteroaggregative *Escherichia coli* may have a high tropism for the human colon, which was ratified by the higher degree of adherence on the rabbit colonic mucosa. Finally, data indicated that *in vitro* organ culture of intestinal mucosa from rabbit may be used to elucidate the enteroaggregative *Escherichia coli* pathogenesis.

HEADINGS – *Escherichia coli*. Intestinal mucosa, ultrastructure. Organ culture techniques. Rabbits. Scanning electron microscopy.

INTRODUCTION

EAEC is an emerging diarrheal pathotype described in the diarrheagenic *E. coli* group (DEC) and has been characterized by the aggregative adherence (AA) pattern where bacteria adhered to the HEp-2 cells (line derived from human laryngeal carcinoma) in a similar arrangement of "stacked bricks"[17].

Associated with cases of acute and persistent diarrheal disease EAEC affect children and adults in all regions of the world, patients in developing countries infected with human immunodeficiency virus (HIV), travelers from industrialized countries visiting less developed regions and residents of industrialized countries with foodborne gastroenteritis outbreaks[6].

Several putative virulence factors are involved in the pathogenesis, which remains uncertain due to it is complexity. It was suggested a three-stage model: i) adhesion of bacteria to the intestinal mucosa; ii) mucus secretion stimulated by bacteria forming a biofilm, which could favor the persistent colonization; and iii) production of toxins and inflammatory response involved in the damage to the intestinal mucosa[11].

Most of the knowledge about the interactions of bacteria and intestinal organs has been obtained from studies with human biopsies collected during surgical intervention or from samples obtained from elderly individuals at autopsy[8]. In order to elucidate the mechanisms of EAEC pathogenesis, models of study in organ culture *in vitro* (IVOC) from intestinal fragments from different portions of the small or/and large intestines of pediatric patients[1,2,10,18,23] and of adults[2,15,23] have been developed. Here we propose a more feasible animal study model in order to apply our understanding on the pathogenesis mechanism of EAEC strains.

METHODS

Bacterial strains

EAEC strains were isolated from the faeces of children under two years of age with acute diarrhea coming from the urban area of Rio de Janeiro, Brazil. Diarrhea was defined as the occurrence of one or more elimination of liquid faeces in a period of 24 hours[20]. As a positive control, the prototype strain EAEC 042, isolated from children with diarrhea in outbreak in Peru[16] was used to infect the ileal and colonic fragments. As a negative control, non-infected intestinal fragments were used. The EAEC strains were previously characterised by DNA hybridization probe[3] or by polymerase chain reaction (PCR)[21] for putative virulence factors of EAEC[7,20]. Adhesion assays in intestinal cell lines after 6 hours of incubation was used to characterize the aggregative pattern (Table 1). All strains were stored at -70°C in Trypticase Soy Broth (TSB, Merck) supplemented with 20% glycerol.

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TABLE 1. Enteroaggregative *Escherichia coli* (EAEC) and prototype strains and their serotypes, phylogenetic groups, virulence factors and adherence patterns

Strains	Serotype [a]	Phylogenetic Group	Virulence factors [b]	Adherence Patterns[c]	
				Caco-2	T84
H92/3	O86:H18	D	pAA*, *aggA, aggR, aap, fyuA, irp2, pic*	AA	AA
I18/2	O86:H11	D	pAA*, *aggR, aap, fyuA, irp2*	AA	AA
I34/4	O111:H21	D	pAA*, *agg3A, aggR, aap, astA, fyuA, irp2, pet, pic*	AA	AA
I49/3	O26:H27	A	pAA*, *agg3A, aggR, aap, astA, irp2, pet, pic*	NA	AA
042	O44:H18	D	pAA*, *aafA*, *aggR*, *aap*, *astA*, *fyuA*, *irp2*, *pet*, *pic*	AA	AA

[a] O, somatic antigen polysaccharide; H, flagellar antigen. [b] pAA, aggregative adherence plasmid; *aggA*, aggregative adherence fimbriae I; *aggR*, transcriptional regulator; *aap*, dispersin; *fyuA* and *irp2*, genes involved in iron caption; *pic*, protein involved in colonization; *agg3A*, aggregative adherence fimbriae III; *astA*, thermostable protein; *pet*, plasmid enconded toxin; *aafA*, aggregative adherence fimbriae II. *detection by hybridization with DNA probe. [c] AA, aggregative adherence; NA, non-adherence.

Rabbit selection and preparation

New Zealand white male rabbits (1.0-1.5 kg) supplied by bioterium of Institute Vital Brazil (Rio de Janeiro, RJ) were used to obtain intestinal tissue for experiments. Previously, rabbit faeces were collected, seeded in TSA (Difco lab), Mac Conkey agar (Merck) and CLED agar (Merck) and incubated at 37°C for a period of 24 hours to certify that the animals were not infected with enteropathogens. Samples tested did not show growth of microorganisms. The rabbits were subjected to fasting for 24 hours before experimental procedures. They were observed for any gastrointestinal manifestations and were used only if found to be symptom-free.

Rabbit *in vitro* organ culture adhesion assay

To obtain intestine fragments of ileum and colon, the animals were anesthetized and euthanized by intravenous application Tiopentax (sodium thiopental, 10 mg/kg, Cristália, São Paulo, Brazil). Then the trichotomy of the abdominal region was performed and, subsequently, asepsis of the incision site with iodized alcohol. The gastrointestinal tract was exteriorized through a ventral midline incision and the mucosa was washed with saline and it was made identification of anatomical sites of the intestinal tract.

The adhesion assays were performed as previously described[10,14] with some modifications. Briefly, 2- to 3-mm^2 fragments of tissues were oriented mucosal surface upward on sterile foam squares placed into a 6-well plate (about 35-mm-diameter). The foam was saturated with Dulbecco's Modified Eagle's Medium (DMEM; Gibco) supplemented with 10% fetal calf serum (FCS) and 0.5% D-mannose. The level of DMEM medium was adjusted to cover the biopsy specimens with a thin film of medium by capillary action.

Bacterial strains were inoculated in 3 mL of TSB (Merck) and incubated for 18 hours at 37ºC and aliquots of 50 µL standardized bacterial suspensions (approximately 10^7 CFU mL^{-1}) was inoculated on the mucosal surface of the fragments and the 6-weel tissue culture plate was then incubated at 37˚C in an atmosphere with 5% CO_2 for 6 hours. The culture medium was changed completely every 2 hours to maintain pH and nutrient levels, without reinoculation with the bacterial culture. After these incubation periods, biopsy samples were washed and prepared for scanning electron microscopy (SEM). EAEC strains were tested with three intestinal fragments of each intestinal region.

Tissue processing

The fragments collected were washed with 0.9% sterile saline and 0.1M sodium cacodylate buffer, pH 7.2 and fixed with 2.5% glutaraldehyde and 2% formaldehyde in 0.1 M sodium cacodylate buffer, pH 7.2. Later, the fragments were washed three times in 0.1 M sodium cacodylate buffer, pH 7.2 and dehydrated through a graded series of ethanol solutions (30%, 50%, 70%, 90% and 100%). Then, the fragments were transferred to baskets and subjected to the drying apparatus chambre (Bal-Tec CPD 030) by the critical point of carbon dioxide method, to be performed replacing absolute ethanol by carbon dioxide. Subsequently, specimens were mounted on stubs with the vili upward with the aid of a stereoscopic microscope, coated with gold in sputter (Balzers Union FL-9496) to become conductors. The observations were performed in the Ultrastructure Cellular Laboratory Hertha Meyer of the *Universidade Federal do Rio de Janeiro* (LCU, UFRJ) in scanning electron microscope (QUANTA 250, FEI) operating at 15 Kv.

Ethical considerations

The study protocol used was approved by Ethic Committee (*Comitê de Ética Para o Cuidado e Uso de Animais Experimentais*) of Biology Institute Roberto Alcântara Gomes of *Universidade do Estado do Rio de Janeiro* (UERJ) n° CEA/236/2008.

RESULTS

EAEC strains interaction with ileal mucosa fragments

Analysis by SEM of non-infected ileum showed that fragments presented the mucosa preserved (Figure 1A). The prototype EAEC 042 strain adhered to the ileal mucosa in the aggregative characteristic pattern of "stacked bricks" (Figure 1C).

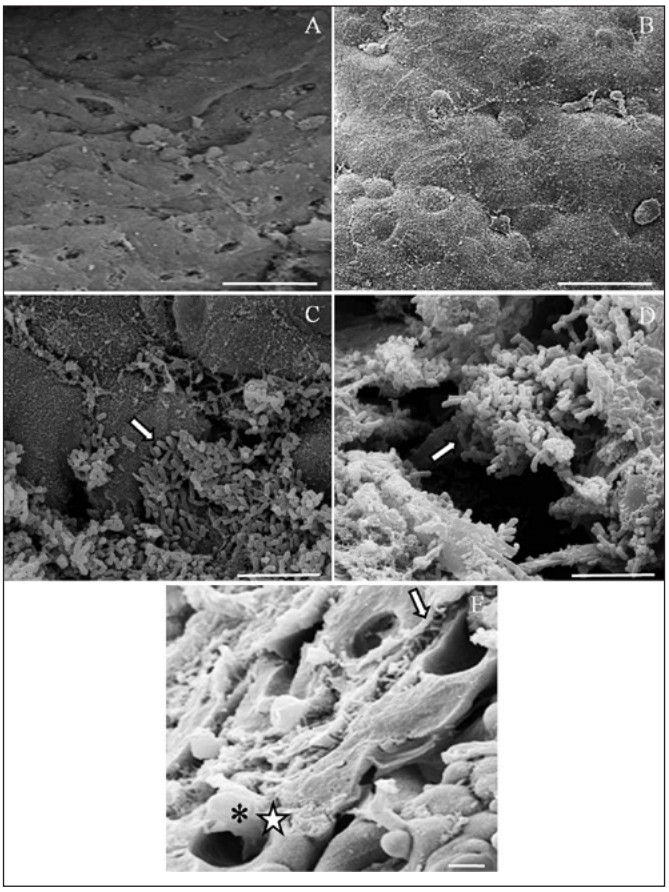

FIGURE 1. A) (1000X; bar- 10 µm), B) (1000X; bar- 10 µm), C) (5000X; bar-10 µm), D) (5000X; bar-10 µm) and E) (1000X; bar- 5 µm). Micrograph of the intestinal rabbit mucosa *ex vivo,* after 6 hours of incubation, observed by scanning electron microscopy. Mucosa preserved on the ileum (A) and on the colon (B) epithelium non-infected. Infected tissue with EAEC 042 showed large bacterial aggregates in a "stacked bricks" pattern (⟶) adhered to the epithelium of the ileum (C) or forming a thick three--dimensional biofilms on the colon (D). The opened and dilated crypts (☆) with discharge of mucus (*) showed in the colon (E).

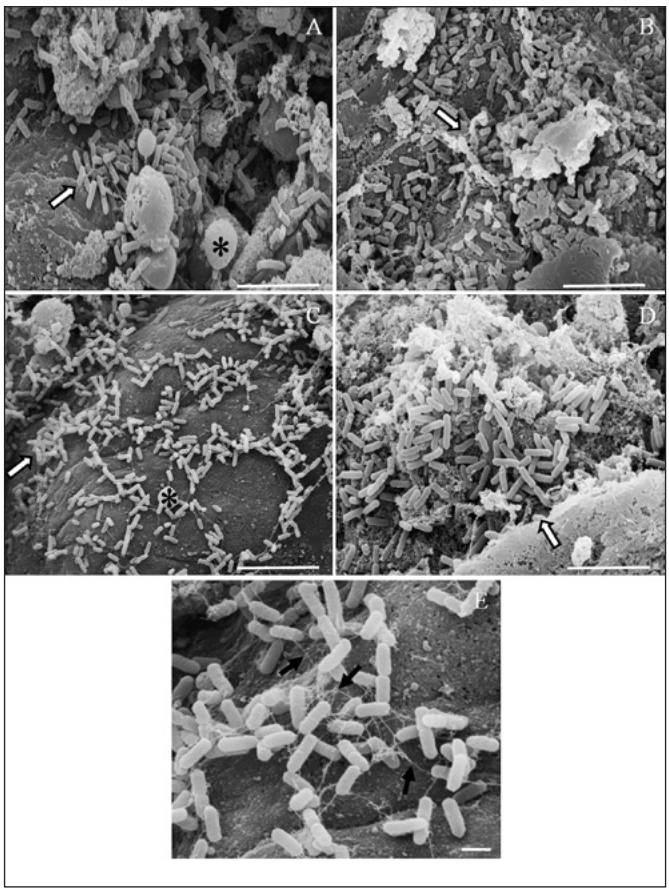

FIGURE 2. A) (3500X; bar- 10 µm), B) (3500X; bar- 10 µm), C) (3500X; bar-10 µm), D) (5000X; bar-10 µm) and E) (5000X; bar- 1 µm). Micrograph of the EAEC strains interaction with intestinal rabbit mucosa *ex vivo,* after 6 hours of incubation, observed by scanning electron microscopy. Interaction with I34/4 strain with bacterial aggregates (⟶) and isolated points of mucus (*) above the ileum (A) and colonic (B) epithelium. Interaction with I49/3 strain with bacterial aggregates (⟶) and isolated points of mucus (*) directly on the ileum epithelium (C) and intense adherence with large bacterial aggregates on the colonic epithelium (D). Ileal mucosa showed threadlike structures (⟶) mediating the link between bacteria-bacteria and bacteria-cell (E).

The intestinal fragments infected with the EAEC strains revealed bacterial aggregates as observed in cell lines Caco-2, cell line of human colorectal adenocarcinoma with phenotypic characteristics of the ileum, adhered directly on the epithelium with isolated points of mucus produced above the mucosa (Figures 2A and 2C). Although the I49/3 strain did not display the standard AA in Caco-2, this was presented in IVOC rabbit model.

Threadlike structures (Figure 2E) were found in all strains, except the positive control, in greater numbers in the ileum compared to colon fragments apparently mediating bacteria-bacteria and bacteria-epithelium interactions**.**

EAEC strains interaction with colonic mucosa fragments

Analysis by SEM of the colonic fragments non-infected showed mucosa preserved (Figure 1B). The prototype EAEC 042 strain adhered to the colonic mucosa in the aggregative characteristic pattern of "stacked bricks" and were able to form a thick three-dimensional biofilm (Figure 1D). Also it was observed the opening of colonic crypts with discharge of mucus (Figure 1E).

The intestinal fragments infected with the EAEC strains revealed intense adherence with large bacterial aggregates as observed in cell lines T84, cell line of human colon carcinoma with phenotypic characteristics of the colon, adhered directly on the colonic epithelium (Figures 2B and 2D).

The analysis of adherent bacteria by SEM revealed the highest number of bacteria adherent in the colon fragments when compared to the ileum fragments.

DISCUSSION

Since EAEC has been isolated from several patients suffering from acute or chronic diarrhea in a number of regions of the world and from various socioeconomic strata your research is also challenging. In addition to this the genetic heterogeneity of the pathotype which considerably complicates our understanding of the pathogenicity, the therapeutic approaches and international surveillance[11].

The study of EAEC in several models, such as in the *in vitro*/*in vivo* assays in animals[22,23], in the *in vitro* organ culture in cellular lines[1,9,18] and with human intestinal fragments[1,2,10,15,18,23] has been widely used by more faithfully mimic the EAEC action in the host.

Thus, IVOC represents ideally a good model for study of the bacterial pathogenesis in intestinal explants which remain structurally intact for periods of hours in culture[10]. This technique has utilized human tissue biopsies, tissue fragments extracted during colonoscopy and even of animals.

In the present study, the EAEC strains challenged in the *ex vivo* rabbit model were able to adhere to both intestinal fragments, ileum and colon, in the analysis by SEM, and they formed bacterial aggregates with a similar pattern of "stacked bricks" adhered directly on the epithelium with isolated points of mucus in some strains. Still, it was possible to infer a higher number of bacteria adherent to colon fragments when compared to the ileum fragments.

Studies conducted by Yamamoto et al.[23] and Nataro et al.[18] are consistent with ours. Yamamoto et al.[23] showed that the EAEC strain 0127:H2 exhibited lower levels of adherence in the jejunal and ileal mucosa and higher levels of adherence to colonic mucosa fixed in formalin. Already Nataro et al.[18] from jejunal, ileum and colon mucosa explants obtained from three pediatric patients revealed that the number of bacteria adherent to colon specimens was significantly higher than the adhering bacteria to mucosal samples of small intestine.

The adhesion of EAEC to the large intestine but not to the mucosa of human small intestine, not only demonstrates that EAEC possess properties enteroadherent, but also suggests that this may be the preferred site of colonization of this pathotype. Still, the lack of adhesion to ileal mucosa could be due to a lack of receptors for EAEC[15]. Additionally, Kang et al.[13] suggested that the degree of cell differentiation and maturation may also affect the receptor concentration and such differences in receptor expression may have an important role in determining the EAEC binding site.

Andrade et al.[2] analyzing the interaction of EAEC strains with pediatric and adults human intestinal fragments of ileum and colon, demonstrated that all EAEC strains adhered to both regions evaluated on a layer of thick mucus and sometimes directly to the mucosa. In all cases in which the strains were adherent to the mucosa in significant numbers, they did into aggregates with the pattern of "stacked bricks" similar to that observed in HEp-2 cell culture.

In the present study threadlike structures were observed in all strains, except the positive control, in greater numbers in the ileum compared to colon fragments. The *aag* and *agg3* genes encode AAF/I and AAF/III, respectively, and are long and flexible fimbriae which are involved in the cohesion of bacterial aggregates and binding with intestinal cells[4,5]. This may explain why these structures were observed in our strains and was not observed in EAEC 042 strain, which has a short and rigid fimbriae.

Our results are consistent with studies conducted by Andrade et al.[2] that observed non-characterized fimbrial structures on bacterial surface interacted with the human ileum fragments, apparently mediating bacteria-bacteria and bacteria-cell interactions. Pereira et al.[19] observed that bacterial aggregates forming biofilms in abiotic surface were mediated by non-bundle forming, flexible pili and that the increased adherence might be mediated by putative F pili expressed by EAEC strains. The I18/2 strain not possess any of the genes described for the fimbriae AAFs. Possibly the visualized threadlike structures could be a novel aggregative adherence fimbria variant (AAF/V) as suggested recently by Jonsson et al.[12]. Further investigation is needed to better understand the main role of these structures in the pathogenesis of these heterogeneous pathotype, once that a wide diversity of adhesive structures include uncharacterized nonfimbrial and fimbrial adhesins.

In conclusion, all challenged EAEC strains adhered to both intestinal fragments, with the highest tropism in the colon, ratifying the use of the IVOC model as a tool for studying the pathogenesis of this pathotype. Finally, the present study is pioneer in the use of rabbit IVOC to evaluate the pathogenic role of EAEC strains.

Authors' contributions

Braga RLL: survey execution, writing of text. Pereira ACM: survey execution; reviewing of text. Santos PA: survey execution. Freitas-Almeida AC: reviewing of text. Rosa ACP: project coordinator, reviewing of text.

Braga RLL, Pereira ACM, Santos PA, Freitas-Almeida AC, Rosa ACP. Modelo *ex vivo* de epitélio intestinal de coelho aplicado ao estudo de colonização por *Escherichia coli* enteroagregativa. Arq Gastroenterol. 2017;54(2):130-4.

RESUMO – Contexto – A síndrome diarréica é considerada um grave problema de saúde pública em todo o mundo e é considerada uma das principais causas de morbidade e mortalidade nos países em desenvolvimento. A elevada incidência de *Escherichia coli* enteroagregativa nas síndromes diarreicas a classificou como um patógeno emergente de infecções gastrintestinais. Depois de décadas de estudo, sua patogênese ainda é incerta e tem sido investigada usando principalmente modelos *in vitro* de adesão em linhagens celulares. **Objetivo** – O presente estudo investigou a interação de cepas de *Escherichia coli* enteroagregativa isoladas de diarreia infantil com mucosa ileal e colônica de coelho *ex vivo*, utilizando o modelo de cultura de órgão *in vitro*. **Métodos** – Os ensaios de adesão *in vitro* utilizando tecido cultivado foram realizados com as cepas co-incubadas com fragmentos intestinais de íleo e de cólon durante um período de 6 horas. Cada cepa foi testada em três fragmentos intestinais para cada região. Os fragmentos foram analisados por microscopia eletrônica de varredura. **Resultados** – Através da microscopia eletrônica de varredura observamos que todas as cepas aderiram a mucosa ileal e colônica de coelho, com o padrão de aderência agregativo típico de "tijolos empilhados" no epitélio. Entretanto, o maior grau de adesão foi observado na mucosa do cólon. Estruturas filiformes foram encontradas em maior número no íleo em comparação com o cólon. **Conclusão** – Esses dados mostraram que *Escherichia coli* enteroagregativa pode ter um maior tropismo para o cólon humano, o que foi ratificado pelo maior grau de aderência na mucosa do cólon de coelho. Finalmente, os dados indicaram que a cultura de órgão *in vitro* da mucosa intestinal de coelho pode ser utilizado para elucidar a patogênese de *Escherichia coli* enteroagregativa.

DESCRITORES – *Escherichia coli*. Mucosa intestinal, ultraestrutura. Técnicas de cultura de órgãos. Coelhos. Microscopia eletrônica de varredura.

REFERENCES

1. Abe CM, Knutton S, Pedroso MZ, Freymüller E, Gomes TAT. An enteroaggregative *Escherichia coli* strain of serotype O111:H12 damages and invades cultured T84 cells and human colonic mucosa. FEMS Microbiol Lett. 2001;203: 199-205.
2. Andrade JAB, Freymüller E, Fagundes-Neto U. Adherence of enteroaggregative *Escherichia coli* to ileal and colonic mucosa: an *in vitro* study utilizing the scanning electron microscopy. Arq Gastroenterol. 2011;48:199-204.
3. Baudry B, Savarino SJ, Vial P, Kaper JB, Levine MM. A sensitive and specific DNA probe to identify enteroaggregative *Escherichia coli*, a recently discovered diarrheal pathogen. J Infect Dis. 1990;161:1249-51.
4. Bernier C, Gounon P, Le Bouguénec C. Identification of an aggregative adhesion fimbria (AAF) type III-encoding operon in enteroaggregative *Escherichia coli* as a sensitive probe for detecting the AAF-encoding operon family. Infect Immun. 2002;70:4302-11.
5. Czeczulin JR, Balepur S, Hicks S, Phillips A, Hall R, Kothary MH, et al. Aggregative adherence fimbria II, a second fimbrial antigen mediating aggregative adherence in enteroaggregative *Escherichia coli*. Infect Immun. 1997;65: 4135-45.
6. Estrada-Garcia T, Navarro-Garcia F. Enteroaggregative *Escherichia coli* pathotype: a genetically heterogeneous emerging foodborne enteropathogen. FEMS Immunol Med Microbiol. 2012;66:281-98.
7. França FLS, Wells TJ, Browning DF, Nogueira RT, Sarges FS, Pereira AC, et al. Genotypic and phenotypic characterisation of enteroaggregative *Escherichia coli* from children in Rio de Janeiro, Brazil. PLoS One. 2013;8:e69971.
8. Gerritsen J, Smidt H, Rijkers GT, de Vos WM. Intestinal microbiota in human health and disease: the impact of probiotics. Genes Nutr. 2011;6:209-40.
9. Harrington SM, Strauman MC, Abe CM, Nataro JP. Aggregative adherence fimbriae contribute to the inflammatory response of epithelial cells infected with enteroaggregative *Escherichia coli*. Cell Microbiol. 2005;7:1565-78.
10. Hicks S, Candy DC, Phillips AD. Adhesion of enteroaggregative *Escherichia coli* to pediatric intestinal mucosa *in vitro*. Infect Immun. 1996;64:4751-60.
11. Jensen BH, Olsen KEP, Struve C, Krogfelt KA, Petersen AM. Epidemiology and clinical manifestations of enteroaggregative *Escherichia coli*. Clin Microbiol Rev. 2014;27:614-30.
12. Jonsson R, Struve C, Boisen N, Mateiu RV, Santiago AE, Jenssen H, et al. Novel aggregative adherence fimbria variant of enteroaggregative *Escherichia coli*. Infect Immun. 2015;83:1396-405.
13. Kang G, Pulimood AB, Mathan MM, Mathan VI. Enteroaggregative *Escherichia coli* infection in a rabbit model. Pathology. 2001;33:341-6.
14. Knutton S, Lloyd DR, McNeish AS. Adhesion of enteropathogenic *Escherichia coli* to human intestinal enterocytes and cultured human intestinal mucosa. Infect Immun. 1987;55:69-77.
15. Knutton S, Shaw RK, Bhan MK, Smith HR, McConnell MM, Cheasty T, et al. Ability of enteroaggregative *Escherichia coli* strains to adhere *in vitro* to human intestinal mucosa. Infect Immun. 1992;60:2083-91.
16. Nataro JP, Scaletsky ICA, Kaper JB, Levine MM, Trabulsi LR. Plasmid-mediated factors conferring diffuse and localized adherence of enteropathogenic *Escherichia coli*. Infect Immun. 1985;48:378-83.
17. Nataro JP, Kaper JB, Robins-Browne R, Prado V, Vial PA, Levine MM. Pattens of adherence of diarrheagenic *Escherichia coli* to HEp-2 cells. Pediatr Infect Dis J. 1987;6:829-31.
18. Nataro JP, Hicks S, Phillips AD, Vial PA, Sears CL. T84 cells in culture as a model for enteroaggregative *Escherichia coli* pathogenesis. Infect Immun. 1996;64:4761-8.
19. Pereira AL, Silva TN, Gomes AC, Araújo AC, Giugliano LG. Diarrhea-associated biofilm formed by enteroaggregative *Escherichia coli* and aggregative *Citrobacter freundii*: a consortium mediated by putative F pili. BMC Microbiol. 2010;10:57.
20. Rosa ACP, Mariano AT, Pereira MAS, Tibana A, Gomes TAT, Andrade JRA. Enteropathogenicity markers in *Escherichia coli* isolated from infants with acute diarrhea and healthy controls in Rio de Janeiro, Brasil. J Med Microbiol. 1998;47:781-90.
21. Schmidt H, Knop C, Franke S, Aleksic S, Heesemann J, Karch H. Development of PCR for screening of enteroaggregative *Escherichia coli*. J Clin Microbiol. 1995;33:701-5.
22. Tzipori S, Montanaro J, Robins-Browne RM, Vial P, Gibson R, Levine MM. Studies with enteroaggregative *Escherichia coli* in the gnotobiotic piglet gastroenteritis model. Infect Immun. 1992;60:5302-6.
23. Yamamoto T, Endo S, Yokota T, Echeverria P. Characteristics of adherence of enteroaggregative *Escherichia coli* to human and animal mucosa. Infect Immun. 1991;59:3722-39.

ORIGINAL ARTICLE

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Screening of wild fruit trees with gastroprotective activity in different experimental models

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ABSTRACT – Background – Given the increase of people with gastrointestinal disorders, the search for alternative treatments with fewer side effects is vital, as well as the demand for food or plants that can help protect the stomach. **Objective** – The aim of this study was to evaluate the gastroprotective action of the extracts of wild fruit trees of *Myrcianthes pungens* (guabiju); *Inga vera* Willd. (ingá-banana) and *Marlierea tomentosa* Cambess. (guarapuruna) in *in vivo* pharmacological models. **Methods** – The different parts of the fruits were separately subjected to a process of extraction by methanol. Two experimental pharmacological models were conducted in mice; the gastric ulcer model induced by non-steroidal anti-inflammatory (indomethacin), and the gastric ulcer model induced by ethanol/HCl, which allowed us to evaluate the gastroprotective activity of the extracts at a dose of 250 mg/kg. Subsequently, the total lesion area (mm^2) and relative lesion area (%) were determined. **Results** – The results showed significant gastroprotective activity against the aggressive agents used – ethanol and indomethacin – for all the extracts tested. **Conclusion** – It is assumed that the fruits have bioactive compounds such as antioxidant substances that act on the prostaglandin levels, protecting them from the damage caused by ethanol and indomethacin. These results prompt further studies to isolate and identify the active properties.

HEADINGS – Gastrointestinal diseases. Phytochemicals. Ethanol. Indomethacin.

INTRODUCTION

Dietary patterns have undergone drastic changes in recent years, due to the replacement of fresh foods by processed foods, and unfortunately, these changes have resulted in an increasing number of people becoming affected by diseases of the digestive tract, such as gastritis, ulcers and carcinomas[11,13].

The development of gastritis, ulcers and other gastric disorders is not well understood. They may be associated with endogenous factors such as autoimmune diseases and gastric hypersecretion and exogenous factors such as stress, alcohol, caffeine, nonsteroidal anti-inflammatory drug (NSAID), infection by *Helicobacter pylori*, among others[7].

Given the large number of people affected by gastritis and the possibility of the evolution to clinical ulcer, the search for new drugs and alternative treatments with fewer adverse side effects is vital, as well as the demand for food or plants that can help protect the stomach[11].

Several experimental studies have highlighted the functional benefits of fruits, vegetables and plants, due to the presence of bioactive compounds that can be used in the prevention and treatment of various diseases[3,9,11,17].

Considering the evidence of promising active principles, such as flavonoids and terpenes, present in wild fruit trees, we have selected the following species to evaluate the gastroprotector potential: *Myrcianthes pungens, Marlierea tomentosa* and *Inga vera*.

Myrcianthes pungens popularly known as "guabiju", belongs to the Myrtaceae family. This plant is found mainly in South America, where it is used in popular medicine to regularize intestinal functions[2,6].

Marlierea tomentosa Cambess, a tree that grows in the coastal forests of Brazil, is popularly known as "guarapuruna" and also belongs to the Myrtaceae family, which is known to present flavonoids and terpenes with analgesic properties[1,12,14].

Inga vera Willd, popularly known as "ingá-banana", grows in the Atlantic Forest and areas of riparian vegetation. It belongs to the family Fabaceae and subfamily Mimosoideae, with about 40 genera and 350-400 species[5].

The aim of this study was to evaluate the gastroprotective action of the extracts of wild fruit trees of *Myrcianthes pungens* (guabiju); *Inga vera* Willd. (ingá-banana) and *Marlierea tomentosa* Cambess. (guarapuruna) in vivo pharmacological models.

METHODS

Plant material and preparation of extracts

Selected wild fruit trees were collected in February 2013 in the state of Santa Catarina, Brazil and identified by Dr. Oscar B. Iza (*Universidade do Vale do Itajaí*).

Leaves and seeds from *Myrcianthes pungens*, pulp and seeds from *Inga vera*, peel and seeds from *Marlierea tomentosa*, were cut into small pieces and extracted by maceration with methanol at room temperature for approximately seven days. The resulting macerates were filtered and concentrated under reduced pressure, using a rotatory evaporator, to yield the respective crude methanol extracts.

Declared conflict of interest of all authors: none
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Drugs, reagents, and solvents

Indomethacin and cimetidine were purchased from Sigma-Aldrich (St. Louis, MO, USA). All the other reagents and solvents used were of analytical grade.

Evaluation of gastroprotective activity

Male Balb/c mice (20-35 g) were provided by the Central Animal House of the Universidade do Vale do Itajaí (UNIVALI) (Itajaí, SC, Brazil). The animals were housed in groups of five, in standard cages, at room temperature (22 ± 2°C) with 12 h dark/12 h light cycles, and received food and water *ad libitum*.

Twelve hours prior to the experiments, they were transferred to the laboratory and given only water *ad libitum*. In all the experiments, the animals were kept in cages with wide-mesh raised floors to prevent coprophagy. The animals used in the present study were housed and cared for in accordance with the Federal Government legislation on animal care. Also, the experiments were authorized by the Ethical Committee for Animal Care of the *Universidade do Vale do Itajaí* (process number 041/13).

Ethanol/HCl-induced ulcer

The experiment was performed according to the method described by Mizui and Doteuchi[15], with some modifications. After 12 h of fasting, 48 animals were randomly divided into 8 different groups of 6 animals each and pre-treated orally with cimetidine (positive control – 100 mg/kg), vehicle (negative control - distilled water) and the methanol extracts from each part of the plants at a dose of 250 mg/kg. All treatments were administered by gavage.

One hour after treatment, all animals received 0.1 mL / 10 g (body weight) of a 0.3 mol/L HCl in 60% ethanol solution (ethanol/HCl) to induce gastric ulcer. Another hour later, the animals were sacrificed by cervical dislocation, and the stomachs removed and opened along the greater curvature. The stomachs were gently rinsed with water to remove the gastric contents and blood clots, for subsequent scanning. The images obtained were analyzed using specific "EARP" software to measure each lesion point. The results were expressed as total lesion area (mm^2) and relative lesion area (%).

Nonsteroidal anti-inflammatory drug-induced ulcer

Experiments were carried out according to the method described in Rainsford[18], with a few modifications. After 12 h of fasting, the animals were randomly divided into different groups of six animals each and pre-treated orally with cimetidine (positive control – 100 mg/kg), vehicle (negative control – distilled water) and the methanol extracts from each part of the fruits, at a dose of 250 mg/kg. All treatments were administered orally.

One hour after treatment, all the mice received indomethacin (100 mg/kg, p.o.) to induce gastric ulcer. Twelve hours after treatment with indomethacin, the animals were sacrificed by cervical dislocation, and the stomachs removed and opened along the greater curvature. The images were scanned and analyzed using image analysis software EARP to determine the number and size of the lesions. The results are expressed as total lesion area (mm^2) and relative lesion area (%).

Statistical analysis

The data are reported as mean ± standard error of the mean (SEM) and were compared using one-way analysis of variance (ANOVA), followed by Dunnett's pairwise test, and P values <0.05 were considered significant.

RESULTS

The gastroprotective potential of *M. pungens*, *M. tomentosa* and *I. vera* extracts was initially demonstrated by a significant reduction in damaged areas of the stomach when compared with the negative control (Table 1) in rats with ethanol-induced ulcer, a necrotizing agent.

TABLE 1. Effect of oral administration of plant extracts in mice (n=6) subjected to induction of acute ulcer ethanol

Treatments	Dose	Total area of lesion (mm^2)	Relative area of lesion (%)
Negative control	250 mg/kg	56.472 ± 9.315	10.615 ± 1.273
M. tomentosa (peel)	250 mg/kg	5.029 ± 2.36**	0,99 ± 0.47**
M. tomentosa (seed)	250 mg/kg	6.195 ± 2.52**	1.591 ± 0.62*
M. pungens (seed)	250 mg/kg	9.212 ± 5.57**	0.731 ± 0.41**
M. pungens (leaves)	250 mg/kg	29.529 ± 11.37*	2.797 ± 0.67*
I. vera (seed)	250 mg/kg	4.823 ± 2.27**	0.94 ± 0.39**
I. vera (pulp)	250 mg/kg	3.868 ± 1.94**	0.407 ± 0.12**
Positive control	100 mg/kg	3.79 ± 1.01**	1.782 ± 0.62*

Results as mean ±SEM for six rats or mice. Statistical comparison was performed using ANOVA followed by Dunnett's post test. *P<0.05 and **P<0.01 compared with the negative control group.

All the extracts were analyzed separately, as the lesion area on the methodology of induction of acute ulcer by ethanol. *I. vera* extracts showed gastroprotective effect in both studied parts (pulp and seed), which had significant differences when compared to the negative control and to the extracts of *M. pungens* (leaves and seeds) (Figure 1).

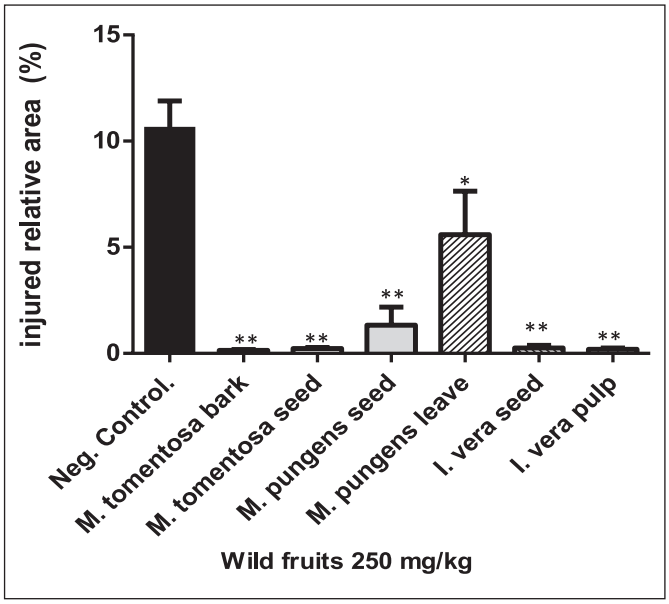

FIGURE 1. Gastroprotective effect of plant extracts (250 mg/kg) in mice in the ethanol-induced acute ulcer methodology. Results as mean ±SEM for six rats or mice. Statistical comparison was performed using ANOVA followed by Dunnett's post test. *P<0.05 and **P<0.01 compared with the negative control group.

Table 2 shows the gastroprotective activity of the plant extracts, in which the total lesion area and relative lesion area were reduced at the dose of 250 mg/kg. All the extracts had significantly lower values when compared with the negative control. It is possible that the extracts possess protective action through the production or maintenance of the mucus against the damage induced by this NSAID.

TABLE 2. Effect of oral administration of plant extracts in acute ulcer induced by NSAID (indomethacin) in mice (n=6)

Treatments	Dose	Total area of lesion (mm²)	Relative area of lesion (%)
Negative control	250 mg/kg	2.781 ± 0.88	1.06 ± 0.21
M. tomentosa (peel)	250 mg/kg	0.583 ± 0.14	0.143 ± 0.03**
M. tomentosa (seed)	250 mg/kg	0.886 ± 0.16	0.233 ± 0.04**
M. pungens (seed)	250 mg/kg	1.531 ± 0.55	0.334 ± 0.12**
M. pungens (leaves)	250 mg/kg	2.193 ± 0.33	0.531 ± 0.08*
I. vera (seed)	250 mg/kg	1.212 ± 0.58	0.253 0.12**
I. vera (pulp)	250 mg/kg	0.761 ± 0.24	0.193 ± 0.06**
Positive control	100 mg/kg	0.899 ± 0.27	0.230 ± 0.07**

NSAID: nonsteroidal anti-inflammatory drug. Results as mean±SEM for six rats or mice. Statistical comparison was performed using ANOVA followed by Dunnett's post test. *P<0.05 and **P<0.01 compared with the negative control group.

Each plant extract was separately analyzed in relation to the results for relative area of the lesion (%). Extracts of *M. pungens* (leaves and seeds) *M. tomentosa* (peel and seeds) and *I. vera* (pulp and seed) showed gastroprotective activity and obtained significantly better results when compared to the negative control (indomethacin) (Figure 2).

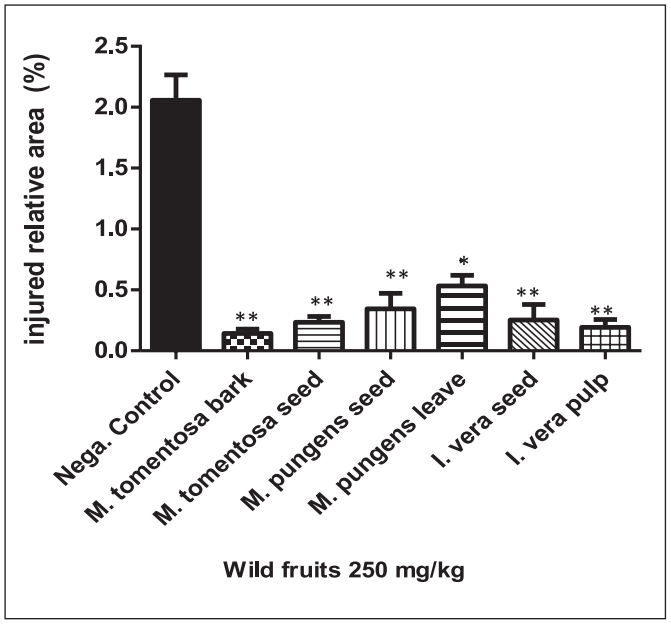

FIGURE 2. Effect of oral administration of plant extracts at a dose of 250 mg/kg and indomethacin (100 mg/kg). Statistical comparison was performed using ANOVA followed by Dunnett's post test. *P<0.05 and **P<0.01 compared with the negative control group.

Administration of the extracts of *M. tomentosa* (peel and seeds) and *I. vera* (pulp and seed) were effective in gastroprotection tests, resulting in a significant decrease in relative area of the lesions when compared with the negative control (Figure 2).

DISCUSSION

The model of acute ethanol-induced ulcers gives significant indications of mechanisms of action of the extracts, which can be associated with antioxidant factors and/or anti-inflammatory factors, and the reduction of acid secretion and mucus production[4,11].

In the ethanol-induced gastric lesions model, there were extensive areas of necrosis and hemorrhage in the gastric mucosa of control animals, which proves the toxicity of this agent. One of the main mechanisms attributed to the toxic effect of ethanol is the alteration in gastric cell homeostasis and tissue damage resulting from its direct action on the mucosa and the formation of free radicals and reactive oxygen species[19,20].

The production of free radicals is controlled by various antioxidants, which may be of endogenous origin (superoxide dismutase) or from the diet, such as polyphenols. Antioxidants are capable of stabilizing or deactivating free radicals before they attack the biological targets of the cell[22].

The results obtained by ethanol-induced induction of acute ulcer assume that the plant extracts studied possess bioactive compounds capable of acting as antioxidants. However, further phytochemical studies are necessary to characterize and quantify these compounds.

Previous studies evidenced the antinociceptive/analgesic activity and the presence of the triterpenes α-amyrin and β-amyrin and some flavonoids in *M. pungens* and *M. tomentosa* leaves[14,16]. There is no scientific evidence on pharmacological activity and phytochemical analysis of *I. vera* species.

In nonsteroidal anti-inflammatory drug-induced ulcer model, indomethacin was the NSAID chosen due to its high ulcerogenic potential when compared with other drugs. Although its mechanism of action is not completely understood, it is believed that indomethacin induces gastric damage, mainly due to its systemic effect, inhibiting COX-1 and COX-2 and reducing the local concentration of prostaglandins (PGs), which are involved in mucus and bicarbonate production[10,23].

Extracts of *M. pungens* (leaves and seeds) *M. tomentosa* (peel and seeds) and *I. vera* (pulp and seed) showed gastroprotective activity and obtained significantly better results when compared to the negative control (indomethacin). It is supposed that the plant extracts contain bioactive compounds with gastroprotective action, demonstrating a decrease in gastric lesions caused by this NSAID[9].

The role of reactive oxygen species is also described for indomethacin-induced lesions. According to Hassan, Martin and Puidg-Parellada[8], 2 hours after oral administration of indomethacin there was sharp increase in the production of superoxide and hydrogen peroxide in the gastric mucosa. Studies have indicated that flavonoids and terpenes, constituents of the plants in question, exhibit pronounced gastroprotective activity in distinct experimental models of ulcer in rat or mice[9,21].

CONCLUSION

This study demonstrates, for the first time, the gastroprotective potential of *M. pungens*, *M. tomentosa* and *I. vera* extracts. All the extracts showed gastroprotective activity for different parts of the plants,

with greater emphasis the pulp of *I. vera*, against the aggressors used – ethanol and indomethacin – being more effective against the first.

It is supposed that the wild species studied possess bioactive compounds, possibly terpenes and flavonoids with antioxidant activity, which could help control the levels of reactive oxygen species in the mucosa, protecting the mucosal damage caused by ethanol and indomethacin. These compounds could be capable of influencing the prostaglandins levels, maintaining mucus and bicarbonate production, and protecting the gastric mucosa. Phytochemical studies are necessary to determine the major compounds responsible for the gastroprotective activity. Studies to determine the antioxidant enzymes involved in the processes related to the protective action of the mucous membrane are also relevant.

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Authors' contributions

All authors contributed in the experimental part and writing of this study.

Nesello LAN, Campos A, Rosa RL, Andrade SF, Cechinel Filho V. Triagem de plantas frutíferas silvestres com ação gastroprotetora em modelos *in vivo*. Arq Gastroenterol. 2017;54(2):135-8.

RESUMO – Contexto – Devido ao aumento de pessoas com distúrbios gastrointestinais, a busca de tratamentos alternativos com menos efeitos colaterais é fundamental, assim como a demanda por alimentos ou plantas que possam ajudar a proteger o estômago. **Objetivo** – O presente estudo teve como objetivo avaliar a ação gastroprotetora dos extratos plantas frutíferas silvestres *Myrcianthes pungen*s (guabiju); *Inga vera* Willd. (ingá-banana) e *Marlierea tomentosa* Cambess. (guarapuruna) em modelos farmacológicos in vivo. **Métodos** – As diferentes partes do fruto foram submetidas separadamente a um processo de maceração em solução metanólica a frio. Foram realizados dois modelos experimentais em camundongos, modelo de úlcera gástrica induzida por anti-inflamatório não-esteroidal (indometacina) e modelo de úlcera gástrica induzida por etanol/HCl, que permitiram avaliar a atividade gastroprotetora dos extratos na dose de 250 mg/kg. Posteriormente, foram determinadas a área total de lesão (mm^2) e a área relativa lesada (%). **Resultados** – Os resultados apresentaram atividade gastroprotetora significativa para todos os extratos estudados frente aos agentes agressores utilizados, etanol e indometacina. **Conclusão** – Supõe-se que os frutos apresentam compostos bioativos, como as substancias antioxidantes, que atuam sobre os níveis de prostaglandinas, protegendo dos danos causados pelo etanol e indometacina. Os resultados encorajam futuros estudos para isolamento e identificação dos princípios ativos dos frutos.

DESCRITORES – Gastroenteropatias. Compostos fitoquímicos. Etanol. Indometacina.

REFERENCES

1. Amaral ACF, Kuster RM, Bessa WS, Barnes RA, Kaplan MAC, Wessjohann LA. Flavonoids and other phenolics from the leaves of two *Marlierea* species (Myrtaceae). Biochem Syst Ecol. 2001;29:653-4.
2. Apel AM, Sobral M, Henriques AT. Composição química do óleo volátil de *Myrcianthes* nativas da região sul do Brasil. Rev Bras Farmacogn. 2006;16:402-7.
3. Asmari AA, Omani SA, Otaibi MA, Abdulaaly AA, Elfaki I, Yahya KA, et al. Gastroprotective effect of minocycline in experimentally induced gastric ulcers in rats. *Int J* Clin Exp Med. 2014;7:586-96.
4. Berté PE, da Silva Lopes J, Comandulli NG, Rangel DW, Monache FD, Cechinel Filho V, et al. Evaluation of the gastroprotective activity of the extracts, fractions, and pure compounds obtained from aerial parts of *Rubus imperialis* in different experimental models. *Naunyn Schmiedebergs Arch Pharmacol. 2014;*387:313-19.
5. Bonjovani MR. Taxa respiratória em sementes recalcitrantes de *Inga vera* Willd. Subsp. *Affinis* (Dc.) T.D. Pennington. [Thesis]. Botucatu: Instituto de Biociências de Botucatu, Universidade Estadual Paulista – UNESP; 2011.
6. Dalla Nora CD, Jablonski A, Rios AO, Hertz PF, Jong EV, Flores SH. The characterization and profile of the bioactive compounds in red guava (*Psidium cattleyanum* Sabine) and guabiju (*Myrcianthes pungens* (O. Berg) D. Legrand). Int J Food Sci Technol. 2014;49:1842-9.
7. Deore AB, Sapakal VD, Dashputre NL, Naikwade NS. Antiulcer activity of *Garcinia indica* linn fruit rinds. J Appl Pharm Sc. 2011;1:151-4.
8. Hassan A, Martin E, Puig-Parellada P. Role of antioxidants in gastric mucosal damage induced by indomethacin in rats. Methods Find Exp Clin Pharmacol. 1998;20:849-54.
9. Klein Júnior LC, Santin JR, Niero R, de Andrade SF, Cechinel Filho V. The therapeutic lead potential of metabolites obtained from natural sources for the treatment of peptic ulcer. Phytochem Rev. 2012;11:567-616.
10. Laine L, Takeuchi K, Tarnawski A. Gastric mucosal defense and cytoprotection: bench to bedside. Gastroenterology. 2008;135:41-60.
11. Lemos M, Santin JR, Klein Júnior LC, Niero R, Andrade SF. Gastroprotective activity of hydroalcoholic extract obtained from the leaves of *Brassica oleracea* var. *acephala* DC in different animal models. J Ethnopharmacol. 2011; 138:503-7.
12. Limberger RP, Sobral M, Henriques AT, Menut C, Bessière JM. Óleos voláteis de espécies de *Myrcia* nativas do Rio Grande do Sul. Quím Nova. 2006;27:916-9.
13. Liu SC, Lin JT, Wang CK, Chen HY, Yang DJ. Antioxidant properties of various solvent extracts from lychee (*Litchi chinenesis* Sonn) flowers. Food Chem. 2009;114:577-81.
14. Messias KLS, Campos Buzzi F, Fischer LGO, Malheiros A, Delle Monache F, Cechinel Filho V. Chemical composition and analgesic activity of the leaves and branches of *Marlierea tomentosa* Camb. Quim Nova. 2008;31:1747-9.
15. Mizui T, Doteuchi M. Effect of polyamines on acidified ethanol-induced gastric lesion in rats. Jpn J Pharmacol. 1983;33:939-45.
16. Nesello LAN, Campos A, Capistrano K, de Campos Buzzi F, Cechinel Filho V. Chemical composition and antinociceptive activity of *Myrcianthes pungens* leaves. IJARNP. 2016;9:14-9.
17. Paguigan ND, Castillo DHB, Chichioco-Hernandez CL. Atividade anti-úlcera de plantas leguminosas. Arq Gastroenterol. 2014;51:64-68.
18. Rainsford KD. Biochemical gastroprotection from acute ulceration induced by aspirin and related drugs. Biochem Pharmacol. 1980;29:1281-9.
19. Repetto MG, Llesuy SF. Antioxidant properties of natural compounds used in popular medicine for gastric ulcers. Brazi J Med Biol Res. 2002;35:523-34.
20. Rozza AL, Moraes Tde M, Kushima H, Tanimoto A, Marques MO, Bauab TM, et al. Gastroprotective mechanisms of *Citrus lemon* (Rutaceae) essential oil and its majority compounds limonene and β-pinene: Involvement of heat-shock protein-70, vasoactive intestinal peptide, glutathione, sulfhydryl compounds, 106 nitric oxide and prostaglandin E2. Chem Biol Interac. 2011;189:82-9.
21. Santin JR, Lemos M, Klein Júnior LC, Niero R, de Andrade SF. Antiulcer effects of *Achyrocline satureoides* (Lam.) DC (Asteraceae) (marcela), a folk medicine plant, in different experimental models. J Ethnopharmacol. 2010;130:334-9.
22. Schwanz M. Avaliação química e biológica de *Maytenus dasyclada* Mart. e *Maytenus cassineformis* Reissek (Celastraceae). [Dissertation]. Porto Alegre: Centro de Ciências Farmacêuticas, Universidade Federal do Rio Grande do Sul; 2012.
23. Suleyman H, Albayrak A, Bilici M, Cadirci E, Halici Z. Different mechanisms in formation and prevention of indomethacin-induced gastric ulcers. Inflammation. 2010;33:224-34.

ORIGINAL ARTICLE

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Impact of Roux-en-Y Gastric Bypass Surgery (RYGB) on metabolic syndrome components and on the use of associated drugs in obese patients

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ABSTRACT – Background – The prevalence of obesity and metabolic syndrome is increasing worldwide and both behavior modification and drug therapy have low adherence. Gastric bypass has shown effective results in both reducing weight and improving comorbidities. **Objective** – To evaluate the impact of Roux-en-Y Gastric Bypass Surgery (RYGB) on both metabolic syndrome components and the use of associated drugs in obese patients. **Methods** – Historical cohort of patients subjected to Roux-en-Y Gastric Bypass Surgery (RYGB) between January 2007 and March 2014 in a private clinic. The sample consisted of 273 obese class II and III individuals, 86.4% of whom were female, with age ≥20 years, followed up for 2 months after surgery. Sociodemographic, anthropometric, biochemical, clinical, and drug-use data were collected from patients' medical records. **Results** – Significant differences were found in weight, body mass index and waist circumference, after 60 postoperative days. Components for metabolic syndrome diagnosis (hypertension P=0.001; hyperglycemia P<0.001; hypertriglyceridemia P=0.006) were reduced after 60 days of postoperative, with the exception HDL-c (P=0.083). There was a significant reduction in the use of antihypertensive (P<0.001), hypoglycemic (P=0.013), lipid lowering (P<0.001), and antiobesity (P=0.010) drugs and increased use of gastroprotective drugs, vitamins, and minerals (P<0.001) after 60 postoperative days. **Conclusion** – Patients subjected to Roux-en-Y Gastric Bypass Surgery exhibited both weight loss and significant improvement not only in metabolic syndrome components (except for HDL-c) but in the use of drugs associated with obesity and metabolic syndrome.

HEADINGS – Metabolic syndrome X. Obesity. Gastric bypass. Drug utilization.

INTRODUCTION

Obesity has been considered a worldwide epidemic, and projections indicate that by 2030, 55 million individuals will die because of obesity[11,13,19]. Developing countries, such as Brazil, are experiencing an alarming growth of obesity and chronic non-communicable diseases. Data from the Ministry of Health show that 50.8% of Brazilian adults (men=54.7% and women=47.4%) are overweight[3]. And both behavior modification therapy and drug therapy have low adherence. So, gastric bypass has shown effective results in both reducing weight and improving comorbidities.

Obesity is closely related with increased risk of morbidity and mortality in 44% of cases of type 2 diabetes (T2DM), 23% of all cases of cardiovascular disease (CVD)[12,20]. In addition, the problem of obesity arises out of the fact that some obese individuals exhibit a set of cardiometabolic risk factors called Metabolic Syndrome (MetS)[19]. Such factors include mainly carbohydrate metabolism disorders (hyperinsulinemia, insulin resistance, T2DM and intolerance of glucose and fats (increased triglycerides and decreased high-density lipoprotein [HDL-c]), abdominal obesity, high blood pressure (hypertension), and coagulation disorders[1].

In addition, studies have shown that treatments that involve lifestyle changes are promising, such as adopting a healthy, balanced diet and regular physical exercise to reduce weight and the components of MetS. However, the literature has recently shown that for obese class II and III MetS patients, neither behavior and lifestyle modification nor even drug therapy show good clinical results because such treatments have relatively poor success and especially poor adherence[9]. Therefore, bariatric surgery is recommended not only for weight loss but also for the treatment of T2DM, hypertension, and dyslipidemias, being the most effective treatment[9].

Roux-en-Y Gastric Bypass Surgery (RYGB) is currently excellent technique due to weight loss and improvement in long-term comorbidities. Most importantly, the RYGB promotes reduced secretion of hormones such as ghrelin, insulin, and leptin, which are related to appetite, weight gain, energy balance, and metabolism and storage, respectively, and play a central role in the reduction of obesity and associated comorbidities[9].

The favorable effects of this gastric surgical intervention on MetS components are evidenced in some studies. For example, in a longitudinal analysis of cardiovascular parameters after gastric bypass surgery, in 937 subjects, Dallal et al.[5] concluded that the procedure resulted in dramatic improvement in cardiovascular

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risk factors. In addition, in a cohort study of 131 diabetic patients who underwent RYGB, Aminian et al.[2] demonstrate a remarkable control of T2DM, dyslipidemias, and hypertension, which is associated with a reduction in the risk of complications such as nephropathy, retinopathy, and CVD. Significant weight loss, and cardiometabolic disease risk reduction, was observed among 633 Hispanic adults[6]. However, studies evaluating the impact of RYGB on drugs' use are still lacking.

In this context, this study aimed to evaluate the impact of RYGB on both MetS components and the use of associated drugs in obese patients.

METHODS

A retrospective cohort study was performed.

Population and sample

The population studied included obese patients who underwent Roux-en-Y Gastric Bypass Surgery (RYGB) in the Integrated Center for Obesity Treatment (*Centro Integrado de Tratamento da Obesidade – CINTRO*) in the city of Porto Alegre between January 2007 and March 2014. The sample consisted of 273 obese class II and class III (morbid) individuals, aged ≥20 years. All patients who underwent Roux-en-Y Gastric Bypass Surgery (RYGB) between January 2007 and March 2014 were included in the study and were monitored for 2 months after surgery. The individuals underwent gastroplasty through gastrojejunal bypass with Roux-en-Y reconstruction[17]. Patients who did not return after the surgery for nutritional and clinical assessments were excluded.

Data collection

All of the information regarding sociodemographic and anthropometric data, clinical and biochemical exams, and drugs used by the patients included in this study, both before and after surgery, were collected from the patients' medical records. The variables obtained were height, weight, body mass index (BMI); MetS components [blood pressure (BP), triglycerides (TG), HDL-c, fasting glucose (FG)]. MetS components were classified in accordance to the Third Report of the National Cholesterol Education Program Adult Treatment Panel NCEP-ATPIII[8] (WC >88 cm for women or >102 cm for men; HDL-c <50 mg/dL for women or <40 m/dL for men; TG >150 mg/dL; BP >130/85 mmHg; FG >110 mg/dL). Drugs used by the patients were obtained and classified by the Anatomical Therapeutic Chemical (ATC) system, which divides the active ingredients into different therapeutic groups according to their sites of action and their pharmacological and chemical characteristics[21]. For this purpose, the 2000 revision and updates were used. In ATC, drugs are classified according to their most important therapeutic use[21].

Statistical analysis

Data were stored in an Excel database and analyzed with statistical software SPSS® 17.0 version. The descriptive analysis was performed using frequency, central tendency, and dispersion measures. The t test for paired samples was used to assess the equality of means of numerical variables in the pre and postoperative periods, and the McNemar test was used to analyze changes in the frequency of categorical variables in the pre and postoperative periods. A 5% level was established to determine the significance of the tests ($P \leq 0.005$).

Ethical considerations

The study was approved by the Ethics Committee on Human Research of the Pontifical Catholic University of Rio Grande do Sul (*Pontifícia Universidade Católica do Rio Grande do Sul* – PUCRS) (protocol nº. 506.428; CAEE 23315213.7.0000.5336). The CINTRO researcher was asked to sign a confidentiality agreement, and the CINTRO coordinator was asked to sign a declaration authorizing the use of medical records in the research, which contained information on all of the participants. All of the patients who entered the cohort from January 2014 to March 2014 signed an informed consent form.

RESULTS

The sample consisted of 273 subjects with mean age of 38.4±11.2 years (range 20-69 years), 236 (86.4%) females and 37 (13.6%) males, with follow-up period of 2 months (60 days) after surgery. The age group with the highest number of patients was 30-39 years, with 102 individuals (37.4%). The majority of the sample consisted of married individuals (63.0%) and with higher education (49.1%) (Table 1).

TABLE 1. General characteristics of obese patients who underwent RYGB

Variables	N (%)
Gender	
Female	236 (86.4)
Male	37 (13.6)
Age group (years)	
20-29	64 (23.4)
30-39	102 (37.4)
40-49	51 (18.7)
≥50	56 (20.5)
Educational level	
Primary school	1 (0.4)
Secondary school	3 (1.1)
Higher education	134 (49.1)
Specialization	119 (43.6)
Masters/Ph.D.	14 (5.1)
Vocational training course	2 (0.7)
Marital status	
Married	172 (63.0)
Single	74 (27.1)
Divorced	20 (7.3)
Widower	7 (2.6)

RYGB: Roux-en-Y gastric bypass surgery

Regarding pre and postoperative results of the anthropometric variables, Table 2 shows that the anthropometric measurements (weight, BMI, and WC) showed a significant difference ($P<0.001$), 60 days after bariatric surgery. The MetS components such as BP [diastolic blood pressure (DBP) and systolic blood pressure (SBP)], triglycerides ($P<0.001$) and fasting glucose ($P=0.005$) showed a significant reduction in their postoperative levels, except for HDL-c ($P=0.112$).

TABLE 2. Comparison of pre and postoperative (60 days after surgery) anthropometric measures, and metabolic syndrome components in obese patients who underwent RYGB

Pre/post operative variables	N	Mean ±SD	Difference		*P**
			Mean ±SE	95% CI	
Pre Weight Post Weight	207	112.70±19.42 95.80±17.14	16.90±0.39	(16.14-17.67)	<0.001
Pre BMI Post BMI	207	41.79±5.14 35.57±4.57	6.22±0.14	(5.95-6.50)	<0.001
Pre WC Post WC	84	120.39±17.18 108.24±15.00	12.15±0.60	(10.97-13.34)	<0.001
Pre SBP Post SBP	97	135.05±19.21 123.20±13.27	11.86±2.00	(7.87-15.84)	<0.001
Pre DBP Post DBP	97	86.29±11.05 80.21±8.16	6.08±1.28	(3.54-8.62)	<0.001
Pre Glucose Post Glucose	55	114.73±42.95 100.40±29.04	14.33±4.84	(4.63-24.03)	0.005
Pre HDL-c Post HDL-c	74	48.12±14.75 45.48±13.35	2.64±1.64	(-0.63-5.91)	0.112
Pre TG Post TG	70	175.16±98.42 109.79±43.21	65.36±11.13	(43.16-87.57)	<0.001

RYGB: Roux-en-Y gastric bypass surgery. *T-test for paired samples, considering the preoperative results compared to postoperative results at 60 days; BMI: body mass index in kg/m^2; WC: waist circumference in cm; SBP: systolic blood pressure in mmHg; DBP: diastolic blood pressure in mmHg; HDL-c: high-density lipoprotein in mg/dL; TG: triglycerides in mg/dL.

Table 3 shows the changes in the frequency of each of the positive criteria for the diagnosis of MetS observed in the preoperative period and 60 days after surgery. The analyses showed that at the assessed range, there was a significant decrease in the frequency of individuals with hypertension (P=0.001), hypertriglyceridemia (P=0.006) and hyperglycemia (P<0.001). Only the frequency of individuals with altered HDL-c levels showed no significant difference (P=0.083).

TABLE 3. Comparison of the frequency of diagnostic criteria for metabolic syndrome in the preoperative period and at 60 days of the postoperative period in obese patients who underwent RYGB

Criteria for metabolic syndrome diagnosis	N	Preoperative N (%)	Postoperative N (%)	*P**
Increased WC	84	84 (100.0)	75 (89.3)	†
Hypertension	117	88 (75.2)	36 (30.8)	0.001
Low HDL-c	74	40 (54.1)	53 (71.6)	0.083
Hypertriglyceridemia	70	37 (52.9)	11 (15.7)	0.006
Hyperglycemia	55	23 (41.8)	10 (18.2)	<0.001

RYGB: Roux-en-Y gastric bypass surgery. *McNemar Test; WC: waist circumference; HDL-c: High-density lipoprotein; † No measures of association are computed for not increased waist circumference, preventing an analysis.

Regarding the use of drugs associated with obesity and MetS, the results showed that after surgery, there was a significant reduction in the use of antihypertensive drugs (P<0.001), antiobesity (P=0.010), hypoglycemic agents (P=0.013), lipid-lowering drugs (P<0.001), among others (Table 4). However, there was a significant increase in the postoperative use of gastroprotective drugs

TABLE 4. Comparison of the frequency of the use of drugs in the pre and postoperative period (60 days after surgery) in obese patients who underwent RYGB

Drugs in use †	N	Preoperative N (%)	Postoperative N (%)	*P**
Antihypertensive	273	156 (57.1)	14 (5.1)	<0.001
Antiobesity	273	9 (3.3)	1 (0.4)	0.010
Drugs used in diabetes	273	29 (10.6)	16 (5.9)	0.013
Cardiac therapy	273	6 (2.2)	0 (0.0)	‡
Drugs for acids related disorders	273	21 (7.7)	159 (58.2)	<0.001
Lipid modifying agent	273	21 (7.7)	1 (0.4)	<0.001
Multivitamins combinations	271	4 (1.5)	262 (96.7)	<0.001
Iron	273	3 (1.1)	126 (46.2)	<0.001

RYGB: Roux-en-Y gastric bypass surgery. *McNemar Test. †The drugs were classified by the Anatomical Therapeutic Chemical (ATC) system recommended by the World Health Organization[21]; ‡No measures of association are computed for cardiac therapy in postoperative, preventing an analysis.

(P<0.001), multivitamin and mineral supplements (P<0.001), and iron alone (P<0.001) (Table 4). After bariatric surgery, a significant reduction was also observed in the number of drugs used by the patients that were associated with obesity and MetS. Before the surgery, the mean ±standard deviation (SD) of drug use was 2.1±1.9 units, which decreased to 0.6±1.0 units after surgery, resulting in a mean difference ±standard error (SE) of 1.4±0.1 (95% CI=1.2-1.7) drugs (P<0.001). The mean ±SD of the use of vitamins, minerals and gastroprotective drugs creased from 0.1±0.4 to 2.3±1.1 units from the pre to the postoperative period, which shows a mean difference ±SE of -2.2±0.1 (95% CI= -2.3 and -2.0), which is also significant (P<0.001).

DISCUSSION

The literature has shown that weight loss decreases MetS markers, thus reducing risk factors for CVD, T2DM, some types of cancer, and skeletal abnormalities, which positively affects individuals' quality of life[22]. However, the literature also suggests that interventions advocating behavioral and lifestyle changes (i.e., diet and exercise) and drug therapy have little success and low adherence over time for obese class II and III patients with comorbidities[9]. In this context, RYGB is important not only for decreasing body weight but also for playing the role of metabolic regulator. RYGB is a mixed surgery that combines malabsorption and restriction of food intake by bypassing a portion of the jejunum.

The results of this study showed that RYGB had significant statistical and clinical impact on weight reduction, BMI, and components of MetS, especially WC, BP, and triglyceride levels, which resulted in a significant reduction in the use of drugs associated with cardiovascular risk. It should be noted that these changes occurred within a short period (60 days after surgery). However, this study did not observe significant changes in HDL-c level, which is an important component of MetS. Conversely, it was found that there was a significant increase in the use of vitamins, minerals, iron, and gastroprotective drugs after surgery, which the literature and clinical practice have demonstrated as the standard treatment for patients who have undergone bariatric surgery[4,10]. It is noteworthy that the absorption of some vitamins and minerals is decreased by several factors: restriction of food intake, change in nutrient absorption, and the use of gastroprotective drugs that change the pH of the stomach and decrease the absorption of iron and calcium. Additionally, gastroprotective drugs are associated with the depletion of some micronutrients, such as iron[13]. Iron and folic acid are micronutrients that are essential for normal metabolism, and deficiencies of these micronutrients may cause health hazards, including the development of chronic diseases such as CVD[10].

Studies have shown that with the weight loss and caloric restriction caused by the RYGB, there is an improvement in energy metabolism, which results in improved regulation of lipid molecules, appetite-regulating hormones, satiety, energy storage, and glycemic control[10]. Thus, our findings corroborate those of the literature, showing a beneficial impact not only in short-term weight reduction but also in the suppression of important cardiovascular risk factors, such as hypertension, increased WC, T2DM, and dyslipidemia, which combined are closely related to the increased mortality rate of obese individuals[15]. Reduction of body-fat mass has an important beneficial impact on metabolism because with the reduction of adipose tissue, especially intra-abdominal tissue, there is a reduction in the inflammatory and oxidative process, which contributes to increased insulin sensitivity and improved regulation of glucose levels, blood pressure, and lipid particles, primarily triglycerides. The hypoglycemic effect of bariatric surgery is most likely due to the improved hormone metabolism associated with diabetes. Bariatric surgery may affect the balance of β cells due to changes in the gastrointestinal tract[16]. In addition, with weight loss and therefore improved insulin sensitivity, musculoskeletal tissue becomes more active, which increases energy consumption. Additionally, weight reduction and increased insulin sensitivity allow for less vasoconstriction and cardiac output, lower retention of sodium/water in renal tubules, and improved regulation of the proliferation of smooth muscle in artery walls. These modifications have a favorable impact on the reduction of blood pressure levels and therefore on hypertension and the use of drugs to treat it. Several studies have reported that weight loss alone is the most effective non-pharmacological intervention for controlling hypertension, and even discrete weight reductions have significantly decreased BP[7]. In addition, due to the caloric restriction promoted by RYGB, the decreased influx of large amounts of fatty acids to the liver will lead to reduced gluconeogenesis, decreased secretion of very low-density lipoprotein (VLDL), and decreased insulin resistance, thus promoting improvements or even healing of comorbidities associated with obesity and MetS components, especially hypertriglyceridemia[4].

The favorable effects of gastric bypass in MetS are shown in some studies, including Aminian et al.[2] and Halperin et al.[11] of obese patients with T2DM; those studies compare bariatric surgery using the bypass technique with the clinical treatment of lifestyle changes in a randomized trial over 1 year. The authors concluded that although both treatments improved patients' quality of life, bariatric surgery has a higher impact on weight loss and improved glycated hemoglobin and cardiometabolic risk levels.

In patients with obesity class I (mild obesity), T2DM and BMI between 30 and 35 kg/m^2 who used oral hypoglycemic agents (and some of whom are insulin-dependent), the bypass technique is effective to improve these parameters. In a study by Lanzarini et al.[14], all patients who underwent bariatric surgery with gastric bypass exhibited improved glycemic control, and 93.6% met the criteria for T2DM remission. This study involves patients who underwent bypass surgery from July 2008 to October 2010 at the University Hospital of Santiago de Chile, with a 24-month follow-up. Although the mean postoperative follow-up was short, it was evident that bariatric surgery may be more effective than the available treatments. Most importantly, studies have shown that obesity is closely related to the development of MetS and to decreased life expectancy. In this regard, a 2009 meta-analysis of 57 prospective studies with 894,576 European and American participants with a mean age of 46±11 years showed that people with BMI between 22.5 and 25 kg/m^2 have lower mortality rates compared to people with BMI greater than 25 kg/m^2. It was found that for every 5 kg/m^2 increase in BMI, overall mortality rate increases by 30%. When associated with other risk factors, this rate increased by 40% in cases of vascular disease and 60%-120% in cases of diabetes and its complications. In terms of years, obesity classes I and II (between 30.0 and 39.9 kg/m^2) decreased life expectancy by 2 to 4 years. In obesity class III (above 40 kg/m^2), the decrease was between 8 and 10 years[18]. This evidence shows the importance of bariatric surgery for these individuals, not only to decrease cardiovascular risk factors and comorbidities but to decrease the mortality rate and thus to increase life expectancy.

This study has some limitations, the most important of which is related to retrospective data collection from medical records. Another limitation of this study relates to it's a short follow-up period, which does not allow to verify the long-term impact of surgery in the investigated parameters. However, cohort studies on the impact of the bypass components of MetS and the use of associated drugs conducted in Brazilian samples are still scarce. In addition, even a short follow-up study showed promising results, demonstrating the effectiveness of bypass in the investigated parameters, which surely will have a beneficial impact on health and quality of life of patients.

CONCLUSION

The results of this study show that RYGB significantly reduces a patient's weight and WC within a short period (60 days after surgery), improving their metabolic profile. These changes contribute to reducing both MetS components and the use and amount of drugs taken for both obesity and MetS. Conversely, it is important to consider that the RYGB procedure and the use of gastroprotective drugs result in lower absorption rates of micronutrients such as iron. Thus, medical and nutritional postoperative follow-up are essential to ensure these patients' continued health and quality of life.

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Authors' contributions

Junges VM: study design, data collection, manuscript writing. Cavalheiro JM: performed bariatric surgery and data collection. Fam EF: clinical evaluation and data collection. Closs VE: statistical analysis, critical review and corrections of manuscript. Moraes JF: statistical analysis, critical review and corrections of manuscript. Gottlieb MG: study design, manuscript writing, critical review and corrections of manuscript.

Junges VM, Cavalheiro JM, Fam EF, Closs VE, Moraes JF, Gottlieb MG. Impacto do bypass gástrico em Y de Roux (RYGB) nos componentes da síndrome metabólica e sobre o uso de drogas associadas em pacientes obesos. Arq Gastroenterol. 2017;54(2):139-44.

RESUMO – Contexto – A prevalência de obesidade e síndrome metabólica é crescente no mundo e tanto a terapia de modificação de estilo de vida quanto a medicamentosa têm baixa adesão. O bypass gástrico tem apresentado resultados eficazes na redução de peso e comorbidades. **Objetivo –** Avaliar o impacto do bypass gástrico em Y de Roux nos componentes da síndrome metabólica e sobre o uso de drogas associadas em pacientes obesos. **Métodos –** Coorte histórica de pacientes submetidos ao bypass gástrico em Y de Roux entre janeiro de 2007 e março de 2014, em clínica privada. A amostra foi composta por 273 indivíduos obesos classe II e III, 86,4% dos quais eram do sexo feminino, idade ≥20 anos, acompanhados por 2 meses após a cirurgia. Dados sociodemográficos, antropométricos, bioquímicos, clínicos e de uso de medicamentos foram coletados nos prontuários dos pacientes. **Resultados –** Foram encontradas diferenças significativas no peso, índice de massa corporal e circunferência da cintura, após 60 dias de pós-operatório. Os componentes para diagnóstico da síndrome metabólica (hipertensão P=0,001; hiperglicemia P<0,001; hipertrigliceridemia P=0,006) foram reduzidos no pós-operatório, com exceção do HDL-c (P=0,083). Houve uma redução significativa no uso de medicamentos anti-hipertensivos (P<0,001), hipoglicêmicos (P=0,013), hipolipemiantes (P<0,001), antiobesidade (P=0,010) e uma maior utilização de gastroprotectores, vitaminas e minerais (P<0,001) após 60 dias de pós-operatório. **Conclusão** – Os pacientes submetidos ao bypass gástrico em Y de Roux exibiram perda de peso e uma melhora significativa, não só em componentes da síndrome metabólica (exceto para o HDL-c), mas também no uso de medicamentos associados à obesidade e à síndrome metabólica.

DESCRITORES – Síndrome X metabólica. Obesidade. Derivação gástrica. Uso de medicamentos.

REFERENCES

1. Alberti K, M. GM, Zimmet P, Shaw J. The metabolic syndrome: a new worldwide definition. Lancet. 2005;366:1059-62.
2. Aminian A, Daigle CR, Romero-Talamas H, Kashyap SR, Kirwan JP, Brethauer SA, et al. Risk prediction of complications of metabolic syndrome before and 6 years after gastric bypass. Surg Obes Relat Dis. 2014;10:576-82.
3. Brasil. Ministério da Saúde. Secretaria de Vigilância em Saúde. Vigitel Brasil 2013. [Internet]. Vigilância de fatores de risco e proteção para doenças crônicas por inquérito telefônico. Brasília - DF: Secretaria de Vigilância em Saúde. 2013; [cited 2015 Jul 6]. Available from: http://portalsaude.saude.gov.br.
4. Courcoulas AP, Christian NJ, Belle SH, Berk PD, Flum DR, Garcia L, et al. Weight change and health outcomes at 3 years after bariatric surgery among individuals with severe obesity. JAMA. 2013;310:2416-25.
5. Dallal RM, Hatalski A, Trang A, Chernoff A. Longitudinal analysis of cardiovascular parameters after gastric bypass surgery. Surg Obes Relat Dis. 2012;8:703-9.
6. De La Cruz-Munoz N, Lopez-Mitnik G, Arheart KL, Livingstone AS, Miller TL, Lipshultz SE, et al. Reduction in cardiometabolic disease risk following gastric bypass surgery among Hispanic adults. Metab Syndr Relat Disord. 2013;11:262-6.
7. Donnelly JE, Blair SN, Jakicic JM, Manore MM, Rankin JW, Smith BK, et al. American College of Sports Medicine Position Stand. Appropriate physical activity intervention strategies for weight loss and prevention of weight regain for adults. Med Sci Sports Exerc. 2009;41:459-71.
8. Executive Summary of the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood cholesterol in Adults (Adult Treatment Panel III). JAMA. 2001;285:2486-97.
9. Finelli C, Padula MC, Martelli G, Tarantino G. Could the improvement of obesity-related co-morbidities depend on modified gut hormones secretion? World J Gastroenterol. 2014;20:16649-64.
10. Gudzune KA, Huizinga MM, Chang HY, Asamoah V, Gadgil M, Clark JM. Screening and diagnosis of micronutrient deficiencies before and after bariatric surgery. Obes Surg. 2013;23:1581-9.
11. Halperin F, Ding SA, Simonson DC, Panosian J, Goebel-Fabbri A, Wewalka M, et al. Roux-en-Y gastric bypass surgery or lifestyle with intensive medical management in patients with type 2 diabetes: feasibility and 1-year results of a randomized clinical trial. JAMA Surg. 2014;149:716-26.

12. Hruby A, Hu FB. The Epidemiology of Obesity: A Big Picture. Pharmacoeconomics. 2015;33:673-89.
13. Ito T, Jensen RT. Association of long-term proton pump inhibitor therapy with bone fractures and effects on absorption of calcium, vitamin B12, iron, and magnesium. Curr Gastroenterol Rep. 2010;12:448-57.
14. Lanzarini E, Csendes A, Gutierrez L, Cuevas P, Lembach H, Molina JC, et al. Type 2 diabetes mellitus in patients with mild obesity: preliminary results of surgical treatment. Obes Surg. 2013;23:234-40.
15. Kones R. Primary prevention of coronary heart disease: integration of new data, evolving views, revised goals, and role of rosuvastatin in management. A comprehensive survey. Drug Des Devel Ther. 2011;5:325-80.
16. Martínez-Moreno JM, Garciacaballero M. Influences of the diabetes surgery on pancreatic β-cells mass. Nutr Hosp. 2013;28:88-94.
17. Silva PRB, Souza MR, Silva EM, Silva SA. Nutritional status and life quality in patients undergoing bariatric surgery. Arq Bras Cir Dig. 2014;27:35-8.
18. Whitlock G, Lewington S, Sherliker P, Clarke R, Emberson J, Halsey J, et al. Body-mass index and cause-specific mortality in 900 000 adults collaborative analyses of 57 prospective studies. Lancet. 2009;373:1083-96.
19. WHO. World Health Organization. [Internet]. Health topics. Obesity; [cited 2015 Jul 6]. Available from: http://www.who.int/topics/obesity/en/.
20. WHO. Global Action Plan - For the prevention and control of noncomunicable diseases 2013-2020. [Internet]. World Health Organization; [cited 2015 Jul 6]. Available from: http://www.who.int/global.
21. WHO. World Health Organization. [Internet]. Collaborating Center for Drug Statistic Methodology, Norwegian Institute of Public Health. The Anatomical Therapeutic Chemical Classification System; [cited 2015 Jul 6]. Available from: http://www.whocc.no/atc_ddd_methodology/.
22. Wirth A, Wabitsch M, Hauner H. The prevention and treatment of obesity. Dtsch Arztebl Int. 2014;111:705-13.

ORIGINAL ARTICLE

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Esophageal motility in men and women evaluated by high-resolution manometry

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ABSTRACT – Background – Esophageal motility has been described in the literature as having differences between men and women. Most of these investigations use the water perfusion method for esophageal manometry. In this investigation the esophageal motility of men and women was compared with high-resolution manometry of the esophagus. **Objective –** To compare the esophageal motility of men and women with the high-resolution manometry method for esophageal manometry, performed in the sitting position. The hypothesis was that men and women have differences in esophageal motility. **Methods** – High-resolution manometry was performed in normal volunteers, 10 men [mean age: 37.5 (8.1) years] and 12 women [mean age: 38.7 (7.5) years], in the sitting position and with 10 swallows of a 5 mL bolus of saline, with an interval of at least 30 seconds between consecutive swallows. We evaluated the integrated relaxation pressure of the lower esophageal sphincter, contraction front velocity, distal contraction integral, distal latency, proximal contraction extension, proximal contraction duration >30 mmHg, proximal contraction duration, proximal contraction integral and maximal upper esophageal sphincter pressure. **Results** – There was no significant difference between men and women in the variables measured. **Conclusion** – There was no difference in esophageal motility of men and women evaluated by the high resolution manometry method, in the sitting position with swallows of a liquid bolus.

HEADINGS – Gastrointestinal motility. Manometry, methods. Sex factors.

INTRODUCTION

Previous publications have shown the possibility of differences between men and women in esophageal motility[11-14]. The results were found using different methods for esophageal motility evaluation, mainly by the water perfusion method. Nowadays high-resolution manometry (HRM) is the best way to perform this evaluation. It can give different parameters from the traditional water perfusion or solid state manometric methods[3,13].

The investigations about gender influence on esophageal motility have described that: integrated relaxation pressure of the lower esophageal sphincter (LES) is higher in women than in men[11]; contractions in proximal esophagus have a higher area under the curve in women[7]; women have an increase in contraction duration in distal esophagus[8]; women have a higher LES pressure, higher distal amplitude, longer distal contraction duration and slower distal velocity of peristaltic contraction[14]. Gender does not influence whether esophageal motility is normal, spastic or with a non-specific motor disorder[1].

The confirmation of difference between men and women on esophageal motility is important in the definition of normal parameters and in the better understanding of esophageal physiology. Esophageal motility has the influence of body position[2,4,6,10,12] and the characteristics of the swallowed bolus[2,5,6,10], and may also have the influence of gender.

A previous evaluation of the effect of gender in esophageal motility with the method of HRM, with the main focus on distal esophagus, described the difference of high integrated relaxation pressure (IRP) in women compared with men as unique[11], being measured in both supine and sitting positions and with a 5 mL saline bolus. In the present investigation our objective was to evaluate, by HRM in the sitting position and swallows of a 5 mL saline bolus, the proximal and distal esophageal motility in men and women, with the hypothesis that the esophageal contraction in women has some differences when compared with the esophageal contraction of men.

METHODS

The esophageal motility of 10 men with mean age 37.5 (8.1) years and 12 women with mean age 38.7 (7.5) years was evaluated (Table 1). Volunteers did not have any symptoms or any gastroenterologic, neurologic, endocrinologic diseases, previous surgery in the digestive tract and no disease at the time of the esophageal

TABLE 1. Men and women involved in the investigation. Mean (SD)

	Men (n=10)	Women (n=12)
Age (years)	37.5 (8.1)	38.7 (7.5)
Weight (kg)	82.8 (13.9)	79.2 (13.3)
Height (m)	1.73 (0.06)	1.61 (0.06)
BMI (kg/m2)	27.6 (3.6)	30.4 (3.8)

BMI: body mass index; SD: standard deviation.

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motility evaluation. The investigation was approved by the Human Research Committee of the University Hospital of Ribeirão Preto and all volunteers gave written informed consent to participate in the investigation.

The evaluation of esophageal motility was done with a 32-channel solid state catheter and the InSIGHT High Resolution Impedance Manometry System (Sandhill Instruments, Highlands Ranch, CO, USA). After the calibration of the catheter at pressures 0 mmHg and 100 mmHg, with at least 6 hours of fasting and with the volunteers sitting on a chair, the manometric catheter was introduced via the nose until the distal channels were inside the stomach, in a position which permits the registration of the pressure from the pharynx to the stomach. After an enough time for the stabilization of the register, each volunteer performed, in the sitting position, 10 swallows of a 5 mL bolus of saline at room temperature, with an interval of at least 30 seconds between swallows. The volunteer performed only one swallow of each 5 mL bolus volume. Double swallows were excluded, and another swallow was performed.

Each examination was analyzed for the integrated relaxation pressure (IRP) of the lower esophageal sphincter, the contraction front velocity (CFV), distal contraction integral (DCI), distal latency (DL), proximal contraction extension (PCE), proximal contraction integral (PCI), proximal contraction duration >30 mmHg (PCD >30 mmHg), proximal contraction duration (PCD), and upper esophageal sphincter (UES) pressure. The method for measurement of each variable was previously described[3,10].

Data were tested for normality using the Shapiro-Wilks test. To test the comparisons between men and women, a two-tailed Student's *t* test was used in normal distribution data and the Mann-Whitney *U* test in data which did not have normal distribution. The results are shown in mean, median, standard deviation (SD). A $P \leq 0.05$ was considered as significant.

RESULTS

There was no difference between men and women in all measurements performed (Table 2).

The IRP in women had a median of 8.2 mmHg (limits: 1–18.1 mmHg), and men a median of 5.0 mmHg (limits: 3.2–12.5 mmHg). Statistical analysis of these results did not find significance (P=0.18, Figure 1).

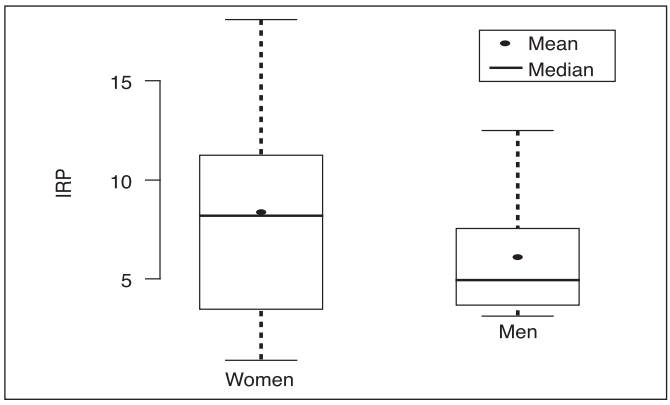

FIGURE 1. Box plot of the integrated relaxation pressure (IRP), in mmHg, of men (n=10) and women (n=12).

DISCUSSION

In this investigation, using HRM and evaluation of the esophageal motility in the sitting position with low viscous liquid bolus, differences between men and women in esophageal motility were not found.

Esophageal motility is different when the individual is in the sitting or in the supine positions. In the sitting position the proportion of abnormal contractions increase[2,10,12], the distal contraction integral and the amplitude of contractions decrease[4,6,11,12]. It could be, at least in part, the explanation for the absence of difference between men and women. Esophageal manometric studies using the water perfusion method were performed in the supine position and in this investigation it was performed in the sitting position. The absence of difference found in the sitting position did not exclude the possibility of difference in another position. However, the results of comparison between men and women in esophageal motility using HRM found a higher integrated relaxation pressure (IRP) in women than in men in both positions[11]. The median of IRP in women was 9.01 mmHg (95th percentile: 4.26-20.73 mmHg) and in men was 7.02 mmHg (95th percentile: 3.26-14.68 mmHg, P=0.04) in the sitting position, and in the supine position it was 8.06 mmHg (95th percentile: 4.04-18.96 mmHg) in women and 7.40 mmHg (95th percentile: 4.16-14.46 mmHg, P=0.04) in men.

TABLE 2. Results of high resolution esophageal manometry in men (n=10) and women (n=12)

	Men		Women		*P* value
	Mean (SD)	Median	Mean (SD)	Median	
IRP (mmHg)	6.0 (3.1)	5.0	8.4 (5.4)	8.2	0.18
CFV (cm/s)	4.9 (2.2)	4.4	5.2 (2.0)	4.4	0.75
DCI (mmHg.s.cm)	1441.6 (1126.3)	1355.3	913.8 (735.8)	803.8	0.15
DL (s)	6.8 (1.0)	6.9	6.8 (0.9)	6.6	0.93
PCE (cm)	4.4 (1.1)	4.4	4.7 (1.2)	4.7	0.57
PCI (mmHg.s.cm)	347.7 (215.6)	312.5	248.8 (170.7)	248.6	0.39
PCD >30mHg (s)	2.0 (0.8)	1.8	1.7 (0.5)	1.7	0.30
PCD (s)	2.6 (0.9)	2.4	2.2 (0.6)	2.3	0.17
Maximal UES Pressure (mmHg)	461.9 (80.9)	457.5	475.6 (93.0)	507.4	0.73

IRP: integrated relaxation pressure; CFV: contraction front velocity; DCI: distal contraction integral; DL: distal latency; PCE: proximal contraction extension; PCI: proximal contraction integral; PCD: proximal contraction duration; PCD: proximal contraction duration; UES: upper esophageal sphincter.

The data of IRP of the present investigation suggested the same interpretation, however the difference did not reach statistical significance (P=0.18). It is possible that the number of subjects included in the groups was not large enough to demonstrate a possible difference. Also, the use of a statistical method which takes in consideration the Bayes factor, which include in the analysis the results of similar experiments, could modify the conclusion[9].

HRM is a modern method for esophageal motility evaluation, however the examination performed in different countries did not found the same results[4]. The type of HRM system, catheter diameter, demographic factors, body position during the test, consistency of the bolus swallowed and esophageal length have influence on the results of the examination[10].

The IRP is an important measure defined in HRM. It represents the mean esophageal gastric transition pressure measured for four contiguous or non contiguous seconds of relaxation in the 10 seconds window following deglutitive upper esophageal sphincter relaxation[10]. An increase in IRP means an outflow obstruction at the esophageal gastric transition[13]. If the difference between men and women is true it means that it is necessary to have a normal upper limit value for men and another for women. This investigation was not able to demonstrate this, however it is suggested that the ideal is to have different sets of normal values taking in consideration the factors that could influence the results[10].

This investigation has limitations. If the number of volunteers was higher the results could reach a more conclusive answer. Different characteristics of bolus swallowed, in terms of volume and consistency, could demonstrated a difference between men and women. It not easy to performed HRM in normal volunteers. It is an invasive examination which causes significant discomfort. What was described is that there is no difference between the esophageal motility of men and women however, if difference exist it is likely that do not have clinical implication.

In conclusion, there is no significant difference in esophageal motility between men and women, evaluated in the sitting position with swallows of liquid low viscous bolus.

Author' contributions

Costa TV had participation in study planning, investigation, data collection and discussion of results, in addition to manuscript preparation and subsequent approval from Arquivos de Gastroenterologia. Dantas RO had participation in study planning, discussion of results and in manuscript preparation and subsequent approval from Arquivos de Gastroenterologia.

Costa TV, Dantas RO. Motilidade do esôfago em homens e mulheres avaliada pela manometria de alta resolução. Arq Gastroenterol. 2017;54(2):145-7.

RESUMO – Contexto – É descrita a existência de diferenças na motilidade de esôfago entre homens e mulheres. A maioria destes trabalhos utilizaram o método de perfusão continua com água para a manometria esofágica. Nesta investigação foi comparada a motilidade do esôfago de homens e mulheres com o método de manometria de alta resolução, realizada na posição sentada e com deglutição de bolo líquido. **Objetivo** – Comparar a motilidade do esôfago em homens e mulheres, na posição sentada, com o método de manometria de alta resolução. A hipótese é que homens e mulheres têm diferenças na motilidade do esôfago. **Métodos** – Manometria de alta resolução foi realizada em voluntários saudáveis, 10 homens [média de idade: 37,7 (8,1) anos] e 12 mulheres [média de idade: 38,7 (7,5) anos], na posição sentada e com 10 deglutições de 5 mL de solução salina. Foram avaliadas a pressão integrada de relaxamento do esfíncter inferior do esôfago, velocidade da contração peristáltica, integral da contração distal, latência distal, extensão da contração proximal, duração da contração proximal >30 mmHg, duração da contração proximal, integral da contração proximal, pressão máxima do esfíncter superior do esôfago. **Resultados** – Não houve diferença significativa entre homens e mulheres nas variáveis medidas. **Conclusão** – Não há diferença entre homens e mulheres na motilidade do esôfago avaliada pelo método de manometria de alta resolução, na posição sentada e com deglutição de um bolo líquido.

DESCRITORES – Motilidade gastrointestinal. Manometria, métodos. Fatores sexuais.

REFERENCES

1. Andrew JM, Heddle R, Hebbard GS, Checklin H, Besanko L Fraser RJ. Age and gender affect likely manometric diagnosis: audit of a tertiary referral hospital clinical esophageal manometry service. J Gastroenterol Hepatol. 2009; 24:125-8.
2. Bernhard A, Pohl D, Fried M, Castell DO, Tutuian R. Influence of bolus consistency and position on esophageal high-resolution manometry findings. Dig Dis Sci. 2008;53:1198-205.
3. Bredenoord AJ, Fox M, Kahrilas PJ, Pandolfino JE, Schwizer W, Smout AJP & the International High Resolution Manometry Working Group. Chicago classification criteria of esophageal motility disorders defined in high resolution esophageal pressure topography. Neurogastroenterol Motil. 2012;24(Suppl.1):57-65.
4. Carmo GC, Jafari J, Sifrim D, Oliveira RB. Normal esophageal pressure topography metrics for data derived from the Sandhill-Unisensor high-resolution manometry assembly in supine and sitting positions. Neurogastroenterol Motil. 2015;27:285-92.
5. Dalmazo J, Aprile LRO, Dantas RO. Esophageal contractions, bolus transit and perception of transit after swallows of liquid and solid boluses in normal subjects. Arq Gastroenterol. 2012;49:250-4.
6. Dalmazo J, Aprile LRO, Dantas RO. Effect of swallowed bolus viscosity and body position on esophageal transit, contraction and perception of transit. Arq Gastroenterol. 2015;52:27-31.
7. Dantas RO, Alves LMT, Cassiani RA. Gender differences in proximal esophageal contractions. Arq Gastroenterol. 2009;46:284-7.
8. Dantas RO, Ferriollli E, Souza MAN. Gender effects on esophageal motility. Braz J Med Biol Res. 1998;31:539-44.
9. Goodman SN. Toward evidence-based medical statistics. 2: The Bayes factor. Ann Inter Med. 1999;130:1005-13.
10. Herregods TVK, Roman S, Kahrilas PJ, Smout AJPM, Bredenoord AJ. Normative values in esophageal high-resolution manometry. Neurogastroenterol Motil. 2015;27:175-87.
11. Jung KW, Jung HY, Myung SJ, Kim SO, Lee J, Yoon IJ, et al. The effect of age on the key parameters in the Chicago Classification: a study using high-resolution esophageal manometry in asymptomatic normal individuals. Neurogastroenterol Motil. 2015;27:246-57.
12. Roman S, Damon H, Pellissier PE, Mion F. Does body position modify the results of esophageal high resolution manometry? Neurogastroenterol Motil. 2010;22:271-5.
13. van Hoeij FB, Bredenoord AJ. Clinical application of esophageal high-resolution manometry in the diagnosis of esophageal motility disorders. J Neurogastroenterol Motil. 2016;22:6-13.
14. Vega KJ, Palácio C, Langford-Legg T, Watts J, Jamal MM. Gender variation in oesophageal motor function: analysis of 129 healthy individuals. Dig Liv Dis. 2010;42:482-4.

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Relationship between nutritional status and the clinical outcomes of patients with and without neoplasms according to multiple correspondence analysis

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ABSTRACT – **Background** – For many years, many studies have reported undesirable outcomes that may occur during the hospital stay of patients diagnosed with malnutrition or even at some nutritional risk. **Objective** – To investigate the relationship between nutritional status and clinical outcomes during hospital stay using the multiple correspondence analysis technique. **Methods** – This cross-sectional study included 600 patients with and without neoplasms. The following data were collected: subjective global assessment, nutritional indicators, nutritional risk screening, anthropometric data (body mass index (BMI), mid-upper arm circumference (MUAC), mid-upper arm muscle circumference (MUAMC), triceps skinfold thickness (TST), recent weight loss (RWL)), and habitual energy intake (HEI/ER <75%). The clinical outcomes of interest were complications, length of hospital stay (LOHS), and death. The data were analyzed by the chi-square or Fisher's exact test at a significance level of 5%. Multiple correspondence analysis was used for the multivariate data analysis. **Results** – The multiple correspondence analysis map for the patients with neoplasms showed that the following characteristics were associated and represented by death, complications, and a greater likelihood of LOHS ≥7 days: underweight according to BMI; TST, MUAC, and MUAMC ≤15th percentile; malnutrition according to the subjective global assessment; at nutritional risk according to the nutritional risk screening; being male; age ≥60 years; and HEI/ER <75%. The multiple correspondence analysis map for the patients without neoplasms showed that the following characteristics were associated and represented by death: underweight according to BMI; TST ≤15th percentile; malnutrition according to the subjective global assessment; and at nutritional risk according to the nutritional risk screening. Complications and LOHS ≥7 days represented the categories male, no recent weight loss, HEI/ER <75%, MUAC and MUAMC ≤15th percentile, TST between the 15th and 85th percentiles, and age <60 years. **Conclusion** – The results of this study confirm an association between unsatisfactory nutritional indicators and undesirable clinical outcomes.

HEADINGS – Nutrition assessment. Nutritional status. Anthropometry. Weight loss. Neoplasms.

INTRODUCTION

Depletion of nutritional status has been associated with many unsatisfactory clinical outcomes in hospitalized patients and in various clinical situations[3,4,14,24,32]. For many years, many studies have reported undesirable outcomes that may occur during the hospital stay of patients diagnosed with malnutrition or even at some nutritional risk[28,31]. These findings have been observed in patients with neoplasms, digestive tract diseases, and other clinical situations[22,24,31].

Hence, malnutrition, nutritional risk, recent weight loss, and low energy intake are considered risk factors for poor clinical outcomes in hospitalized patients, regardless of the underlying disease[3,4,14,22,28,31,32].

Given this hospital setting reality, many studies[3,4,22,28,29,34] have used different nutritional status assessment methods, especially the nutritional risk screening (NRS-2002)[28,29,34], subjective global assessment[22,28,29,34], biochemical parameters[24,29], and anthropometry[3,22,24,29] to investigate the relationship between poor nutritional status and bad clinical outcomes, and many of these methods have been considered good predictors of bad clinical outcomes during hospital stay[24,28]. Yet, other studies have suggested the combination of subjective and objective methods[29] for nutritional status assessment.

The findings of the abovementioned studies have indicated a need of implementing nutritional care actions and nutritional education strategies in the hospital routine, which can contribute to early detection of malnutrition and consequently, increase the effectiveness of actions that reestablish an adequate nutritional status in hospitalized patients[11,12,13]. The objective of this study was to determine the relationship between nutritional status and the clinical outcomes of patients with and without neoplasms during hospital stay using the multiple correspondence analysis technique.

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METHODS

Study characteristics, location, cases, ethical approval, and sample size

This cross-sectional study lasted two years (2014-2015). The sample consisted of 600 hospitalized patients with and without neoplasms from the surgery ward of the Hospital and Maternity Hospital Celso Pierro of the Pontifical Catholic University of Campinas-SP-Brazil. The study site is a general hospital institution that provides clinical care, surgery, elective treatment, and emergency care. All study patients were surgical patients because they were recruited at the surgical ward. The group of patients with neoplasms (N=300) regarded patients submitted to cancer surgery (patients with gastrointestinal tract, pulmonary, and head and neck cancers). The group of patients without neoplasms (N=300) had undergone different types of surgery, such as esophagectomy to treat megaesophagus, other gastrointestinal tract surgeries, cholecystectomy, colectomy, fundoplication to correct esophageal hiatal hernia (patients with gastrointestinal tract diseases, abdominal wall hernias, megacolon, and megaesophagus). The study was approved by the local Research Ethics Committee (protocol no. 393.937).

Inclusion and exclusion criteria

The inclusion criteria were: nutritional assessment within 48 hours of hospital admission, all nutritional assessment data recorded in the medical records of the institution, and age above 20 years. The exclusion criteria were: patients with edema, ascites, or terminal disease; patients undergoing hemodialysis; isolated patients; or patients hospitalized only for tests or clinical investigation. Bedridden patients and patients who could not communicate were also excluded as it was not possible to obtain body weight and energy intake data.

Data collection

The data collected from the medical records included gender, age, length of hospital stay (LOHS), type of disease, complications, mortality, and nutritional indicators to determine nutritional status and nutritional risk.

Variables for nutritional status diagnosis

The following nutritional indicators were analyzed: subjective global assessment (SGA), nutritional risk screening (NRS), anthropometric data, and habitual energy intake (HEI). Nutritional status was classified according to the established cut-off points for each one of these indicators.

1. Subjective global assessment (SGA)

SGA data were analyzed as recommended by Detsky et al.[10]. SGA makes a subjective assessment by scoring weight loss, food intake, and clinical and physical signs of malnutrition, classifying individuals as well-nourished, mildly malnourished, moderately malnourished, and severely malnourished[10]. Patients classified as mildly, moderately, and severely malnourished were considered malnourished.

2. Nutritional risk screening (NRS)

NRS[19,20] is a means of detecting nutritional risk early in hospitalized patients. It has been validated and recommended by the European Society of Clinical Nutrition and Metabolism (ESPEN) and used in many hospitals globally[27,28,29,34]. NRS determines nutritional risk by investigating weight loss, body mass index, low food intake, and disease severity. A score ≥3 indicates nutritional risk, and <3, no nutritional risk[19,20].

3. Anthropometry

Body weight and height, mid-upper arm circumference (MUAC), and triceps skinfold thickness (TST) were collected, and body mass index (BMI) and mid-upper arm muscle circumference (MUAMC) were calculated. The body composition indicators (MUAC, TST, and MUAMC) were classified according to their percentile distribution as recommended by Frisancho[15] and Burr & Phillips[6] for adults and older adults aged >65 years, respectively. Lean mass depletion (LMD) was defined as MUAC and MUAMC equal to or lower than the 15th percentile (≤P15); a percentile above the reference percentile for lean mass was defined as MUAC and MUAMC above the 85th percentile (>P85); and lean mass preservation (LMP) was defined as MUAC and MUAMC between the 15th and 85th percentiles (P15-P85). Fat mass depletion (FMD) was defined as TST equal to or below the 15th percentile (≤P15); excess fat mass (EFM) was defined as TST above the 85th percentile (>P85); and fat mass preservation (FMP) was defined as TST between the 15th and 85th percentiles (P15-P85)[6,15].

BMI was classified as recommended by the World Health Organization[35] and Lipschitz[25] for adults and older adults, respectively. Recent weight loss reported by the patients on hospital admission was also investigated and classified as recent weight loss (yes) and no recent weight loss (no).

4. Assessment of habitual energy intake (HEI)

The patients' habitual energy intake was assessed during an interview on admission about the foods consumed on a typical day. The interview collected the habitual food history, and the type and amount of foods normally consumed. The energy intake was then calculated by nutrition analysis software[33]. Habitual energy intake (HEI) was determined by analyzing habitual food intake data, with subsequent calculation of percent HEI adequacy in relation to individual energy requirement (%HEI/ER). The individual energy requirement was estimated by the equation proposed by Harris & Benedict[17]. This method has already been described in other studies[22]. Later, HEI was expressed as a percentage of the energy requirement. Low energy intake was defined as energy intake below 75% of the individual energy requirement (HEI/ER<75%)[26,31].

Clinical outcomes

The clinical outcomes of interest were complications, LOHS, and death. Complications were defined as postoperative pulmonary and cardiovascular complications, anastomotic fistula, and abdominal abscesses (sepsis). Length of hospital stay (LOHS) was classified as ≤6 days or ≥7 days. An outcome was defined as death when the patient died during hospital stay.

Statistical analysis

The study patients were characterized by descriptive analysis, namely frequency tables for the categorical variables and measures of position and dispersion for the continuous variables. The chi-square test or Fisher's exact test[9,30] verified associations or compared proportions using a significance level of 5%.

Multivariate data analysis included multiple correspondence analysis (MCA), which studies cross-frequency tables (contingency tables) that explore the simultaneous relationships between variables[5,9,16,21]. This exploratory technique uses maps to determine whether the interest groups can be differentiated by their elements (structures subjacent to the variable set). The maps' interpretation was based on the points located approximately on the same direction of the origin and in the same region of space by parameters such as total inertia, the relative contribution to the inertia and supplementary variables[5,9,16,21]. Multiple correspondence analysis was chosen because it allows analyzing all outcomes simultaneously, characterizing the patients' profiles and considering the three outcomes together with the other exploratory variables[5,9,16,21].

RESULTS

The data of 600 hospitalized patients were analyzed by general descriptive analysis, comparing the study variables according to disease group (with or without neoplasms) and presence of complications (Table 1), death (Table 2), and length of hospital stay (LOHS) ≤6 days or ≥7 days (Table 3).

TABLE 1. Descriptive analysis and comparison of the variables by presence of complications in each disease group.

Variables	With neoplasms			Without neoplasms		
	With complications N=39 N(%)	Without complications N=261 N(%)	*P*-value*	With complications N=46 N(%)	Without complications N=254 N(%)	*P*-value*
Gender						
Female	13 (33.3)	90 (34.5)	0.89	17 (37.0)	98 (38.6)	0.83
Male	26 (66.7)	171 (65.5)		29 (63.0)	156 (61.4)	
Age						
<60 years	20 (51.3)	126 (48.3)	0.73	32 (69.6)	138 (54.3)	0.055
≥60 years	19 (48.7)	135 (51.7)		14 (30.4)	116 (45.7)	
NRS						
At risk	21 (53.8)	100 (38.3)	0.065	17 (37.0)	108 (42.5)	0.48
Not at risk	18 (46.2)	161 (61.7)		29 (63.0)	146 (57.5)	
SGA						
Malnourished	18 (46.2)	62 (23.8)	**0.0032**	17 (37.0)	51 (20.1)	**0.012**
Well nourished	21 (53.8)	199 (76.2)		29 (63.0)	203 (79.9)	
BMI						
Excess weight	12 (30.8)	88 (33.7)	0.93	17 (37.0)	92 (36.2)	0.99
Normal weight	18 (46.2)	117 (44.8)		19 (41.3)	105 (41.3)	
Underweight	9 (23.1)	56 (21.5)		10 (21.7)	57 (22.4)	
MUAC						
≤P15	17 (43.6)	96 (36.8)	0.69	22 (47.8)	84 (33.1)	0.15
P15-P85	15 (38.5)	108 (41.4)		17 (37.0)	127 (50.0)	
>P85	7 (17.9)	57 (21.8)		7 (15.2)	43 (16.9)	
TST						
≤P15	7 (17.9)	45 (17.2)	0.94	8 (17.4)	35 (13.8)	0.60
P15-P85	21 (53.8)	135 (51.7)		26 (56.5)	135 (53.1)	
>P85	11 (28.2)	81 (31.0)		12 (26.1)	84 (33.1)	
MUAMC						
≤P15	17 (43.6)	100 (38.3)	0.66	20 (43.5)	96 (37.8)	0.63
P15-P85	17 (43.6)	134 (51.3)		23 (50.0)	132 (52.0)	
>P85	5 (12.8)	27 (10.3)		3 (6.5)	26 (10.2)	
Weight loss						
Yes	19 (48.7)	129 (49.4)	0.93	8 (17.4)	126 (49.6)	<.0001
No	20 (51.3)	132 (50.6)		38 (82.6)	128 (50.4)	
%HEI/ER						
<75%	19 (48.7)	155 (59.4)	0.21	25 (54.3)	143 (56.3)	0.81
≥75%	20 (51.3)	106 (40.6)		21 (45.7)	111 (43.7)	

NRS: nutritional risk screening; SGA: subjective global assessment; BMI: body mass index; MUAC: mid-upper arm circumference; MUAMC: mid-upper arm muscle circumference; TST: triceps skinfold thickness; %HEI/ER: percent habitual energy intake adequacy with respect to the individual energy requirement. * Chi-square test.

TABLE 2. Descriptive analysis and comparison of the variables by death in each disease group

Variables	With neoplasms			Without neoplasms		
	No death N=289 N(%)	Death N=11 N(%)	*P*-value	No death N=289 N(%)	Death N=11 N(%)	*P*-value
Gender						
Female	100 (34.6)	3 (27.3)	0.75 **	112 (38.8)	3 (27.3)	0.54 **
Male	189 (65.4)	8 (72.7)		177 (61.2)	8 (72.7)	
Age						
<60 years	143 (49.5)	3 (27.3)	0.15 *	165 (57.1)	5 (45.5)	0.54 **
≥60 years	146 (50.5)	8 (72.7)		124 (42.9)	6 (54.5)	
NRS						
At risk	113 (39.1)	8 (72.7)	**0.031** **	119 (41.2)	6 (54.5)	0.54 **
Not at risk	176 (60.9)	3 (27.3)		170 (58.8)	5 (45.5)	
ASG						
Malnourished	73 (25.3)	7 (63.6)	**0.0097** **	62 (21.5)	6 (54.5)	0.019 **
Well nourished	216 (74.7)	4 (36.4)		227 (78.5)	5 (45.5)	
BMI						
Excess weight	98 (33.9)	2 (18.2)	0.57 **	106 (36.7)	3 (27.3)	0.79 **
Normal weight	129 (44.6)	6 (54.5)		119 (41.2)	5 (45.5)	
Underweight	62 (21.5)	3 (27.3)		64 (22.1)	3 (27.3)	
MUAC						
≤P15	110 (38.1)	3 (27.3)	0.16 **	102 (35.3)	4 (36.4)	1.00 **
P15-P85	120 (41.5)	3 (27.3)		139 (48.1)	5 (45.5)	
>P85	59 (20.4)	5 (45.5)		48 (16.6)	2 (18.2)	
TST						
≤P15	51 (17.6)	1 (9.1)	0.59 **	41 (14.2)	2 (18.2)	0.83 **
P15-P85	151 (52.2)	5 (45.5)		155 (53.6)	6 (54.5)	
>P85	87 (30.1)	5 (45.5)		93 (32.2)	3 (27.3)	
MUAMC						
≤P15	113 (39.1)	4 (36.4)	1.00 **	111 (38.4)	5 (45.5)	0.81 **
P15-P85	145 (50.2)	6 (54.5)		149 (51.6)	6 (54.5)	
>P85	31 (10.7)	1 (9.1)		29 (10.0)	-	
Weight loss						
Yes	142 (49.1)	6 (54.5)	0.72 *	133 (46.0)	1 (9.1)	**0.026** **
No	147 (50.9)	5 (45.5)		156 (54.0)	10 (90.9)	
%HEI/ER						
<75%	168 (58.1)	6 (54.5)	1.00 **	161 (55.7)	7 (63.6)	0.76 **
≥75%	121 (41.9)	5 (45.5)		128 (44.3)	4 (36.4)	

NRS: nutritional risk screening; SGA: subjective global assessment; BMI: body mass index; MUAC: mid-upper arm circumference; TST: triceps skinfold thickness; MUAMC: mid-upper arm muscle circumference; %HEI/ER: percent habitual energy intake adequacy with respect to the individual energy requirement. * Chi-square test; ** Fisher's exact test.

TABLE 3. Descriptive analysis and comparison of the variables by length of hospital stay in each disease group

Variables	With neoplasms			Without neoplasms		
	TI ≤ 6 days N=143 N(%)	TI ≥ 7 days N=157 N(%)	*P*-value*	TI ≤ 6 days N=143 N(%)	TI ≥ 7 days N=157 N(%)	*P*-value*
Gender						
Female	46 (32.2)	57 (36.3)	0.45	61 (37.2)	54 (39.7)	0.66
Male	97 (67.8)	100 (63.7)		103 (62.8)	82 (60.3)	
Age						
<60 years	73 (51.0)	73 (46.5)	0.43	90 (54.9)	80 (58.8)	0.49
≥60 years	70 (49.0)	84 (53.5)		74 (45.1)	56 (41.2)	
NRS						
At risk	44 (30.8)	77 (49.0)	**0.0013**	54 (32.9)	71 (52.2)	**0.0007**
Not at risk	99 (69.2)	80 (51.0)		110 (67.1)	65 (47.8)	
SGA						
Malnourished	26 (18.2)	54 (34.4)	**0.0015**	27 (16.5)	41 (30.1)	**0.0048**
Well nourished	117 (81.8)	103 (65.6)		137 (83.5)	95 (69.9)	
BMI						
Excess weight	62 (43.4)	38 (24.2)	**0.0021**	62 (37.8)	47 (34.6)	0.29
Normal weight	55 (38.5)	80 (51.0)		71 (43.3)	53 (39.0)	
Underweight	26 (18.2)	39 (24.8)		31 (18.9)	36 (26.5)	
MUAC						
≤P15	44 (30.8)	69 (43.9)	0.059	46 (28.0)	60 (44.1)	**0.013**
P15-P85	64 (44.8)	59 (37.6)		89 (54.3)	55 (40.4)	
>P85	35 (24.5)	29 (18.5)		29 (17.7)	21 (15.4)	
TST						
≤P15	21 (14.7)	31 (19.7)	0.064	19 (11.6)	24 (17.6)	0.29
P15-P85	69 (48.3)	87 (55.4)		89 (54.3)	72 (52.9)	
>P85	53 (37.1)	39 (24.8)		56 (34.1)	40 (29.4)	
MUAMC						
≤P15	50 (35.0)	67 (42.7)	0.14	54 (32.9)	62 (45.6)	0.045
P15-P85	73 (51.0)	78 (49.7)		90 (54.9)	65 (47.8)	
>P85	20 (14.0)	12 (7.6)		20 (12.2)	9 (6.6)	
Weight loss						
Yes	65 (45.5)	83 (52.9)	0.20	76 (46.3)	58 (42.6)	0.52
No	78 (54.5)	74 (47.1)		88 (53.7)	78 (57.4)	
%HEI/ER						
<75%	83 (58.0)	91 (58.0)	0.99	82 (50.0)	86 (63.2)	0.022
≥75%	60 (42.0)	66 (42.0)		82 (50.0)	50 (36.8)	

LOHS: length of hospital stay; NRS: nutritional risk screening; SGA: subjective global assessment; BMI: body mass index; MUAC: mid-upper arm circumference; TST: triceps skinfold thickness; MUAMC: mid-upper arm muscle circumference; %HEI/ER: percent habitual energy intake adequacy with respect to the individual energy requirement. * Chi-square test.

Complications occurred in 39 (13%) patients with neoplasms and 46 (15.3%) patients without neoplasms (Table 1). Comparison of the study variables (gender, age, RWL, BMI, MUAC, TST, MUAMC, SGA, NRS, and % HEI/ER) of non-neoplastic and neoplastic patients with and without complications showed that only SGA differed in patients with neoplasms (P=0.0032) and without neoplasms (P=0.012). In other words, the frequency of malnutrition according to the SGA was higher in patients with complications than in those without complications.

Regarding death (Table 2), comparison of the study variables of non-neoplastic and neoplastic patients showed that SGA differed between survivors and deceased with neoplasms (P=0.0097) and without neoplasms (P=0.019). The prevalence of malnutrition according to the SGA was significantly higher in deceased patients,

regardless of disease group. The same occurred in patients at nutritional risk according to the NRS (P=0.031): the frequency of death was higher in patients with neoplasms who were at nutritional risk according to the NRS. Finally, non-neoplastic survivors lost more weight (P=0.026).

Table 3 shows the comparison of the study variables by LOHS (≤6 days and ≥7 days). The rates of malnutrition according to the SGA and nutritional risk according to the NRS differed significantly in the two disease groups. Malnutrition according to the SGA (P=0.0015) and nutritional risk according to the NRS (P=0.0013) were more prevalent in neoplastic patients with a hospital stay ≥7 days. Malnourished non-neoplastic patients according to the SGA (P=0.0048) and at nutritional risk according to the NRS (P=0.0007) also had longer hospital stays. MUAC (P=0.013) and MUAMC (P=0.045) also differed significantly in non-neoplastic patients, that is, non-neoplastic patients with longer hospital stays (≥7 days) had MUAC and MUAMC ≤P15, indicating lean mass depletion.

The variables included in the multiple correspondence analysis (MCA) were gender, age group, recent weight loss, nutritional status according to BMI, MUAC, MUAMC, TST, SGA, and NRS, and % HEI/ER. The clinical outcomes of interest, such as complications, death, and LOHS ≤6 or ≥7days were used as supplementary variables.

The MCA map for neoplastic patients (Figure 1) showed that the adjusted inertia for the two first dimensions was 93.5%. The first dimension explained 79.7% of data variability, and the categories are mainly organized along this axis. The nutritional status categories according to MUAC, MUAMC, and TST >85th percentile were grouped and not represented by any of the study clinical outcomes. On the other hand, underweight according to BMI; TST, MUAC, and MUAMC ≤P15; malnutrition according to the SGA; nutritional risk according to the NRS; recent weight loss; being male; age ≥60 years; and HEI/ER<75% were associated and represented by death, presence of complications, and closer to a hospital stay ≥7 days. Absence of complications, LOHS ≤6 days, and survival represented the categories of well-nourished according to the SGA; HEI/ER ≥75%; no nutritional risk according to the NRS; no recent weight loss; MUAC, MUAMC, and TST between the 15th and 85th percentiles; age <60 years; being female; and appropriate BMI.

The MCA map for non-neoplastic patients (Figure 2) showed that the adjusted inertia for the two first dimensions was 94.6%. The first dimension explains 68.3% of the data variability, and the categories were organized mainly along this axis. MUAC, MUAMC, and TST >85th percentile and age ≥60 years were grouped and not represented by any of the clinical outcomes. Meanwhile, underweight according to BMI; TST ≤P15; malnutrition according to the SGA; and nutritional risk according to the NRS were associated and represented by death. Recent weight loss; excess weight according to BMI; MUAC and MUAMC between the 15th and 85th percentiles; HEI/ER ≥75%; well-nourished according to the SGA; being female; and no nutritional risk according to the NRS were represented by survivors without complications and with LOHS ≤ 6 days. Complications and LOHS ≥7 days represented the categories being male; no recent weight loss; HEI/ER <75%; MUAC and MUAMC ≤P15; TST between P15 and P85; and age <60 years.

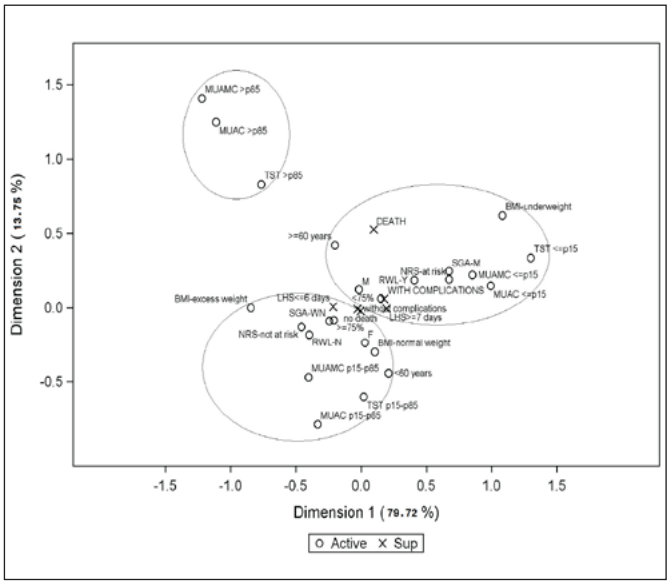

FIGURE 1. Multiple correspondence analysis map (projections on the first 2 dimensions) for neoplastic patients. The eigenvalues and proportion of explained inertia have been corrected with Benzécri/Greenacre formula[26,27]. Active variables: gender (female and male); age group (<60 and ≥60 years); recent weight loss (yes-Y and no-N); BMI (underweight, normal weight, excess weight); MUAC, TST, and MUAMC (≤P15, P15-P85, and >P85); SGA (M and WN); NRS (at risk and not at risk); %HEI/ER (<75% and ≥75%). Sup=supplementary variables: complications (with complications and without complications); death (death and no death); and length of hospital stay (≤6 days and ≥7 days).

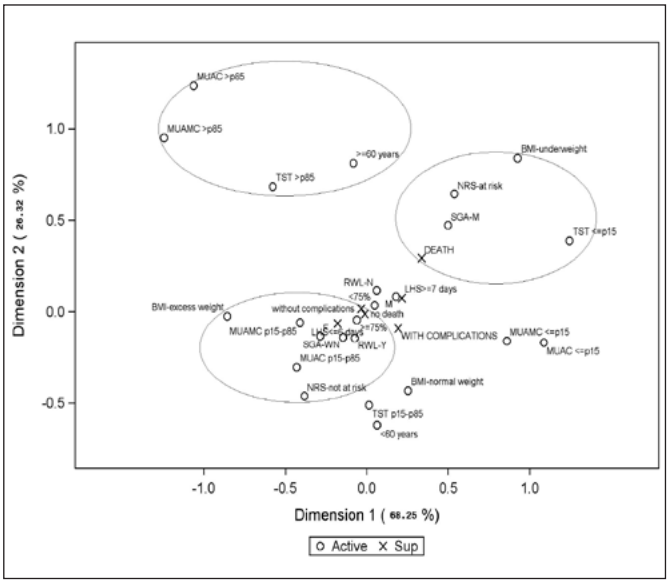

FIGURE 2. Multiple correspondence analysis map (projections on the first 2 dimensions) for non-neoplastic patients. The eigenvalues and proportion of explained inertia have been corrected with Benzécri/Greenacre formula[26,27]. Active variables: gender (female and male); age group (<60 and ≥60 years); recent weight loss (yes-Y and no-N); BMI (underweight, normal weight, excess weight); MUAC, TST, and MUAMC (≤P15, P15-P85, and >P85); SGA (M and WN); NRS (at risk and not at risk); %HEI/ER (<75% and ≥ 75%). Sup=supplementary variables: complications (with complications and without complications); death (death and no death); and length of hospital stay (≤6 days and ≥7 days).

DISCUSSION

This study investigated the nutritional status of a population of patients in a surgical ward using different indicators of nutritional status/risk and its relationship with the clinical outcomes length of hospital stay (LOHS), complications, and death, using multiple correspondence analysis. Many studies have investigated the nutritional status of hospitalized patients and its relationship with the risk factors associated with clinical intercurrences during hospital stay[3,22-24,27,28,31]. These studies have found strong associations between the nutritional status of these patients and the presence of complications, death, and a lengthy hospital stay, and many have established these associations with logistic regression analyses[24,22,28,31]. The current study differs from other studies because it used MCA to investigate the relationship between nutritional status and clinical outcomes. MCA found associations between the study variables and demonstrated how they behave inside the MCA map. The MCA map for neoplastic patients evidenced that the variables death and LOHS ≥7 days were associated with many indicators of nutritional risk according to the NRS, malnutrition according to the SGA, underweight according to BMI, depletion of lean and fat masses according to body composition parameters, and recent weight loss. These findings have been described by other studies using another type of analysis[1,2,22,23,24]. The present study has found that nutritional status-related variables are associated with routine clinical outcomes during hospital stay. As reported by other recent studies[2], malnutrition on hospital admission is prevalent and associated with long hospital stay. A strong association has also been found between nutritional risk assessed by other indices and longer hospital stay in adult patients[7].

The MCA map for neoplastic patients has shown that malnutrition according to the SGA and nutritional risk according to the NRS are associated with complications, death, and LOHS ≥7 days.

Other studies have also shown that patients at nutritional risk according to the NRS have more complications, higher mortality rates, and longer hospital stays[31]. Such findings may suggest that NRS variables can be considered independent predictors of unsatisfactory clinical outcomes[31].

Other nutritional indicators, such as the SGA, BMI, low energy intake (<50%), and handgrip strength, predict malnutrition and are associated with long hospital stays[2].

According to multiple logistic regression, moderate to severe nutritional risk, higher age, and emergency hospitalization increases LOHS and mortality significantly[24]. Many studies[8,18,27,28], have shown the importance of using the NRS to detect nutritional risk early and its association with clinical outcomes; others have used the NRS together with biochemical indicators[8]. A study that assessed the impact of nutritional support on the clinical outcomes of patients at nutritional risk has found lower rates of complications and shorter hospital stays in patients at nutritional risk who receive adequate nutritional support during their stay[18].

The MCA map for non-neoplastic patients has shown that death was associated with nutritional risk according to the NRS, malnutrition according to the SGA, and underweight and fat mass depletion according to TST. On the other hand, HEI/ER <75%; MUAC and MUAMC ≤P15; TST between the 15th and 85th percentiles; and age <60 years were represented by the presence of complications and LOHS ≥7 days in males.

Other recent studies confirmed an association between nutritional status deterioration and longer hospital stays, such as the prospective study conducted by Allard et al.[1], in Canadian hospitals. In said study the authors administered the SGA and measured body weight on hospital admission and discharge, and found, using multivariate analysis, that nutritional status deterioration according to the SGA and weight loss during hospital stay were significantly associated with longer hospitals stays, regardless of other factors, such as disease severity[32].

Using MCA, this study once again confirms the association between malnutrition and nutritional risk in a population representative of surgical ward patients. The study hospital has a team of dietitians and other health professionals that screens the nutritional status of its patients routinely. Although some study patients had good nutritional status and proper weight on admission, other patients were malnourished or at nutritional risk. This reality was observed in a sample of 600 patients from a large university hospital that provides care to patients from the public health care system (SUS) of the municipality and region of a large Brazilian city. One of the disadvantages of cross-sectional studies is the difficulty of establishing a relationship of cause and effect. Moreover, the commonly used multivariate techniques assess one outcome at a time, while MCA allows assessing all outcomes at once. MCA characterizes the patients' profiles and considers the three outcomes together with the other exploratory variables[5,9,16,21].

In conclusion, the data provided by MCA confirmed the association between unsatisfactory nutritional indicators and undesirable clinical outcomes, and may contribute to the nutritional and clinical prognosis of neoplastic and non-neoplastic patients.

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Authors' contributions

Leandro Merhi VA and Aquino JLB contributed to the study design and data analysis. The paper was written by Leandro Merhi VA and Aquino JLB and the authors read and approved the final manuscript.

Leandro Merhi VA, Aquino JLB. Relação entre estado nutricional e desfechos clínicos pela técnica da análise de correspondência múltipla em pacientes com e sem neoplasias. Arq Gastroenterol. 2017;54(2):148-55.

RESUMO – **Contexto** – Muitos estudos já vêm relatando há muitos anos, alguns desfechos indesejáveis que podem se manifestar durante o curso da hospitalização em pacientes diagnosticados com desnutrição ou até mesmo com algum risco nutricional. **Objetivo** – Explorar pela técnica da análise de correspondência múltipla a relação entre o estado nutricional e os desfechos clínicos apresentados no decorrer da internação em pacientes hospitalizados. **Métodos** – Estudo transversal com 600 pacientes com e sem neoplasias. Foram estudados os indicadores nutricionais de avaliação subjetiva global, screening de risco nutricional, antropometria (IMC, circunferência braquial-CB, circunferência muscular do braço-CMB, prega cutânea tricipital-PCT), perda de peso recente e consumo energético habitual (CEH/NE <75%). Como desfechos clínicos, foram considerados a presença de complicações, tempo de internação e óbito. Os dados foram analisados pelo teste qui-quadrado ou exato de Fisher, com nível de significância de 5%. Para a análise multivariada dos dados, utilizou-se a análise de correspondência múltipla. **Resultados** – O mapa fornecido pela análise de correspondência múltipla no grupo de pacientes com neoplasias, mostrou que as categorias de baixo peso pelo IMC, PCT, CB e CMB ≤ ao percentil 15, desnutrido pela avaliação subjetiva global, com risco nutricional pelo screening de risco nutricional, com perda de peso recente, sexo masculino, idade ≥ a 60 anos e CEH/NE <75% se associaram e foram representadas pelo óbito, com complicações e mais próximos do tempo de internação ≥ a 7 dias. O mapa fornecido pela análise de correspondência múltipla no grupo de pacientes sem neoplasias, mostrou que as categorias de baixo peso pelo IMC, PCT ≤ ao percentil 15, desnutrido pela avaliação subjetiva global e com risco nutricional pelo screening de risco nutricional se associaram e foram representadas pelo óbito. Complicações e tempo de internação ≥ 7 dias representaram as categorias de sexo masculino, sem perda de peso recente, CEH/NE <75%, CB e CMB ≤ ao percentil 15, PCT entre percentil 15 e 85 e idade < 60 anos. **Conclusão** – Os resultados deste estudo confirmaram uma associação entre indicadores nutricionais insatisfatórios e desfechos clínicos indesejáveis.

DESCRITORES – Avaliação nutricional. Estado nutricional. Antropometria. Perda de peso. Neoplasias.

REFERENCES

1. Allard JP, Keller H, Jeejeebhoy KN, Laporte M, Duerksen DR, Gramlich L, et al. Decline in nutritional status is associated with prolonged length of stay in hospitalized patients admitted for 7 days or more: A prospective cohort study. Clin Nutr. 2016;35:144-52.
2. Allard JP, Keller H, Jeejeebhoy KN, Laporte M, Duerksen DR, Gramlich L, et al. Malnutrition at Hospital Admission - Contributors and Effect on Length of Stay: A Prospective Cohort Study From the Canadian Malnutrition Task Force. JPEN J Parenter Enteral Nutr. 2016;40:487-97.
3. Amaral TF, Matos LC, Teixeira MA, Tavares MM, Álvares L, Antunes A. Undernutrition and associated factors among hospitalized patients. Clin Nutr. 2010;29:580-5.
4. Beghetto MG, Luft VC, Mello ED, Polanczyk CA. Accuracy of nutritional assessment tools for predicting adverse hospital outcomes. Nutr Hosp. 2009;24:56-62.
5. Benzécri JP. Sur le calcul des taux d'inertie dans l'analyse d'un questionnaire, addendum et erratum à [BIN. MULT.]. Cahiers de l'Analyse des Données. 1979,4,377–8.
6. Burr ML, Phillips MK. Anthropometric norms in the elderly. Br J Nutr. 1984;51:165-9.
7. Caccialanza R, Klersy C, Cereda E, Cameletti B, Bonoldi A, Bonardi C, Marinelli M, Dionigi P. Nutritional parameters associated with prolonged hospital stay among ambulatory adult patients. CMAJ. 2010;182:1843-9.
8. Chen ZY, Gao C, Ye T, Zuo XZ, Wang GH, Xu XS, Yao Y. Association between nutritional risk and routine clinical laboratory measurements and adverse outcomes: a prospective study in hospitalized patients of Wuhan Tongji Hospital. Eur J Clin Nutr. 2015;69:552-7.
9. Conover WJ (1999). Practical Nonparametric Statistics. 3ª ed. John Wiley & Sons Inc. Nova Iorque.
10. Detsky AS, McLaughlin JR, Baker JP, Johnston N, Whittaker S, Mendelson RA, Jeejeebhoy KN. What is subjective global assessment of nutritional status? JPEN J Parenter Enteral Nutr. 1987;11:8-13.
11. Diez-Garcia RW, de Sousa AA, Proença RP, Leandro-Merhi VA, Martinez EZ. Gauging food and nutritional care quality in hospitals. Nutr J. 2012;11:66.
12. Diez-García RW, Martínez EZ, Penaforte FRO, Japur CC. Hospital nutritional care: propositions endorsed by the scientific Community. Nutr Hosp. 2015;32:1353-61.
13. Duerksen DR, Keller HH, Vesnaver E, Allard JP, Bernier P, Gramlich L, Payette H, Laporte M, Jeejeebhoy K. Physicians' perceptions regarding the detection and management of malnutrition in canadian hospitals: results of a canadian malnutrition task force survey. Journal of Parenteral and Enteral Nutrition. 2015;39:410-7.
14. Fontes D, Generoso SV, Correia MITD. Subjective global assessment: a reliable nutritional assessment tool to predict outcomes in critically ill patients. Clin Nutr. 2014;33:291-5.
15. Frisancho AR. Anthropometric standards for the assessment of growth and nutritional status. Michigan: The University of Michigan Press; 1990.
16. Greenacre MJ. Multiple and Joint Correspondence Analysis. In: Greenacre MJ and Blasius J, eds., Correspondence Analysis in the Social Sciences, London: Academic Press, 1994.
17. Harris J, Benedict F. A biometric study of basal metabolism in man. Washington D.C. Carnegie Institute of Washington. 1919.
18. Jia ZY, Yang J, Tong DN, Peng JY, Zhang ZW, Liu WJ, Xia Y, Qin HL. Screening of nutritional risk and nutritional support in general surgery patients: a survey from Shanghai, China. Int Surg. 2015;100:841-8.
19. Kondrup J, Allison SP, Elia M, Vellas B, Plauth M. ESPEN guidelines for nutrition screening 2002. Clin Nutr. 2003;22:415-21.
20. Kondrup J, Rasmussen HH, Hamberg O, Stanga Z, ESPEN Working Group. Nutritional risk screening (NRS 2002): a new method based on a analysis of controlled clinical trials. Clin Nutr. 2003;22:321-36.
21. Le Roux B, Rouanet H. Multiple Correspondence Analysis: Quantitative applications in the social sciences, volume 163, California, SAGE, 2009.
22. Leandro-Merhi VA, Aquino JLB, Chagas JFS. Nutrition status and risk factors associated with length of hospital stay for surgical patients. JPEN J Parenter Enteral Nutr. 2011;35:241-8.
23. Leandro-Merhi VA, Aquino JLB. Comparison of nutritional diagnosis methods and prediction of clinical outcomes in patients with neoplasms and digestive tract diseases. Clin Nutr. 2015;34:647-51.
24. Lee H, Cho YS, Jung S, Kim H. Effect of nutritional risk at admission on the length of hospital stay and mortality in gastrointestinal cancer patients. Clin Nutr Res. 2013;2:12-8.
25. Lipschitz DA. Screening for nutritional status in the elderly. Prim Care. 1994;22:55-67.
26. Mimiran P, Hosseinpour-Niazi S, Mehrabani HH, Kavian F, Azizi F. Validity and reliability of a nutrition screening tool in hospitalized patients. Nutrition. 2011;27:647-52.
27. Raslan M, Gonzalez MC, Dias MCG, Nascimento M, Castro M, Marques P, Segatto S, Torrinhas RS, Cecconello I, Waitzberg DL. Comparison of nutritional risk screening tools for predicting clinical outcomes in hospitalized patients. Nutrition. 2010;26:721-6.
28. Raslan M, Gonzalez MC, Torrinhas RSMM, Ravacci GR, Pereira JCR, Waitzberg DL. Complementarity of Subjective Global Assessment (SGA) and Nutritional Risk Screening 2002 (NRS 2002) for predicting poor clinical outcomes in hospitalized patients. Clin Nutr. 2011;30:49-53.
29. Ryu SW, Kim, IH. Comparison of different nutritional assessments in detecting malnutrition among gastric cancer patients. World J Gastroenterol. 2010;16:3310-7.
30. SAS System for Windows (Statistical Analysis System), version 9.4. SAS Institute Inc, 2002-2012, Cary, NC, USA.
31. Sorense J, Kondrup J, Prokopowicz J, Schiesser M, Krahenbbuhl L, Meier R, et al. EuroOOPS: an international, multicentre study to implement nutritional risk screening and evaluate clinical outcome. Clin Nutr. 2008;27:340-9.
32. Tangvik RJ, Tell GS, Eisman JA, Guttormsen AB, Henriksen A, Nilsen RM, Oyen J, Ranhoff AH. The nutritional strategy: four questions predict morbidity, mortality and health care costs. Clin Nutr. 2014;33:634-41.
33. Universidade Federal de São Paulo. Escola Paulista de Medicina. Programa de Apoio a Nutrição (NUTWIN) – programa de computador, versão 1.5. São Paulo: UNIFESP/ EPM; 2002.
34. Velasco C, Garcia E, Rodríguez V, Frias L, Garriga R, Álzarez J, Garcia-Peris P, León M. Comparison of four nutritional screening tools to detect nutritional risk in hospitalized patients: a multicentre study. Eur J Clin Nutr. 2011;65:269-74.
35. World Health Organization (WHO). Obesity: Preventing and managing the global epidemic - Report of a WHO Consultation on obesity. Geneva: WHO, 1998.

ORIGINAL ARTICLE

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Personality traits, anger and psychiatric symptoms related to quality of life in patients with newly diagnosed digestive system cancer

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ABSTRACT – Background – The presence of psychiatric symptoms, anger, and personality characteristics are factors that affect the quality of life of newly diagnosed digestive system cancer patients. **Objective** – This study aims to identify which stable characteristics of the individual's personality interfere with quality of life, even when reactive emotional characteristics of falling ill are controlled. **Methods** – A cross-sectional study was performed at the Oncology Clinic (*Hospital das Clínicas*), Marília/SP, Brazil, in which 50 adult patients with digestive system cancer and diagnosed less than 6 months answered the State-Trait Anger Expression Inventory, Temperament and Character Inventory, Hospital Anxiety and Depression Scale and WHOQOL-BREF. Multiple regression was performed to verify if quality of life was related to stable characteristics of the subject's personality (anger trait, temperament and character) after controlling to the transient emotional aspects (anger state, psychiatric symptoms). **Results** – The quality of life psychological health score was higher in presence of self-directedness character and reward dependence temperament and quality of life environment score was higher in presence of self-directedness character and lower in presence of harm avoidance temperament. **Conclusion** – The psychological well-being and the adaptive needs to the environment that favoring a better quality of life were reinforced mainly by the self-directedness character; which means that patients more autonomous cope better with the disease. On the other hand, the harm avoidance temperament (meaning the patient has fear of aversive situations) impaired the adaptive capacity to deal with the changes of the day-to-day imposed by the disease. Understanding these personality traits is important to the health professionals drive the patient to more successful treatment.

HEADINGS – Digestive system neoplasms. Personality. Anxiety. Depression. Quality of life.

INTRODUCTION

It is known that cancer is the second cause of death in the world[39] being the colorectal neoplasm the third kind of cancer in men and the second in women[38]. Due to this high prevalence of the disease is important to comprehend the psychological aspect of the disease.

The prognosis and treatment response that a cancer patient has suffers direct interference of psychosocial aspects[3,35], and environmental conditions[14].

From the point of view of quality of life (QOL), it is noted that the oncologic patient suffers from some changes that can generate a dependence on their formal and informal caregivers, harming the physical, psychological, social and spiritual aspects of the newly diagnosed individual[17] .Thus, patients with cancer of the oral cavity, oropharynx, hypopharynx and larynx have a high prevalence of depressive symptoms and low functional and psychological level at the beginning of treatment, which may be predictors of increased severity of symptoms and malfunction of these post-treatment states[22]. Patients with gastrointestinal cancer show a decrease in QOL due to the presence of anguish, anxiety and depression symptoms[41,42] and those with laryngeal cancer and who underwent total or partial laryngectomy also present a deficit in social and emotional functions in QOL scores[9] and in people with gastric cancer, hopelessness is observed as a major impact on QOL[34].

In fact, emotional reactions to cancer are important aspects in understanding the patient's QOL. A study[31] with 68 newly diagnosed head and neck cancer patients were consecutively selected to declare their perceptions about the disease and the own quality of life; it was concluded that the more concerned the patient was with the physical symptoms the more intense was the emotional reactions related to the disease, and the lower was the QOL scores. Other study[12] with 180 hepatic and colon cancer patients that answered about stressors aspects, coping and QOL related to the disease showed that patients with more active personality and more resilient had higher psychologic well-being and QOL.

Being passive and active attitudes as coping strategies, it is important to identify which feelings are evolved in QOL aspects, mainly in newly diagnosed patients. It is known that the impact of the diagnosis of cancer can cause damage to anger control, with the expression of negative affect[37], and that 12% of gastric cancer subjects showed anger at facing the disease situation[33]. Another factor with little scientific information regards to the influence

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that personality characteristics of the subject and its cognitive and behavioral form of conflict situations coping act for a psychological adjustment of the individual. The personality traits of patients with gastric cancer can interfere with QOL[40]; it is observed that personality type D, which is characterized by traits of negative affectivity and social inhibition, was also investigated in patients with colorectal cancer showing that this type of personality interferes negatively on the QOL[32]. Along the same lines, temperament characteristics and character have not demonstrated clearly interfering factors in anxiety and depression[6].

Clinically, these factors are not presented in a fragmented way, i.e., feelings, emotions and personality of the newly diagnosed cancer patient form one single set in the patient. The importance of understanding how these factors work together in newly diagnosed patients with digestive system cancer is to develop more information that may sustain better psychological treatment to these patients. This study aims to identify which stable characteristics of the subject's personality (trace of anger, temperament and behavior), therefore the most difficult aspects to be modified interfere in various fields of quality of life, controlling the transitional emotional characteristics reactive to the disease (state of anger, depressive and anxious symptoms) that interfere with the expression of the patient's personality.

METHODS

This is a survey study developed between July of 2011 and November of 2012 at the Oncology Clinic, a multiprofessional facility from the *Hospital das Clínicas* of the *Faculdade de Medicina de Marília* (FAMEMA), in Marilia – Sao Paulo, Brazil. The hospital attends the Marilia region that comprises 62 nearby cities being the reference to patients with high complexity diseases as cancer.

The sample size was estimated to have at least 40 patients, to guarantee 10 patients (25% of the included patients)[29] with minor psychiatric symptoms. It would improve the statistical analysis quality. They were selected by convenience, including all patients that comprise the inclusion criteria: have digestive cancer diagnosed for less than 6 months and over 18 years old. The exclusion criteria were the patients have a mini-mental score below of the minimum to the own educational level to avoid patients with cognitive impairment[10], as well as the ones who did severe clinical conditions to respond to the questionnaires (either hospitalized or in home care). From a population of 792 patients listed in the facility with digestive cancer, 120 were included in the study but 10 had severe clinical conditions to respond to the questionnaires being excluded. Thus, all the 110 eligible patients from the study sample were equally invited to participate during their scheduled visit to the clinic. Sixty patients (50%) answered a part of the study protocol but then refused to continue alleging it was too much difficult (17 patients) or too long enough (43 patients). Only 50 patients completed the protocol (Figure 1).

Two psychologists were trained to apply the instruments; to not explain the questionnaires in order to avoid bias of answers, and to read the questions to the patients in case they have difficulty. The patients were approached by them before consultation or medical procedure for which had already been scheduled. After filling out the Informed Consent, they answered the mini-exam of the mental state[10] and a questionnaire to identify the sociodemographic and clinical characteristics and the instruments already validated for use in Brazil:

1. Hospital Anxiety and Depression Scale – HADS[7] was applied to identify symptoms of anxiety and depression. There are two subscales, one about anxiety symptoms (diagnostic sensitivity of 93.7% and specificity of 72.6%) and other about depressive symptoms (diagnostic sensitivity of 84.6% and specificity of 90.3%), each ranging from 0 to 21, wherein the higher the score, the greater the presence of symptoms; and the cut 8/9 represents the absence or presence of symptoms, respectively.

2. State-Trait Anger Expression Inventory – STAXI[5] was applied to identify trait components, state and direction of anger. There are six subscales that comprise: a personality trait domain of chronic anger, an anger state domain related to the current situation, and four domains on the direction of anger (which are not used in this study).The results of the chronic anger trait domain and rage state domain are expressed in scores and categorized according to percentiles, where scores below 25 represents that the subject uses excessive denial defenses and repression of feelings of anger, many times unacceptable; percentiles between 25 and 75 indicates that the score is in the range considered normal for these elements; and score above 75 indicates that the subject is very inclined to experience or express feelings of anger to a degree that may interfere with interpersonal relationships, leading him to predispose to develop physical and mental disorders.

3. Temperament and Character Inventory – TCI[20,25] was applied to describe personality characteristics through four temperament factors (novelty seeking - the behavior in response to new stimuli; harm avoidance - the inhibition of behavior in response

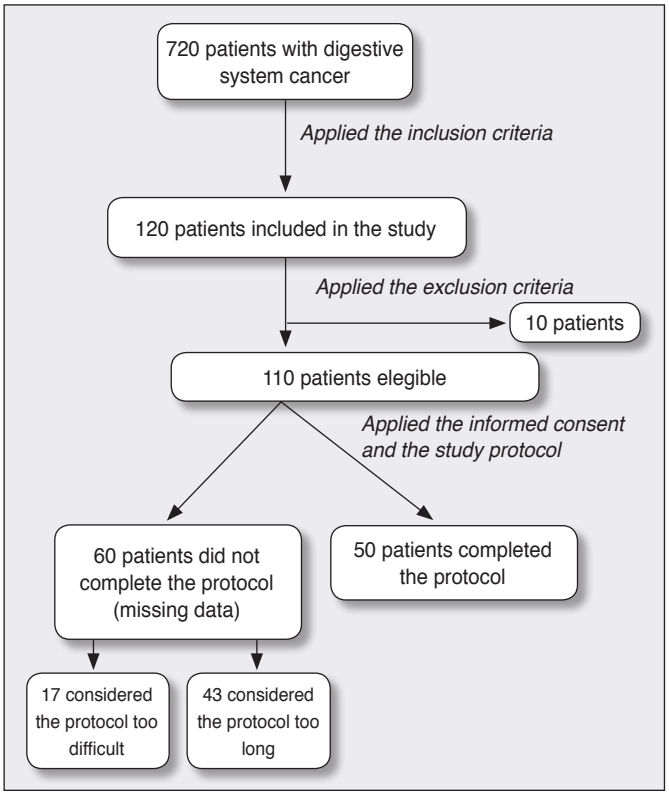

FIGURE 1. Sample description

to punishment; reward dependence – the need for rewards, and persistence – the ability to maintain behavior despite frustration or fatigue) and three of character (self-directedness – the autonomy of subject, defining his characteristics of responsibility, confidence and direction in relation to a goal; cooperativeness – the conception of the subject in relation to society, to other, and self- transcendence – the system of the individual's ideals in relation to the universe and to God). The confiability of the Brazilian version were satisfatory (alpha- Cronbach of 0.74 to novelty seeking, 0.81 to harm avoidance, 0,71 to reward dependency, 0.43 to persistence, 0.90 to self-directeness, 0.80 to cooperativeness, and 0.92 to self -transcendence)[20,25] . As in STAXI, the result is expressed in scores and categorized according to percentile; the lower or higher the score of each factor, the less or more representative Will be the presence of this factor, without flexibility, showing inadequacy in the modulation of the subject before the presented reality.

4. WHOQOL-Bref[19] that assesses the quality of life related to health in 4 domains: physical (how the subject deals with pain, energy, sleep, mobility, activities of daily living, dependence on medication/treatment and capacity to work), psychological (how the subject deals with positive and negative feelings, ability to think and learn, self-esteem and body image, and spirituality), social relationships (how the subject deals with personal relationships, social support and sexual activity) and environment (how the subject deals with their physical safety, home environment, financial resources, leisure and transport). Each domain has a result expressed in percentage: the higher the percentage, the better quality of life in that domain.

A descriptive analysis was performed through the calculation of absolute and relative frequencies of the variables, in addition to measures of central tendency (mean, median) and dispersion (standard deviation). To assess the relationship between the stable variables of personality (trace of anger, temperament and behavior), transient emotional states (state of anger, depressive and anxious symptoms) and domains of quality of life, we used Spearman correlation test, considering measures from 0.4 as strong correlation coefficient[1].

Finally, four multiple hierarchical regression method enter were performed to verify if each outcome variable (QOL domains: physical, psychological, environment and social relationship) was stable characteristics of the subject's personality (anger, temperament and character) after controlling to the transient emotional aspects (state of anger, depression and anxiety symptoms). To avoid spurious results, each model of regression had the independent and control variables defined by the statistical significant results of the Spearman correlation.

The significance level used in the analyzes was $P<0.05$ and the analysis was performed using Statistical Package for Social Sciences (SPSS) version 20.0. The study was approved by the ethics and research committees of FAMEMA (protocol 465/11) and *Universidade Federal de São Paulo* – UNIFESP (Protocol 1580/11).

RESULTS

Description of the sample and psychosocial variables

The patients were, on average, 56±11 years (33-74 years range); in a 16 to 180 days variation, cancer diagnosis time had an average of 114±46 days. Other clinical and demographics characteristics of newly diagnosed patients with digestive tract cancer are described in Table 1.

TABLE1. Clinical and demographics characteristics of newly diagnosed patients with digestive system cancer

Characteristics	N (%)
Gender: male	33 (66.0)
Live with a partner	38 (76.0)
Scholarship level: up to elementary school	21 (42.0)
Work situation: patient is retired or have a medical license	27 (54.0)
Infradiaphragmatic portion of the tumor in digestive tract	34 (68.0)
Stage degree of the tumor III or IV	38 (76.0)
Presence of comorbidities as hypertension and diabetes	14 (28.0)
Use of psychoactive substances (alcohol and tobacco)	9 (18.0)
Use of psychiatric medication (antidepressives and ansyolitics)	10 (20.0)

Table 2 showed the central tendency of the stable and transitional emotional characteristics of subjects' personality and the quality of life.

TABLE 2. Stable and transitional emotional characteristics of personality of newly diagnosed patients with digestive system cancer

	Median	Range
Stable characteristics of personality		
Chronic anger trait	17.0	10.0-40.0
Novelty seeking temperament	14.5	6.0-26.0
Harm avoidance temperament	15.0	4.0-26.0
Reward dependence temperament	13.0	4.0-23.0
Persistence temperament	5.0	1.0-8.0
Cooperativeness character	33.0	12.0-41.0
Self-transcendence character	21.5	7.0-32.0
Self-directedness character	32.0	14.0-44.0
Transient characteristics related to falling ill		
Anger state	10.0	10.0-29.0
Anxious symptoms	4.00	0-15
Depressive symptoms	0	0-16
Quality of life domains		
Physical health	61%	21%-79%
Psychological health	63%	17%-79%
Social relationships	67%	17%-92%
Environment	69%	34%-100%

Assessment of stable personality characteristics and transient emotional states related to falling ill

Table 3 shows the study of correlation between QOL domains and the personality characteristics and transient states of patients with newly diagnosed digestive system cancer. Although several correlations have shown to be statistically significant, the coefficient found showed a higher correlation than or equal to 0.40 only for some aspects related to psychological health and the environment. The patient's psychological health was correlated with two characteristics of stable personality traits, reward dependence tempera-

TABLE 3. Spearman's correlation coefficients between domains of quality of life and variables of anger, temperament and character, anxious and depressive symptoms

	Quality of life			
	Physical Health	**Psychological Health**	**Social Relationships**	**Environment**
Stable characteristics of personality				
Chronic anger trait	0.01	-0.26	-0.26	-0.33*
Novelty seeking temperament	0.07	0.12	-0.04	-0.05
Harm avoidance temperament	-0.22	-0.37**	-0.21	-0.44**
Reward dependence temperament	-0.07	0.51**	0.22	0.19
Persistence temperament	0.28*	0.16	0.12	0.24
Cooperativeness character	0.04	0.33*	0.35*	0.33*
Self-transcendence character	0.06	0.02	0.22	0.05
Self-directedness character	0.13	0.43**	0.23	0.45**
Transient characteristics related to falling ill				
Anger state	-0.31*	-0.22	-0.15	-0.40**
Anxious symptoms	-0.28*	-0.18	-0.06	-0.28
Depressive symptoms	-0.32*	-0.38**	-0.25	-0.29*

* $P<0.05$; ** $P<0.01$

ment and self-directedness character (r=0.51 and 0.43 respectively; $P<0.01$). QOL due to environment domain was correlated with two stable characteristics, harm avoidance temperament and self-directedness character (r=-0.44 and 0.45 respectively, $P<0.01$) and a transient characteristic and emerged from falling ill, the state of anger (r=-0.40, $P<0.01$). The presence of depressive symptoms correlated with almost all domains (except social relationships), although with coefficients lower than 0.4.

In order to assess which personality factors impact on QOL, according to the results already shown in Table 3, it was decided to build three blocks of linear regression models to explain QOL, one block with respect to psychological health and two blocks in relation to the environment. The depressive symptoms score was the control variable for QOL in both domains, and the environment was also controlled by the score state of anger in response to falling ill. Table 4 shows the results.

1. Both self-directedness character and reward dependence temperament are associated to quality of life related to psychological health of patients with newly diagnosed cancer of the digestive system, even after the control for depressive symptoms score, i.e., regardless of the patient presenting depressive symptoms or not, patients with higher score of self-directedness character had 42.2% more psychological health than patients with lower score. The same occurred to reward dependence (patients with higher score of reward recompense temperament had 32.3% more psychological health than patients with lower score). The variability of psychological health controlled by depression was correlated by self-directedness by 38% and by reward dependence by 31%.

2. Both self-directedness character and harm avoidance temperament are associated to the quality of life related to the environ-

TABLE 4. Predictive study of personality factors on the quality of life related to psychological health and environment, controlled for the presence of depressive symptoms and anger state

	Independent variable	**Standardized coefficients**		**95.0% CI for beta**	
		Beta	**Sig.**	**Lower bound**	**Upper bound**
Dependent variable: psychological health	SD score	.422	.001	.351	1.240
Control variable: depressive symptoms	RD score	.323	.010	.267	1.890
Dependent variable: environment	SD score	.349	.011	.156	1.142
Control variable: depressive symptoms	HA score	-.324	.018	-1.436	-.142
Dependent variable: environment	SD score	.415	.005	.244	1.301
Control variable: reactive anger state	HA score	-.389	.010	-1.660	-.233

SD: self-directedness; RD: reward dependence; HA: harm avoidance.

ment of the newly diagnosed patient, even after the control for the depressive symptoms score, i.e., regardless of the patient present depressive symptoms or not, patients with higher score of self-directedness had 34.9% more quality of life related to the environment than patients with lower score. The opposite occurred to harm avoidance: patients with higher score of harm avoidance had 32.4% less quality of life related to the environment than patients with lower score. The variability of the environment controlled by depression was related to self-directedness by 21% and harm avoidance by 20%.

3. Both self-directedness character as harm avoidance temperament are associated to quality of life related to the environment of the newly diagnosed patient, even after controlling for state of anger score in response to falling ill, i.e., regardless of the patient to be angry or not, patients with higher score of self-directedness had 41.2% more quality of life related to the environment than patients with lower score. The opposite occurred to harm avoidance: patients with higher score of harm avoidance had 38.9% less quality of life related to the environment than patients with lower score. The variability of environment controlled by anger was related to self-directedness by 14% and by harm avoidance by 12%.

DISCUSSION

Patients diagnosed with cancer of the digestive system for less than 6 months showed personality traits that favored, and others that have harmed their quality of life, regardless of the emotions triggered by falling ill. Besides the impact that personality aspects have on the psychological health of the patient, it was realized that the quality of life related to environment was one aspect that emerged as susceptible at the time of the disease.

Environment has been an important aspect in quality of life of cancer patients in spite of the length of the disease. Comparing 309 colorectal cancer patients in Germany with 15 months of diagnostic and general population at same demographic characteristics, Arndt et al.[2] identified a similar quality of life between groups but financial aspects were more impaired with the disease. The environment quality of life domain relates to how the subject deals with their physical safety, home environment, financial resources, leisure and transportation[19]; it is then learned that this domain explores the impact that a critical situation in life has on the day-to-day life of the individual and his need to have access to medical treatment. In the first six months of a serious disease, the impact of the disease affects the individual in many ways, but even considering patients in more advanced stages of digestive system cancer, the need for reorganization his daily life is reflected in the change of the environment.

People with a predominance of self-directedness character, that is, with autonomy and greater appropriation of their responsibilities, with confidence and able to drive toward a goal (treatment), felt with more quality of life to deal with new routine. It is important to note that this capability was not impaired by feelings of depression or anger at the disease. The self-directedness character was also a determining factor for the psychological health of the patient, i.e., feeling autonomous helped the patient to deal with their positive and negative feelings, and felt self-esteem, regardless of the presence of depressive symptoms. These feelings related to self-esteem can be found as characteristics of personality[27]. Besides feelings, other aspects can be affected by cancer, as the social field of interpersonal relationships (spouses, family, friends), labor and productive life[2,15]. Experiencing the process of being ill with cancer may lead to consequences or even interrupt the routine, directly impacting their quality of life.

Quality of life related to the environment was impaired in patients with harm avoidance temperament, i.e., patients that inhibit certain behaviors after feeling punished by something or someone, independent of depressive symptoms or anger in the face of the disease. These patients show signs of negative feelings, insecurity and fear of threatening and aversive situations[13]. Thus, when experiencing the disease and treatment, this type of patient attempts to distance himself from the problem, and tends to minimize symptoms for a longer period of time than others[13].

Another aspect found in our study was the state of anger present at the moment of treating the disease, which showed relatively low score. Jukulsen et al.[23] studied the role of anger in 153 patients with cancer in the first 8 months of diagnostic, prospectively. They showed that low scores of anger is normal but more important is the quality of anger that the patient expresses; which means, anger expression and anger control are positive to improve quality of life, and anger inhibition decreases it. Other study with 637 colorectal cancer patients showed that denial or repression of anger in patient's life story is a mark to this kind of cancer[24].

From a clinical point of view, even with low score of anger state, the objective of studying was attended: the role of stable characteristics personality in these patients is relevant to some aspects of the quality of life of the newly diagnosed patient with digestive tract cancer. This is a problem because these characteristics are more difficult to be modified. The health professional who attend these patients need to understand their difficulty to deal with the diagnostic. Chao[11] suggests more attention to the psychosocial needs of patients at the beginning of the adaptation process in order to strengthen them and support them emotionally for the return of their daily activities more quickly. It is understood that, as it is a chronic disease, patients need to live with their clinical situation according to their emotional possibilities[4]. Staying autonomous can foster greater involvement and responsibility with their health during treatment in this new life stage[36]. Due to the impairment caused by the disease and its stigma, it is still observed that this can mean deprivation of everyday sociability, segregation, and disruption of daily, occupational activities, and the absence from work. Thus, the access to goods and services are made more difficult[28,30]. During and after treatment, it is noteworthy, there may be possible loss of muscle mass causing functional impairment to the patient[16].

Furthermore, treatment with chemotherapy, surgery and radiation therapy cause loss of strength and damage to normal tissues, and therefore there may be a loss of autonomy[8,26]. These changes bring psychological distress and the change in daily activities may be slowed with certain actions by the family and multidisciplinary team who serves them. This patient can benefit when he realizes that social support and emotional and physical support can help him change his habits improving his health[13,18]. This type of care interferes positively in coping mechanisms, which provides autonomy by clarifying and promoting his quality of life according to his living conditions[21].

Some limitations of the study must be considered. Although the sample size was adequate to our purpose, it was collected by convenience and not randomically. Fifty-five percent of the eligible patients refused to participate in the study and it was no possible

to control the missing data. The instruments together were too long to these patients what was the main reason to they decline to participate in the study. Other point to be considered is the low symptomology of depression and presence of anger state, what can be explained by three reasons: in fact a low presence of symptoms; the use of antidepressants what could minimize the symptoms; or a failure to collect data from who was more depressive or anger. Finally, the cross-sectional design does not allow more comprehension about the impact of the diagnostic on QOL (in the future more data will be present; this paper is a preliminary report of a longitudinal study with these patients)."

CONCLUSION

In this study there was a predominance of male patients with digestive tract cancer in infradiaphragmatic portion; they had diagnostic time of 4 months; presented factors as reward dependence and self-directedness character that favoring a better quality of life related to psychological health. This psychological well-being was reinforced by the self-directedness character; which means that patients more autonomous cope better with the disease. The adaptive needs to the environment that favoring a better QOL were also reinforced by the self-directedness character. On the other hand, the harm avoidance temperament (meaning the patient has fear of aversive situations) impaired the adaptive capacity to deal with the changes of the day-to-day imposed by the disease. Understanding these personality traits is important to the health professionals drive the patient to more successful treatment using strategies that help the patient to adhere well to the treatment.

Authors' contributions

Honorato NP: developed the research design, collected data and wrote the paper. Abumusse LVM: participated in discussion section. Coqueiro DP: participated in discussion section, statistical analysis, table elaboration. Citero VA: developed the research design and wrote the paper, statistical analysis, table elaboration.

Honorato NP, Abumusse LVM, Coqueiro DP, Citero VA. Traços de personalidade, raiva e sintomas psiquiátricos relacionados à qualidade de vida em pacientes com câncer do sistema digestório recém-diagnosticado. Arq Gastroenterol. 2017;54(2):156-62.

RESUMO – **Contexto –** A presença de sintomas psiquiátricos, raiva, e características de personalidade são fatores que interferem na qualidade de vida do paciente com câncer do sistema digestório recém-diagnosticado. **Objetivo –** Este estudo objetiva identificar que características estáveis da personalidade interferem na qualidade de vida, mesmo quando controladas as características emocionais reativas ao adoecer. **Métodos** – Um estudo transversal foi realizado na Clínica de Oncologia (Hospital das Clínicas), Marília/SP Brasil, no qual foram avaliados 50 pacientes adultos com câncer digestivo diagnosticados há menos de 6 meses que responderam ao Inventário de Expressão de Raiva Traço-Estado, Inventário de Temperamento e Caráter, Escala Hospitalar de Ansiedade e Depressão e WHOQOL-BREF. Regressão múltipla foi aplicada para verificar se a qualidade de vida estava relacionada com as características estáveis de personalidade (traço de raiva, temperamento, caráter) após controlar os resultados para a presença de aspectos emocionais transitórios (estado de raiva, sintomas psiquiátricos). **Resultados –** O escore de saúde psicológica da qualidade de vida foi maior na presença de caráter de autodirecionamento e do temperamento de dependência de gratificação; o escore de meio-ambiente da qualidade de vida foi maior na presença de caráter de autodirecionamento, e menor na presença de temperamento de evitação ao dano. **Conclusão** – O bem-estar psicológico e as necessidades adaptativas ao meio-ambiente que favorecem uma melhor qualidade de vida foram reforçados principalmente pelo caráter de autodirecionamento. Por outro lado, o temperamento de evitação ao dano prejudica a capacidade adaptativa de lidar com as mudanças diárias, impostas pela doença. Compreender estes traços de personalidade é importante para que os profissionais de saúde conduzam o paciente por um tratamento de maior sucesso.

DESCRITORES – Neoplasias do sistema digestório. Personalidade. Ansiedade. Depressão. Qualidade de vida.

REFERENCES

1. Altman DG. Practical statistics for medical research. Ed. Chapman & Hall/CRC. London, 1991, pg. 295.
2. Arndt V, Merx H, Stegmaier C, Ziegler H, Brenner H. Quality of life in patients with colorectal cancer 1 year after diagnosis compared with the general population: a population-based study. J Clin Oncol. 2004;22:4829-36.
3. Bausewein C. [Thinking from the end - Does the diagnosis "cancer" make a difference to patients and carers?]. Z Evid Fortbild Qual Gesundhwes. 2013;107:136-9.
4. Bertan FC, Castro EK. [Quality of life, anxiety and depressions indicators and sexual satisfaction in adult patients with cancer]. Rev Salud Soc. 2010;1:76-88.
5. Biaggio AMB. Ansiedade, raiva e depressão na concepção de C. D. Spielberger [Anxiety, Angerand Depression According to the Concept of C.D. Spielberger]. Ver Psiquiatr Clín. 1999;25:291-3.
6. Bonacchi A, Miccinesi G, Guazzini M, Rossi A, Bacci S, Toccafondi A, et al. Temperament and character traits associated with health-related quality of life in cancer patients. Tumori. 2012;98:377-84.
7. Botega NJ, Bio MR, Zomignani MA, Garcia Jr C, Pereira WA. [Mood disorders among medical in-patients: a validation study of the hospital anxiety and depression scale (HAD)]. Rev Saúde Pública. 1995;29:355-63.
8. Bovio G, Bettaglio R, Bonetti G, Miotti D, Verni P. [Autonomy of the elderly patient with cancer: the right to know the diagnosis]. Acta Paul Enferm. 2007;20:509-13.
9. Braz DS, Ribas MM, Dedivitis RA, Nishimoto IN, Barros APB. Quality of life and depression in patients undergoing total and partial laryngectomy. Clinics (Sao Paulo). 2005;60:135-42.
10. Brucki SMD, Nitrini R, Caramelli P, Bertolucci PHF, Okamoto IH. [Suggestions for Mini Mental State Exam in Brazil]. Arq Neuropsiquiatr. 2003;61:777-81.
11. Chao HL, Tsai TY, Livneh H, Lee HC, Hsieh PC. Patients with colorectal cancer: relationship between demographic and disease characteristics and acceptance of disability. J Adv Nurs. 2010;66:2278-86.
12. Cheng C, Chan NY, Chio JH, Chan P, Chan AO, Hui WM. Being active or flexible? Role of control coping on quality of life among patients with gastrointestinal cancer. Psychooncology. 2012;21:211-8.
13. Cloninger CR. A systematic method for clinical description and classification of personality variants: a proposal. Arch Gen Psychiatry. 1987;44:573-88.
14. Costanzo ES, Sood AK, Lutgendorf SK. Biobehavioral influences on cancer progression. Immunol Allergy Clin North Am. 2011;31:109-32.
15. Engel J, Kerr J, Schlesinger-Raab A, Eckel R, Sauer H, Hölzel D. Quality of life in rectal cancer patients: a four-year prospective study. Ann Surg. 2003;238:203-13.
16. Fearon K, Strasser F, Anker SD, Bosaeus I, Bruera E, Fainsinger RL, et al. Definition and classification of cancer cachexia: an international consensus. Lancet Oncol. 2011;12:489-95.

17. Felder-Puig R, Formann AK, Mildner A, Bretschneider W, Bucher B, Windhager R, et al. Quality of life and psychosocial adjustment of young patients after treatment of bone cancer. Cancer. 1998;83:69-75.
18. Finfgeld-Connett D. Clarification of social support. J Nurs Scholarsh. 2005;37:4-9.
19. Fleck MPA, Louzada S, Xavier M, Chachamovich E, Vieira G, Santos L, Pinzon V. [Application of the Portuguese version of the abbreviated instrument of quality life WHOQOL-bref]. Rev Saúde Pública. 2000;34:178-83.
20. Fuentes D, Tavares H, Camargo CHP, et al. In: Gorenstein C, Andrade LHSG, Zuardi AW. [Clinical Assessment Scales in Psychiatry and Psychopharmacology]. São Paulo: Lemos Editorial; 2000. p. 363-76.
21. Gozzo TO, Panobianco MS, Clapis MJ, de Almeida, AM. [Dermatological Toxicity in Women With Breast Cancer Undergoing Chemotherapy Treatment]. Rev Latinoam Enferm. 2010;18:681-7.
22. Graeff A, Leeuw JR, Ros WJ, Hordijk GJ, Blijham GH, Winnubst JA. Pretreatment factors predicting quality of life after treatment for head and neck cancer. Head Neck. 2000;22:398-407.
23. Julkunen J, Gustavsson-Lilius M, Hietanen P. Anger expression, partner support, and quality of life in cancer patients. J Psychosom Res. 2009;66:235-44.
24. Kune GA, Kune S, Watson LF, Bahnson CB. Personality as a risk factor in large bowel cancer: data from the Melbourne Colorectal Cancer Study. Psychol Med. 1991;21:29-41.
25. Moreira DF. [Pathological gambling: neuroimaging, neuropsychology and personality analyses] [dissertation]. São Paulo: Universidade de São Paulo; 2005. 143 p.
26. Muscaritoli M, Anker SD, Argilés J, Aversa Z, Bauer JM, Biolo G, et al. Consensus definition of sarcopenia, cachexia and pre-cachexia: joint document elaborated by Special Interest Groups (SIG) "cachexia anorexia in chronic wasting diseases" and "nutrition in geriatrics". Clin Nutr. 2010;29:154-9.
27. Myers DG, Diener E. Who is happy? Psychol Sci. 1995;6:10-9.
28. Pedro ICS, Rocha SMM, Nascimento LC. [Social support and social network in family nursing: reviewing concepts]. Rev Latinoam Enferm. 2008;16:324-7.
29. Pinto RN, Chaves AC, Lourenço MT, Mari J de J. Information needs of needs of recently diagnosed cancer patients in Brazil. Information needs of recently diagnosed cancer patients in Brazil. Int J Psychiatry Med. 2004;34:319-29.
30. Rodrigues JSM, Ferreira NMLA. [Structure and functionality of the social support network for adults with cancer]. Acta Paul Enferm. 2012;25:781-7.
31. Scharloo M, Baatenburg de Jong RJ, Langeveld TP, van Velzen-Verkaik E, Doornop den Akker MM, Kaptein AA. Quality of life and illness perceptions in patients with recently diagnosed head and neck cancer. Head Neck. 2005;27:857-63.
32. Shun SC, Hsiao FH, Lai YH, Liang JT, Yeh KH, Huang J. Personality trait and quality of life in colorectal cancer survivors. Oncol Nurs Forum. 2011;38:E221-8.
33. Sun DZ, Liu L, Jiao JP, Wei PK, Jiang LD, Xu L. Syndrome characteristics of traditional Chinese medicine: summary of a clinical survey in 767 patients with gastric cancer. Zhong Xi Yi Jie He Xue Bao. 2010;8:332-40.
34. Svedlund J, Sullivan M, Liedman B, Lundell L. Relationship of tumor burden and patients' minimization of distress in facing surgery for gastric cancer. Psychosomatics. 2005;46:233-43.
35. Thomas SP, Groer M, Davis M, Droppleman P, Mozingo J, Pierce M. Anger and cancer: an analysis of the linkages. Cancer Nurs. 2000;23:344-9.
36. Visentin A, Labronici L, Lenardt MH. Autonomia do paciente idoso com câncer: o direito de saber o diagnóstico. Acta Paul Enferm. 2007;20:509-13.
37. White VM, English DR, Coates H, Lagerlund M, Borland R, Giles GG. Is cancer risk associated with anger control and negative affect? Findings from a prospective cohort study. Psychosom Med. 2007;69:667-74.
38. World Health Organization. Cancer [Internet]. Geneva: WHO; 2016. [cited 2016 Jan 25]. Available from: http://www.who.int/cancer/en/
39. World Health Organization. Cancer [Internet]. Geneva: WHO; 2015. [cited 2016 Jan 25]. Available from: http://www.who.int/mediacentre/factsheets/fs297/en/
40. Yamaoka K, Shigehisa T, Ogoshi K, Haruyama K, Watanabe M, Hayashi F, Hayashi C. Health-related quality of life varies with personality types: a comparison among cancer patients, non-cancer patients and healthy individuals in a Japanese population. Qual Life Res. 1998;7:535-44.
41. Yan H, Sellick K. Quality of life of Chinese patients newly diagnosed with gastrointestinal cancer: a longitudinal study. Int J Nurs Stud. 2004;41:309-19.
42. Zhou FL, Zhang WG, Wei YC, Xu KL, Hui LY, Wang XS, Li MZ. Impact of comorbid anxiety and depression on quality of life and cellular immunity changes in patients with digestive tract cancers. World J Gastroenterol. 2005;11:2313-8.

ORIGINAL ARTICLE

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Assessing the sleep quality and depression-anxiety-stress in irritable bowel syndrome patients

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ABSTRACT – Background – Irritable bowel syndrome (IBS) is one of the most common functional gastrointestinal disorders with chronic abdominal pain and altered bowel habit without any organic reason. Sleep disorders may be associated to IBS. **Objective** – We aimed to assess sleep disturbances and depression-anxiety-stress in IBS patients. **Methods** – In this analytical cross sectional study from November 2013 to May 2014, A total of 123 IBS patients were recruited by simple random sampling. IBS was diagnosed using ROME-III criteria. Demographic and basic data were driven from all patients then Pittsburg Sleep Quality Index questionnaire was utilized to estimate sleep quality and DASS (depression anxiety stress scale) questionnaire was filled out for depression, anxiety and stress. **Results** – The mean age of patients was 29±9, where 48 cases (39%) were male. Twelve cases (10%) had a background disease. Types of IBS in patients were included 38% diarrhea, 42% constipation and 20% mixed. From all IBS patients 87 (71%) cases had depression, 97 (79%) patients stress, 94 (76%) patients had anxiety. Seventy-six (62%) cases of IBS patients had poor sleep quality. Simultaneously employing predictors demonstrate that gender, background disease, and type of IBS did not statistically significant. On the other hand, depression (P=0.034, OR=2.35), anxiety (P=0.011, OR=3.022), and stress (P=0.029, OR=2.77) were significantly effect on sleep quality in poor sleepers. **Conclusion –** Many of IBS patients is suffering from poor sleep quality. It seems that sleep disorder should be considered and treated in this patients.

HEADINGS – Irritable bowel syndrome. Sleep wake disorders. Depression. Anxiety.

INTRODUCTION

Psychological disorders have an important role in many diseases like irritable bowel syndrome (IBS), widely involving individuals with any ethnicity and gender (5%-20% of general populations)[2,30]. It is often associated with other disorders, hence, the patient may have to undergo expensive tests and treatments, as a result the disease also compels burden to societies. On the other hand, IBS influence on patients' quality of life[7,17,23,28,31].

IBS is one of the most functional gastrointestinal disorders manifesting with cramping abdominal pain, discomfort and also defecation disorders (constipation and/or diarrhea)15[15]. Onset of IBS is more likely to occur as a post infection disease or stressful life event, but other etiological factors are still unknown[6,29].

Sleep disturbances is a common complaint in IBS patients causing some psychological disorders including neurosis, anxiety, and depression that are more prevalent in IBS patients correlated with sleep quality[3,4,8,14,16,18,26,28,]. High prevalence of sleep disturbances has been observed among IBS patients[9,24,27,32], a remarkable increase in rapid-eye-movement (REM) sleep is the only unusual pattern demonstrated in the patients[11,25].

Assessing sleep quality associated with the mentioned psychological disorders in IBS patients has been investigated in few studies[20,24], but the results are controversial whether poor sleep quality related to the underlying mood disturbances in IBS patients[5,10]. The present study discuss existence of the association in the patients using the Pittsburgh Sleep Quality Index (PSQI)[19] and depression anxiety stress scale (DASS)[1] which Persian versions has been adapted and confirmed. Moreover, the severity of IBS, the obtained scores, and demographic features are also analyzed to explore interactions.

METHODS

A cross sectional study was carried out between November 2013 to May 2014. A total of 123 proved IBS patients (Rome III criteria) were recruited by convenience sampling from an academic hospital clinic and two private clinics. During the time of study all IBS patients were asked to fill DASS and PSQI Questionnaires, having taken informed consent.

Two trained nurses interviewed the participants about demographic features and explain instruction of the questionnaires. Then, depression, anxiety, stress and sleep quality information was collect via the self-administered questionnaire of DASS and PSQI.

PSQI has 19 questions and seven components including sleep quality, sleep latency, sleep duration, habitual sleep efficiency, and

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sleep disturbances, use of sleep medication, and daytime dysfunction over the last months. Poor sleep quality defined as score less than 5 globally while the total score is 21. PSQI was translated to Persian and it was reported suitable questioner in Iranian population with acceptable validity and reliability[3,19]. The other one, DASS, composed of 42 questions to evaluate depression, anxiety and stress[1].

Patients those had gastrointestinal symptoms were examined by a gastroenterologist. Proven diagnosis of IBS using ROME-III criteria was necessary. Patients with comorbidity of a psychiatric disorder or organic bowel disease and incomplete questionnaires were excluded.

Statistical analysis

Univariate logistic regression applied for evaluating the association between independent variables and sleep quality. Multivariate logistic regression was used to evaluate simultaneous effect of these variables on sleep quality. In another analysis depression, anxiety and stress divided into four severity grades to compare groups. *P*-value 0.05 was considered as statistical significance.

RESULTS

One hundred and twenty-three IBS patients were assessed. The mean age was 29.7±9.8 and 39% was male. About 10% of cases had a background disease. Moreover, about 38% of patients were constipation dominant, 42.3% were diarrhea dominant and 19.5% suffered from mixed type (constipation-diarrhea). The majority of IBS patients had depression anxiety and stress (Table 1). Classification of DASS scores by its guideline showed that of 123 IBS patients, 70.72% had depression, 75.63% had anxiety, and 78.86% had stress.

TABLE 1. Demographic characteristics

Characteristic	Frequency n(%)
Gender	
Male	48 (39.0%)
Female	75 (61.0%)
Background disease	
Yes	12 (9.8%)
No	111 (90.2%)
IBS type	
Constipation	47 (38.2%)
Diarrhea	52 (42.3%)
Mixed	24 (19.5%)
Sleep quality	
Poor	28 (22.8%)
Good	95 (77.2%)
Depression	
Yes	87 (70.7%)
No	36 (29.3%)
Anxiety	
Yes	94 (76.4%)
No	29 (23.6%)
Stress	
Yes	97 (78.9%)
No	26 (21.1%)

IBS: irritable bowel syndrome

Moreover, severity of depression, anxiety, and stress in poor sleepers was extremely more than good sleepers (Table 2, Figure 1-3). Totally, seventy-six cases (61.8%) of IBS patients had poor sleep quality.

TABLE 2. Severity of depression, anxiety and stress irritable bowel syndrome patients

Psychological condition	Frequency n (%)
Depression (123 persons)	
Normal	36 (29.3%)
Mild	16 (13.0%)
Moderate	27 (21.9%)
Severe	21 (17.1%)
Very severe	23 (18.7%)
Anxiety (119)	
Normal	29 (24.4%)
Mild	12 (10.1%)
Moderate	17 (14.3%)
Severe	22 (18.5%)
Very severe	39 (32.7%)
Stress (123)	
Normal	26 (21.1%)
Mild	13 (10.6%)
Moderate	21 (17.1%)
Severe	40 (32.5%)
Very severe	23 (18.7%)

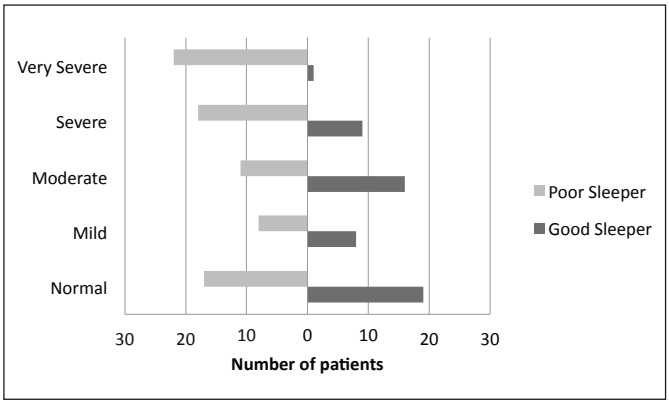

FIGURE 1. Severity of depression by quality of sleep

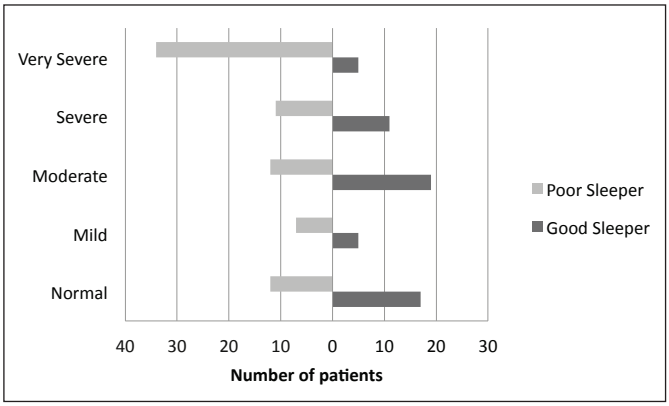

FIGURE 2. Severity of Anxiety by quality of sleep

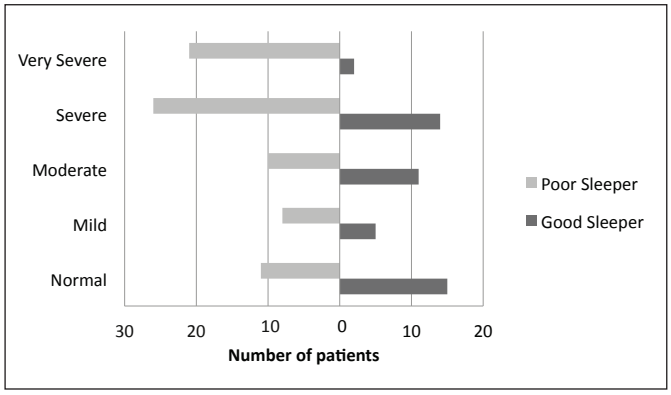

FIGURE 3. Severity of stress by quality of sleep

The sleep quality score in our patients ranged from 1 to 17 with the mean of 7.2±3.5. Twenty-eight (22.7%) were poor sleepers. There was no correlation between PSQI and type of IBS (r=0.038, *P*=0.674).

Simultaneously employing predictors demonstrate that gender, background disease, and type of IBS did not statistically significant. On the other hand, depression (*P*=0.034, OR=2.35), anxiety (*P*=0.011, OR=3.022), and stress (*P*=0.029, OR=2.77) were significantly effect on sleep quality in poor sleepers.

DISCUSSION

Along with the aim of the study to assess sleep quality by utilizing depression, anxiety, and stress in IBS patients, majority of IBS patients suffered from poor sleep quality which fairly associated with the psychological conditions.

Poor sleep quality has been demonstrated in IBS patients; a Turkish studies has indicated that 36% of IBS patients were poor sleepers in compare with healthy volunteers (18%) using PSQI[33]. However, the present study does not compare IBS patients with other groups, our participants were also 61.8% poor sleepers.

In Iranian population, the prevalence of anxiety and depression has been estimated, 35% and 16%, respectively, using general health questionnaire (GHQ-28)[18]. Another study estimated the prevalence of anxiety and depression in a local Iranian population were 20.8 and 21%[22]. Frequency of mood and anxiety disorders in IBS patients evaluated in several studies and Frequency of 5%-15% for depression and 16%-19% for Anxiety have been reported[12,21]. These values of depression and anxiety are significantly more than general population[2,12,13]. While, using PSQI in IBS patients, the present study, have been dramatically calculated depression (71%), anxiety (76%), and stress (79%). Discrepancy might be because of type of questionnaire and data collection but we suggest doing more studies about this subject.

Direct association of IBS and emotional disorders such as anxiety, depression, and stress causing sleep disturbances has been a controversial issue, some studies have proved that sleep disturbances was totally independent from the bowel problems especially IBS[32]. Here, the results were confirmed the independency of IBS and sleep quality.

This study has some limitations. First, we didn't have control group. Second, we didn't investigate objective sleep quality because there is no sleep lab in our university.

CONCLUSION

Our results confirm few previous studies and indicated that IBS patients have poor sleep quality and it is independent from the other factors. So we must consider and treat this problem in IBS patients. And also we must pay attention to depression more than the other psychological disorders in these patients because it can worsen sleep quality.

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Authors' contributions

Baniasadi N and Dehesh MM suggested the initial conception, and gathered the information with the help of Hayatbakhsh Abbasi M and Oghabian Z. Mohebbi E did the data analysis and interpretation. Dehesh MM wrote the manuscript and then all the authors edited and confirmed the final version.

Baniasadi N, Dehesh MM, Mohebbi E, Hayatbakhsh Abbasi M, Oghabian Z. Avaliação da qualidade do sono e de depressão, ansiedade e estresse em pacientes com síndrome do intestino irritável. Arq Gastroenterol. 2017;54(2):163-6.

RESUMO – Contexto – A síndrome do intestino irritável (SII) é um dos transtornos gastrointestinais funcionais mais comuns, com dor abdominal crônica e alteração do hábito intestinal sem motivo orgânico aparente. Distúrbios do sono podem estar associados à SII. **Objetivo** – Avaliar distúrbios do sono e sinais de depressão, ansiedade e estresse em pacientes com SII. **Métodos** – Através de estudo analítico transversal, observou-se entre de novembro de 2013 e maio 2014, um total de 123 pacientes com SII, recrutados por amostragem aleatória simples. A SII foi diagnosticada usando-se os critérios de Roma III. Dados demográficos e básicos foram obtidos de todos os pacientes e o questionário de índice de qualidade de sono de Pittsburg foi utilizado para estimar a qualidade do sono; o questionário DASS (escala de depressão ansiedade stress) foi preenchido para depressão, ansiedade e stress. **Resultados** – A média de idade dos pacientes foi de 29±9 anos, sendo 48 (39%) do sexo masculino. Doze (10%) tinham alguma doença associada. Nos subtipos de SII foram incluídos 38% com diarreia, 42% com constipação e 20% de forma alternada. Do total, 87 (71%), pacientes tinham depressão, o estresse foi observado em 97 (79%) e 94 (76%) tinham ansiedade. Setenta e seis (62%) pacientes com SII tinham sono de má qualidade. A análise de preditores, empregados simultaneamente, demonstrou que o gênero, a doença associada e o tipo de SII não foram estatisticamente significantes. Por outro lado, depressão (*P*=0,034, OR=2,35), ansiedade (*P*=0.011, OR=3.022) e stress (*P*=0,029, OR=2,77) contribuíram significativamente no efeito da má qualidade do sono. **Conclusão** – A maioria dos pacientes com SII tem sono de má qualidade. Recomenda-se que o distúrbio do sono deva ser considerado e tratado nestes pacientes.

DESCRITORES – Síndrome do intestino irritável. Transtornos do sono-vigília. Depressão. Ansiedade.

REFERENCES

1. Asgharimoghadam MA SF, Dibajnia P, Zanganeh J. Preliminary assessment of realibility and validity depression, anxiety, and stress scale (DASS) in nonclinical samples. Daneshvar Medical Journal. 2008;15:23-8.
2. Bellini M, Tosetti C, Costa F, Biagi S, Stasi C, Del Punta A, et al. The general practitioner's approach to irritable bowel syndrome: from intention to practice. Dig Liver Dis. 2005;37:934-9.
3. Benca RM, Okawa M, Uchiyama M, Ozaki S, Nakajima T, Shibui K, Obermeyer WH. Sleep and mood disorders. Sleep Med Rev. 1997;1:45-56.
4. Budavari AI, Olden KW. Psychosocial aspects of functional gastrointestinal disorders. Gastroenterol Clin North Am. 2003;32:477-506.
5. Buysse DJ, Reynolds CF, Monk TH, Berman SR, Kupfer DJ. The Pittsburgh Sleep Quality Index: a new instrument for psychiatric practice and research. Psychiatry Res. 1989;28:193-213.
6. Chang L. The role of stress on physiologic responses and clinical symptoms in irritable bowel syndrome. Gastroenterology 2011;140:761-5. e5.
7. Creed F. The relationship between psychosocial parameters and outcome in irritable bowel syndrome. Am J Med. 1999;107:74-80.
8. Cremonini F, Camilleri M, Zinsmeister AR, Herrick LM, Beebe T, Talley NJ. Sleep disturbances are linked to both upper and lower gastrointestinal symptoms in the general population. Neurogastroenterol Motil. 2009;21:128-35.
9. Elsenbruch S, Thompson JJ, Harnish MJ, Exton MS, Orr WC. Behavioral and physiological sleep characteristics in women with irritable bowel syndrome. Am J Gastroenterol. 2002;97:2306-14.
10. Francis C, Morris J, Whorwell P. The irritable bowel severity scoring system: a simple method of monitoring irritable bowel syndrome and its progress. Aliment Pharmacol Ther. 1997;11:395-402.
11. Heitkemper M, Jarrett M, Burr R, Cain KC, Landis C, Lentz M, Poppe A. Subjective and objective sleep indices in women with irritable bowel syndrome. Neurogastroenterol Motil. 2005;17:523-30.
12. Janssens KA, Zijlema WL, Joustra ML, Rosmalen JG. Mood and anxiety disorders in chronic fatigue syndrome, fibromyalgia, and irritable bowel syndrome: results from the LifeLines Cohort Study. Psychosom Med. 2015;77:449-57.
13. Kanuri N, Cassell B, Bruce S, White KS, Gott BM, Gyawali CP, Sayuk GS. The impact of abuse and mood on bowel symptoms and health-related quality of life in irritable bowel syndrome (IBS). Neurogastroenterol Motil. 2016;28:1508-17.
14. Levy RL, Olden KW, Naliboff BD, Bradley LA, Francisconi C, Drossman DA, Creed F. Psychosocial aspects of the functional gastrointestinal disorders. Gastroenterology 2006;130:1447-58.
15. Longstreth GF, Thompson WG, Chey WD, Houghton LA, Mearin F, Spiller RC. Functional bowel disorders. Gastroenterology. 2006;130:1480-1491.
16. Luscombe FA. Health-related quality of life and associated psychosocial factors in irritable bowel syndrome: a review. Qual Life Res. 2000;9:161-76.
17. Maxion-Bergemann S, Thielecke F, Abel F, Bergemann R. Costs of irritable bowel syndrome in the UK and US. Pharmacoeconomics. 2006;24:21-37.
18. Modabbernia M-J, Mansour-Ghanaei F, Imani A, Mirsafa-Moghaddam SA, Sedigh-Rahimabadi M, Yousefi-Mashhour M, et al. Anxiety-depressive disorders among irritable bowel syndrome patients in Guilan, Iran. BMC Res Notes. 2012;5:112.
19. Moghaddam JF, Nakhaee N, Sheibani V, Garrusi B, Amirkafi A. Reliability and validity of the Persian version of the Pittsburgh Sleep Quality Index (PSQI-P). Sleep Breath. 2012;16:79-82.
20. Morin CM, Gibson D, Wade J. Self-reported sleep and mood disturbance in chronic pain patients. Clin J Pain. 1998;14:311-4.
21. Mykletun A, Jacka F, Williams L, Pasco J, Henry M, Nicholson GC, Kotowicz MA, Berk M. Prevalence of mood and anxiety disorder in self reported irritable bowel syndrome (IBS). An epidemiological population based study of women. BMC Gastroenterol. 2010;10:88.
22. Noorbala A, Yazdi SB, Yasamy M, Mohammad K. Mental health survey of the adult population in Iran. Br J Psychiatry. 2004;184:70-3.
23. Nyrop K, Palsson O, Levy R, Von Korff M, Feld AD, Turner MJ, Whitehead WE. Costs of health care for irritable bowel syndrome, chronic constipation, functional diarrhoea and functional abdominal pain. Aliment Pharmacol Ther. 2007;26:237-48.
24. Okami Y, Kato T, Nin G, Harada K, Aoi W, Wada S, et al. Lifestyle and psychological factors related to irritable bowel syndrome in nursing and medical school students. J Gastroenterol. 2011;46:1403-10.
25. Orr WC. Gastrointestinal functioning during sleep: a new horizon in sleep medicine. Sleep Med Rev. 2001;5:91-101.
26. Robert JJ, Elsenbruch S, Orr WC. Sleep-related autonomic disturbances in symptom subgroups of women with irritable bowel syndrome. Dig Dis Sci. 2006;51:2121-7.
27. Rotem AY, Sperber AD, Krugliak P, Freidman B, Tal A, Tarasiuk A. Polysomnographic and actigraphic evidence of sleep fragmentation in patients with irritable bowel syndrome. Sleep. 2003;26:747-52.
28. Sperber AD, Tarasiuk A. Disrupted sleep in patients with IBS—a wake-up call for further research? Nat Clin Pract Gastroenterol Hepatol. 2007;4:412-3.
29. Spiller R, Garsed K. Postinfectious irritable bowel syndrome. Gastroenterology. 2009;136:1979-88.
30. Videlock EJ, Chang L. Irritable bowel syndrome: current approach to symptoms, evaluation, and treatment. Gastroenterol Clin North Am. 2007;36:665-85.
31. Whitehead WE, Palsson OS, Levy RR, Feld AD, Turner M, Von Korff M. Comorbidity in irritable bowel syndrome. Am J Gastroenterol. 2007;102:2767-76.
32. Wu J, Song Z, Xu Y, Zhang YM, Shen RH. [Probe into sleep quality in the patients with irritable bowel syndrome]. Zhonghua Nei Ke Za Zhi. 2010;49:587-90.
33. Yıldırım O, Alçelik A, Canan F, Aktaş G, Sit M, İşçi A, Yalçın A, Yılmaz EE. Impaired subjective sleep quality in irritable bowel syndrome patients with a Type D personality. Sleep Biol Rhythms. 2013;11:135-8.

REVIEW

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Efficacy of tacrolimus for induction of remission in patients with moderate-to-severe ulcerative colitis: a systematic review and meta-analysis

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ABSTRACT – Background – There is evidence that shows that calcineurin inhibitors may be useful for the treatment of severe ulcerative colitis. However, evidence regarding the efficacy of tacrolimus for remission induction in this setting is scarce. **Objective** – To develop a systematic review on the existing evidence regarding the clinical efficacy of tacrolimus for the induction of remission in patients with moderate-to-severe ulcerative colitis. **Methods** – A literature search was undertaken from 1966 to August 2016 using MEDLINE, Embase, LILACS and the Cochrane Library. The following MeSH terms were used: "Inflammatory Bowel Diseases" or "Ulcerative Colitis" and "Calcineurin Inhibitors" or "Tacrolimus" or "FK506". Studies performed in adult ulcerative colitis patients that evaluated the clinical efficacy of tacrolimus for the induction of remission were considered for revision. A meta-analysis was performed with those included studies that were also placebo-controlled and randomized. Clinical response as well as clinical remission and mucosal healing were evaluated. **Results** – Overall, 755 references were identified, from which 22 studies were finally included. Only two of them were randomized, placebo-controlled trials. A total of 172 patients were evaluated. A significantly lower risk of failure in clinical response was found for tacrolimus versus placebo [RR 0.58 (0.45-0.73)]; moreover, a lower risk of failure in the induction of remission was also found versus placebo [RR 0.91 (0.82-1)]. **Conclusion** – Tacrolimus seems to be a valid therapeutic alternative for the induction of remission in patients with moderate-to-severe ulcerative colitis.

HEADINGS – Inflammatory bowel diseases. Tacrolimus. Calcineurin inhibitors.

INTRODUCTION

Inflammatory Bowel Disease (IBD) is an immunological condition that carries a significant morbidity as well as mortality[1]. It has been classified as Crohn's Disease (CD) or Ulcerative Colitis (UC). Even though these entities share a common ground, they exhibit differences in terms of clinical presentation and therapeutic alternatives. During the last few years, there has been a significant development of scientific evidence showing the benefit of biological therapy – mainly anti-TNF α agents – in both scenarios[7].

Nevertheless, approximately 30% of patients with moderate-to-severe disease, whether they are CD or UC patients, do not respond to anti-TNF α therapy (primary failure)[3] and a significant proportion of patients (13% to 25% per year of treatment) may develop resistance and hence loss of efficacy to this type of treatment (secondary failure)[27,33]. This is why alternative therapeutic strategies are needed for these patients so that clinical conditions are improved and potentially serious complications can be avoided.

There is some evidence that show the efficacy of calcineurin inhibitors for the treatment of patients with severe UC[18]. Due to the effect that these drugs exert on calcineurin, they can inhibit the transcription of the Interleukin-2 gene – among others – which is necessary for the activation of T lympochytes[2]. As a consequence, although the quality of the available evidence is far from ideal, cyclosporine has been used in this clinical context; much less evidence is available in the CD scenario.

Bearing this in mind, a class-effect on moderate-to-severe UC could be inferred; consequently, the use of other calcineurin inhibitors such as tacrolimus has been proposed. Tacrolimus has the advantages of oral administration, a well-known safety profile and the previous experience of use in other clinical settings[31]. However, the evidence of the efficacy of its use in moderate-to-severe UC is scarce.

As a consequence, we sought to carry out a systematic review of the available evidence on this matter, with a meta-analysis considering randomized controlled trials.

METHODS

A literature search was carried out from 1966 to August 2016 using the following databases: MEDLINE, Embase, LILACS and The Cochrane Library. The search strategy included the following MeSH terms: "Inflammatory Bowel Disease" or "Ulcerative Colitis" and "Calcineurin Inhibitors" or "Tacrolimus" or "FK-506". Also, we reviewed the bibliographic references of the papers identified as potentially relevant. Additionally, we manually reviewed the abstracts of the Digestive Disease Week and United European Gastroenterology Week from 2010 to 2016.

The two authors performed bibliographic search in an independent manner. Potentially relevant abstracts were reviewed to check if they fulfilled inclusion criteria. These criteria were: a) studies that addressed the efficacy of tacrolimus in more than five UC patients; b) studies performed in adult population. For meta-

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analysis performance, studies which fulfilled the aforementioned criteria and were randomized controlled trials were included. PRISMA recommendations were followed for this purpose.

Authors' findings were then compared. If there was disagreement regarding the inclusion of a study, this would be discussed until consensus was reached. If data duplication was suspected, the authors of the studies would be contacted to exclude this situation.

The methodological quality of the included studies would be evaluated following the Evidence-Based Gastroenterology Sterring Group recommendations[29]. In addition, Jadas score was estimated for each study.

Outcome measures were: clinical response, clinical remission after induction treatment and, if available, mucosal healing rate.

Meta-analysis was performed using REVMAN software (Review Manager Version 5.1. Copenhagen: The Nordic Cochrane Center, The Cochrane Collaboration, 2011). Heterogeneity was estimated by means of I2 test and chi square test. If no significant heterogeneity was found, a fixed-effect model would be used for meta-analysis; otherwise, a random-effect model would be preferred. Outcome measures were described as Relative Risks (RR) with their corresponding 95% Confidence Interval (95%CI). Additionally, their corresponding Number Necessary to Treat (NNT) were calculated.

RESULTS

Overall, 755 potentially relevant citations were identified; 48 of them were furtherly chosen for analysis. Figure 1 shows the flow diagram which describes the reasons for citations exclusion. Finally, 22 studies[4-6,9-17,20-25,28,30,32,33] that fulfilled eligibility criteria were included. Only two studies were randomized controlled trials[24,25] (Ogata 2006 and Ogata 2012); these were included for meta-analysis.

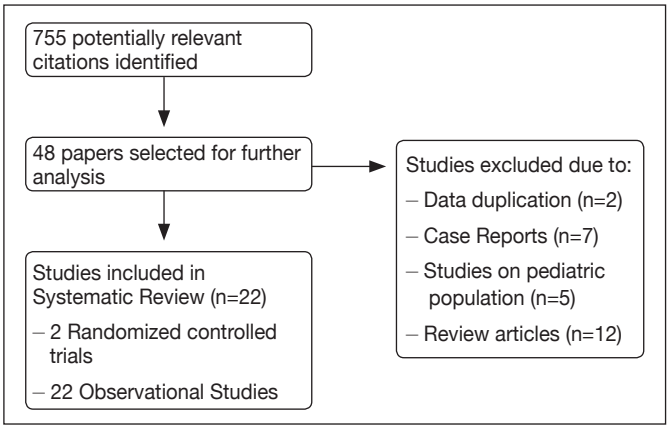

FIGURE 1. Flow-Chart showing the selection process of the studies included in the systematic review.

Table 1 describes the main features of the non-controlled studies that evaluated the efficacy of tacrolimus in patients with UC. The population included in these studies are rather similar – moderate-to-severe UC patients which are steroid-dependent or steroid-refractory– but certain discrepancy was found in the way that clinical response and clinical remission were defined: some studies used Truelove Witts criteria, whereas others used Lichtiger score. Some minor differences in terms of follow up time were also found. It is worth mentioning that only one of these studies[14] (Ikeya 2015) considered mucosal healing as an outcome. The tacrolimus dosage was adjusted to obtain a serum level between 10 to 15 ng/mL. Overall, 609 patients were included in non-controlled studies: in many of these cases, colectomy was delayed but not avoided.

TABLE 1. Characteristics of observational studies included in the systematic review

Author	Year	Country	UC severity and distribution	Remission definition (and time considered)	Number of patients	Interventions	Concomitant treatments	Results
Fellermann[10]	2002	Germany	Moderate-to-severe colitis/ steroid-refractory	Truelove-Witts Score. Remission evaluation in 2 weeks. Follow up for 6 months	38	IV Tacrolimus (0.01-0.02 mg/kg/day) or P.O. (0.1-0.2 mg/kg/day)	Antibiotics; aminosalicilates; steroids; thiopurines	28.94% (11/38) = clinical remission in 2 weeks. 16-month colectomy rate= 34%
Hogenauer[13]	2003	Austria	Moderate-to-severe colitis/ steroid-refractory	Truelove Witts Score. Evaluation up to week 12	9	Tacrolimus 0.15 mg/kg/day (adjusted by serum target level: 10-20 ng/mL)	Steroids; thiopurines	66.66% (6/9) = clinical remission in 12 weeks. 3/9= 33.33% of colectomy on follow up
Baumgart[4]	2006	Germany	Steroid-dependent or refractory (57% pancolitis)	Modified Clinical Activity Index up to 30 days	40	Tacrolimus 0.1 mg/kg/day (adjusted by serum target level: 4-8 ng/mL)	Steroids	45% (18/40) = clinical remission in 30 days; 77.5% (31/40) of clinical response. 22.5% colectomy on follow up
Ng[22]	2007	England	Steroid-dependent moderate-to-sevre colitis or previous failure to thiopurines or Infliximab	Truelove Witts score up to 4 weeks	6	Tacrolimus 0.1 mg/kg/day (adjusted by serum target level: 5-10 ng/mL)	Aminosalicilates; steroids; thiopurines; probiotics	50% (3/6)= clinical remission in 4 weeks; 66.66% (4/6) of clinical response
Benson[5]	2007	USA	Steroid-dependent or refractory moderate-to-severe colitis	Clinical variables and progression to colectomy up to 29 weeks	32	Tacrolimus 0.2 mg/kg/day (adjusted by serum target level:10-12 ng/mL)	aminosalicilates; steroids; thiopurines	9.37% (3/32)= clinical remission; 68.75% (22/32) of clinical response. 37.5%= colectomy on follow up

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Yamamoto[32]	2008	Japan	Moderate-to-severe colitis refractory to other treatments (81.5% pancolitis)	Truelove Witts Score up to 30 days	27	Tacrolimus 0.1 mg/kg/day (adjusted by serum target level:10-15 ng/mL)	aminosalicilates; steroids; thiopurines	70.4% (19/27)= clinical remission in 30 days; 77.8% (21/27) of clinical response. 26.9%= colectomy on follow up
Thin[30]	2012	Australia	Moderate-to-severe colitis refractory to other treatments	Colitis Activity Index up to 30 days	24	Tacrolimus 0.1 mg/kg/day (adjusted by serum target level:8-12 ng/mL)	aminosalicilates; steroids	37.5% clinical remission (9/24); 58.3% (14/24) clinical response
Schmidt[28]	2013	Germany	Steroid-refractory Moderate-to-severe colitis	Lichtiger score up to 12 weeks; need for colectomy	130	Tacrolimus 0.1 mg/kg/day	aminosalicilates; steroids; thiopurines	72% (94/130) clinical remission; 14% (18/130)= colectomy on follow up
Inoue[15]	2013	Japan	Moderate-to-severe pancolitis without steroid treatment	Lichtiger score. Mayo score for mucosal healing up to 4 weeks.	11	Tacrolimus 0.1 mg/kg/day (adjusted by serum target level:10-15 ng/mL)	aminosalicilates; thiopurines	72.7% (8/11) clinical remission; 100% (11/11) clinical response
Miyoshi[21]	2013	Japan	Steroid-dependent or refractory moderate-to-severe colitis	Lichtiger score. Mayo score for mucosal healing up to 12 weeks.	51	Tacrolimus initial dose= 5 mg/day; then, oral Tacrolimus (adjusted by serum target level: 10-15 ng/mL)	aminosalicilates; steroids; thiopurines	39.2% (20/51)= clinical remission; 62.74% (32/51)= clinical response
Landy[17]	2013	England	Moderate-to-severe colitis refractory to other treatments	Truelove Witts Score up to 6 months	25	Tacrolimus 0.1 mg/kg/day (adjusted by serum target level:5-10 ng/mL)	aminosalicilates; steroids; thiopurines	20% (5/25)= clinical remission; 24% (6/25)= clinical response
Boschetti[6]	2014	France	Moderate-to-severe colitis refractory to other treatments	UC-DAI at 4 and 12 weeks	30	Tacrolimus 0.1-0.2 mg/kg/day (adjusted by serum target level:10-15 ng/mL)	steroids; thiopurines	47% (14/30)= clinical remission; 70% (21/30)= clinical response in 4 weeks
Hiraoka[11]	2015	Japan	Colitis moderada-severa cortico-dependiente/ refractaria	Lichtiger score up to 2-3 weeks	47	Tacrolimus 0.05-0.15 mg/kg/day (adjusted by serum target level: 10-15 ng/mL)	aminosalicilates; steroids; thiopurines	87% (41/47) showed remission and/or clinical response
Ikeya[14]	2015	Japan	Moderate-to-severe colitis	Colitis Activity Index. Mayo score for mucosal healing up to 12 weeks	44	Tacrolimus 0.1 mg/kg/day (adjusted by serum target level:10-15 ng/mL)	aminosalicilates; steroids; thiopurines	86.4% (38/44)= clinical response; 65.9% (29/44)= clinical remission; 43.8% mucosal healing
Kawakami[16]	2015	Japan	Steroid-dependent or refractory moderate-to-severe colitis	Lichtiger score up to 4 weeks	49	Tacrolimus 0.1 mg/kg/day (adjusted by serum target level:10-15 ng/mL)	aminosalicilates; steroids; thiopurines	89.6%= clinical response; 75.6%= clinical remission in 4 weeks
Minami[20]	2015	Japan	Moderate-to-severe colitis refractory to other treatments	Mayo score up to 8 weeks	22	Tacrolimus 0.1 mg/kg/day (adjusted by serum target level:10-15 ng/mL)	aminosalicilates; steroids; thiopurines	90.9% (20/22)= clinical response; 63.63% (14/22)= clinical remission in 8 weeks
Hiraoka[12]	2015	Japan	Moderate-to-severe colitis refractory to other treatments	CAI	24	Tacrolimus 0.1 mg/kg/day (adjusted by serum target level:10-15 ng/mL)	aminosalicilates; steroids; thiopurines	79% (19/24)= remission and/or response
Endo[9]	2016	Japan	Steroid-dependent or refractory moderate-to-severe colitis	CAI up to 8 weeks	47	Tacrolimus 0.1 mg/kg/day (adjusted by serum target level:10-15 ng/mL)	aminosalicilates; steroids; thiopurines	68.08% (32/47)= clinical response; 55.31% (26/47)= clinical remission. 14.89% (7/47)= colectomy on follow up
Yamamoto[33]	2016	Japan	Steroid-dependent or refractory moderate-to-severe colitis	UC-DAI up to 12 weeks	50	Tacrolimus 0.1 mg/kg/day (adjusted by serum target level:10-15 ng/mL)	aminosalicilates; steroids; thiopurines	62% (31/50) = clinical response; 40% (20/50)= clinical remission
Nuki[23]	2016	Japan	Moderate-to-severe colitis	UC-DAI up to 10 weeks	21	Tacrolimus 0.1 mg/kg/day (adjusted by serum target level:10-15 ng/mL)	aminosalicilates; steroids; thiopurines	85.7% (18/21)= clinical response; 66.7% (14/21)= clinical remission in 10 weeks

UC: ulcerative colitis. DAI: disease activity index; CAI: colitis activity index.

Only two studies[24,25] (Ogata 2006 and Ogata 2012) were randomized controlled trials. The features of these trials are shown in Table 2. Overall, 127 UC patients were included for meta-analysis. Table 3 shows the methodological analysis of these two trials. As witnessed in Figure 2, tacrolimus significantly increased the risk of clinical response versus placebo [RR of clinical response failure 0.58 (0.45-0.73)], with a NNT of 3. Figure 3 highlights the RR of failure of clinical remission versus placebo, which was 0.91 (0.82-1), with a NNT of 10. Mucosal healing, as shown in Figure 4, was also significantly favoured by tacrolimus treatment [RR 0.59 (0.46-0.74)] with a NNT of 3. According to Egger test, a low risk of publication bias was found (P>0.5).

TABLE 2. Characteristics of randomized controlled trials included in meta-analysis

Author	Year	Country	UC severity and distribution	Remission definition (and time considered)	Number of patients	Interventions	Concomitant treatment
Ogata[25]	2006	Japan (17 centers)	Moderate-to-severe left colitis or pancolitis	DAI Score <2 with every subitem <1. Clinical response: a decrease of at least 4 points in DAI. Evaluation in 2 weeks and then open follow up up to week 12. Mucosal healing evaluated	65 patients randomized; 60 patients finished study: 19 in Tacrolimus group (high-dosage), 21 in Tacrolimus low-dosage group and 20 in placebo group	0.05 mg/kg b.i.d. (adjusted to serum target levels of 5-10 ng/mL or 10-15 ng/mL) versus placebo	Aminosalicilates and/or oral/IV steroids
Ogata[24]	2012	Japan	Moderate-to-severe, steroid-refractory left colitis or pancolitis	DAI Score <2 with every subitem <1. Clinical response: a decrease of at least 4 points in DAI. Evaluation in 2 weeks and then open follow up up to week 12. Mucosal healing evaluated	62 patients aleatorizados; 30 a rama placebo y 32 a rama tacrolimus	0.5-1 mg b.i.d. (adjusted to serum target levels of 10-15 ng/mL) versus placebo	Aminosalicilates and/or oral/IV steroids

UC: ulcerative colitis. DAI: disease activity index.

TABLE 3. Methodological analysis of randomized controlled trials included in meta-analysis

ID Study	Concealed random allocation	Blinding of patients and medical team	Similar interventions between groups	Complete follow up	Intention to treat analysis	Jadad score
Ogata 2006[25]	Not clear	Yes	Yes	Yes	No	4
Ogata 2012[24]	Yes	Yes	Yes	Yes	Yes	5

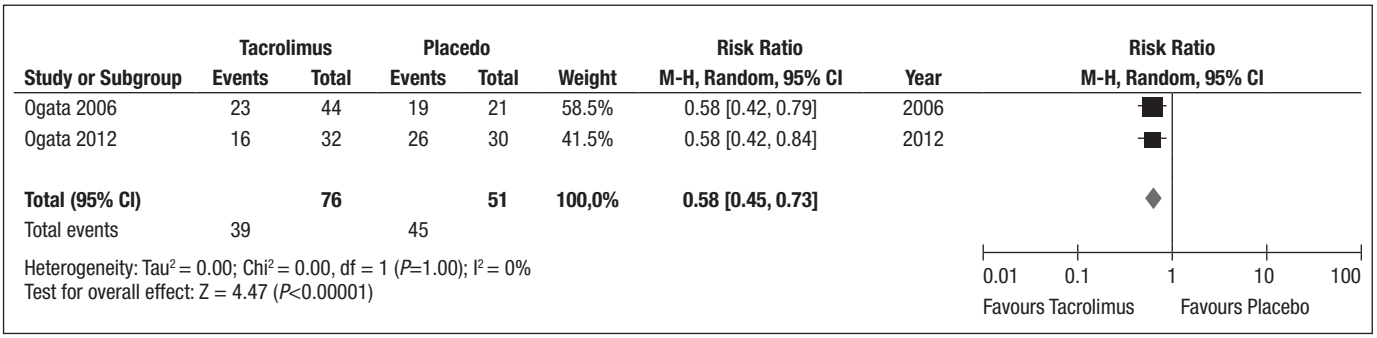

FIGURE 2. Forest Plot showing meta-analysis on clinical response of tacrolimus versus placebo.

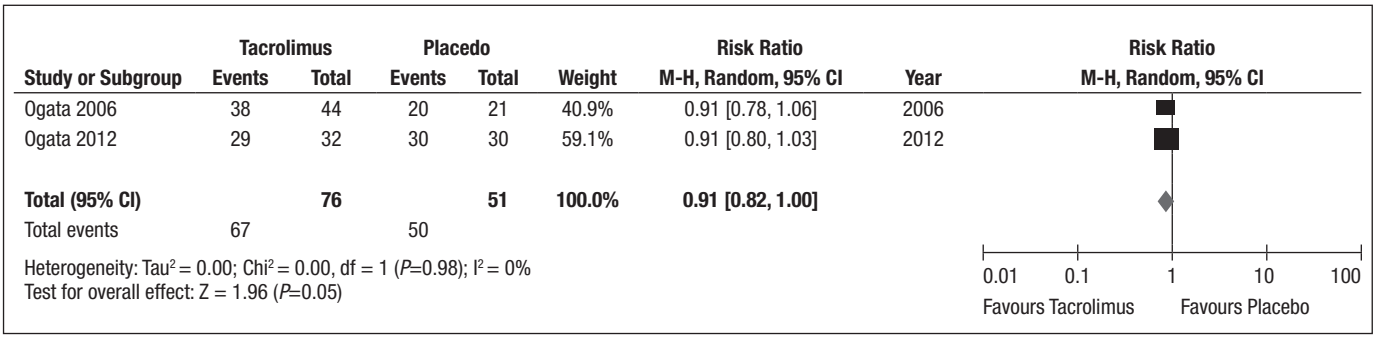

FIGURE 3. Forest Plot showing meta-analysis on clinical remission of tacrolimus versus placebo.

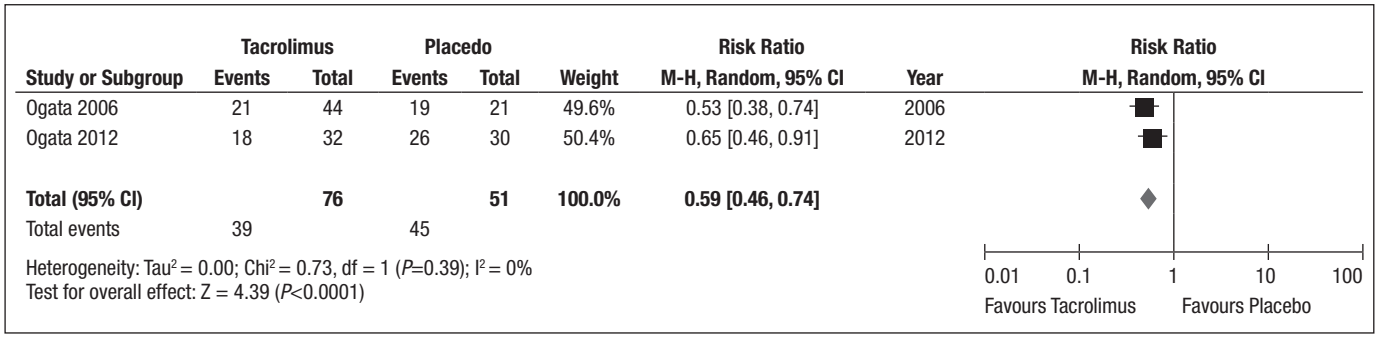

FIGURE 4. Forest Plot showing meta-analysis on mucosal healing of tacrolimus versus placebo.

The most commonly reported severe adverse events were gastroenteritis and sepsis. When considering only the randomized controlled trials, there was not a significant increase in the risk of serious adverse events versus placebo [RR 3.38 (0.17-68.91)].

DISCUSSION

According to the results observed in our systematic review, tacrolimus therapy could be a valid alternative in the context of moderate-to-severe UC that have not responded to other treatments or who are regarded as cortico-dependent or refractory.

As mentioned before, evidence on the efficacy of calcineurin inhibitors derives mainly from the experience of cyclosporine as a rescue therapy in the setting of severe UC. In a pioneer study, Lichtiger et al.[18] showed that endovenous cyclosporine was useful to avoid colectomy in patients with severe UC flares after systemic steroid therapy failure. D'Haens et al.[8] concluded that cyclosporine is a valid alternative to intravenous steroids in severe flares. Additionally, controlled studies have shown that cyclosporine may be equivalent to Infliximab in this type of clinical scenario[18]. Nevertheless, it should be reminded that cyclosporine has a non-neglectable adverse event profile and there is much less evidence apart from its parenteral use.

Tacrolimus is a well-known calcineurin inhibitor that is widely used as immunosupresant therapy in the context of transplant or even for the treatment of autoinmmune conditions[31]. Tacrolimus has the advantage that it can be administered orally and that its serum concentration can be easily measured and adjusted to reach the adequate level in each case. The initial experience in UC patients was originated in referral centers from Japan, and then it was adopted by some centers in Europe. According to what is witnessed in uncontrolled studies, tacrolimus shows a high proportion of clinical response in the short-term, with a low NNT; however, when clinical remission is considered, such proportion is lower and more variable.

It is worth mentioning, according to what is observed from uncontrolled studies, the relatively high proportion of patients who would eventually require colectomy in spite of receiving tacrolimus[4,5,10,13,28,32] (Fellermann 2002, Hogenauer 2003, Baumgart 2006, Benson 2007, Yamamoto 2008, Schmidt 2013). As a consequence, it would seem like tacrolimus could delay the need for colectomy in a selected group of patients – those moderate-to-severe UC patients with prior failure to other therapeutic alternatives. It should also be mentioned that no study has considered tacrolimus therapy in patients with less severe disease.

There is a scarcity of high-quality trials to know the real impact of tacrolimus in the management of UC. As a matter of fact, as shown by this review, there has only been published two controlled trials[24,25] (Ogata 2006 and Ogata 2012), with a relatively low number of patients and a rather short follow up time. One strength of these two trials is that mucosal healing was included as an outcome, which is known to be a relevant prognostic factor and a therapeutic target, particularly when it comes to UC[26]. It is also worth mentioning that all studies assessed the efficacy of oral tacrolimus for the continued treatment of UC patients.

Evidence on the efficacy of calcineurin inhibitors in the setting of CD is more scarce: there are only a few controlled trials, mainly on patients with perianal disease, and few cohort studies[19]. Nevertheless, the relatively few alternatives in severe CD may turn tacrolimus into a valid therapeutic option, regardless of the few scientific evidence available.

Some limitations should be mentioned. First of all, there were few studies included for meta-analysis, so the conclusions should be cautiously interpreted. We decided not to perform meta-analysis with observational uncontrolled studies, because this represents a high risk for relevant biases, which could in turn produce a distortion in the conclusions derived from higher-quality studies. Last but not least, it is worth mentioning that most observational studies were retrospective, with the logical limitations that this implicate.

In conclusion, tacrolimus seems to show in both uncontrolled studies as well as in placebo controlled trials, a significant efficacy to induce clinical response and remission in moderate-to-severe UC. However, more evidence is undoubtfully needed to fully estimate the magnitude of its benefit.

Authors' contributions

Juan Lasa: bibliographic review, data analysis, statistical analysis, manuscript review. Pablo Olivera: bibliographic review, data analysis, manuscript elaboration.

Lasa J, Olivera P. Eficácia do tacrolimus para indução de remissão em pacientes com colite ulcerosa moderada a grave: uma revisão sistemática e meta-análise. Arq Gastroenterol. 2017. [ahead of print].

RESUMO – **Contexto** – Há evidências que mostram que os inibidores de calcineurina podem ser úteis para o tratamento da colite ulcerativa severa. No entanto, há poucos dados sobre a eficácia do tacrolimus para indução de remissão neste cenário. **Objetivo** – Desenvolver uma revisão sistemática sobre evidências existentes sobre a eficácia clínica do tacrolimus para a indução de remissão em pacientes com colite ulcerosa de moderada a grave. **Métodos** – Realizada pesquisa bibliográfica de 1966 a agosto de 2016 usando MEDLINE, Embase, LILACS e Biblioteca Cochrane. Foram utilizados os seguintes termos MeSH: "doenças inflamatórias intestinais" ou "colite ulcerativa " e "inibidores da calcineurina" ou "tacrolimo" ou "FK506". Foram considerados para revisão estudos que avaliaram a eficácia clínica do tacrolimus para a indução de remissão em pacientes adultos com colite ulcerosa. Uma meta-análise foi realizada com esses estudos incluídos que também fossem controlados por placebo e randomizados. Avaliou-se a resposta clínica, bem como remissão clínica e a cicatrização da mucosa. **Resultados** – No total, 755 referências foram identificadas, dos quais 22 estudos foram finalmente incluídos. Apenas dois deles eram experimentações randomizadas e, placebo-controlada. Um total de 172 pacientes foram avaliados. Verificou-se um risco significativamente menor de falha na resposta clínica para tacrolimus versus placebo [RR 0.58 (0.45-0,73)]; Além disso, um menor risco de falha na indução da remissão também foi encontrado versus placebo [RR 0,91 (0,82-1)]. **Conclusão** – Tacrolimus parece ser uma alternativa terapêutica válida para a indução de remissão em pacientes com colite ulcerosa moderada a grave.

DESCRITORES – Doenças inflamatórias intestinais. Tacrolimo. Inibidores de calcineurina.

REFERENCES

1. Abraham C, Cho JC. Inflammatory bowel disease. N Engl J Med. 2009;361:2066-78.
2. Actis GC, Volpes R, Rizzetto M. Oral microemulsion cyclosporine to reduce steroids rapidly in chronic active ulcerative colitis. European J Gastroenterol Hepatol. 1999;11:905-8
3. Allez M, Karmiris K, Louis E, et al. Report of the ECCO pathogenesis workshop on anti-TNF therapy failures in inflammatory bowel diseases: Definitions, frequency and pharmacological aspects. J Crohn's Colitis. 2010;4:355-66.
4. Baumgart DC, Pintoffl J, Sturm A, Wiedenmann B, Dignass AU. Tacrolimus is safe and effective in patients with severe steroid-refractory or steroid-dependent inflammatory bowel disease- a long-term follow up. Am J Gastroenterol. 2006;101:1048-56.
5. Benson A, Barrett T, Sparberg M, Buchman AL. Efficacy and safety of tacrolimus in refractory ulcerative colitis and crohn's disease: a single center experience. Inflamm Bowel Dis. 2008;14:7-12.
6. Boschetti G, Nancey S, Moussata D, Stefanescu C, Roblin X, Chauvenet M, Stroeymeyt K, Bouhnik Y, Flourie B. Tacrolimus induction followed by maintenance monotherapy is useful in selected patients with moderate-to-severe ulcerative colitis refractory to prior treatment. Dig Liv Dis. 2014;46:875-80.
7. Costa J, Magro F, Caldeira D, Alarcão J, Sousa R, Vaz-Carneiro A. Infliximab Reduces Hospitalizations and Surgery Interventions in Patients With Inflammatory Bowel Disease: A Systematic Review and Meta-analysis. Inflamm Bowel Dis. 2013;19:2098-110.
8. D'Haens G, Lemmens L, Geboes K, Vandeputte L, Van Acker F, Mortelmans L, Peeters M, Vermeire S, Penninckx F, Nevens F, Hiele M, Rutgeerts P. Intravenous cyclosporine versus intravenous corticosteroids s single therapy for severe attacksof ulcerative colitis. Gastroenterology. 2001;120:1323-9.
9. Endo K, Onodera M, Shiga H, Kuroha M, Kimura T, Hiramoto K, Kakuta Y, Kinouch Y, Shimosegawa T. A comparison of short nd long-term therapeutic outcomes of infliximab – versus tacrolimus-based strategies for steroid-refractory ulcerative colitis. Gastroenterol Res Prac 2016;3162595.
10. Fellermann K, Tanko Z, Herrlinger KR, Witthoeft T, Homann N, Bruening A, Ludwig D, Stange EF. Response of refractory colitis to intravenous or oral tacrolimus (FK506). Inflamm Bowel Dis. 2002;8:317-24.
11. Hiraoka S, Kato J, Moritou Y, Takei D, Inokuchi T, Nakarai A, Takahasi S, Harada K, Okada H, Yamamoto K. The earliest trough concentrarionredicts the dose of tacrolimus required for remission induction therapy in ulcerative colitis patients. BMC Gastroenterol. 2015;15:53.
12. Hiraoka S, Inokuchi T, Takei D, Nakarai A, Sugihara Y, Morito Y, Takahashi S, Harada K, Okada H, Yamamoto K, Kato J. Tacrolimus therapy for outpatients with refractory ulcerative colitis. Gastroenterology. 2015;148:(Suppl 1):870-1.
13. Hogenauer H et al. Effect of oral tacrolimus (FK506) on steroid-refractory moderate/severe ulcerative colitis. Aliment Pharmacol Ther. 2003;18:415-23.
14. Ikeya K, Sugimoto K, Kawasaki S, Iida T, Maruyama Y, Watanabe F, Hanai H. Tacrolimus for remission inductionn ulcerative colitis: Mayo endoscopic subscore 0 and 1 predicts long-term prognosis. Dig Liv Dis. 2015; 47:365-71.
15. Inoue T, Murano M, Narabayashi K, Okada T, Nouda S, Ishida K, et al. The efficacy of oral tacrolimus in patients with moderate/severe ulcerative colitis not receiving concomitant corticosteroide therapy. Intern Med. 2013;52:15-20.
16. Kawakami K, Inoue T, Murano M, Narabayashi K, Nouda S, Ishida K, et al. Effects of oral tacrolimus s a rapid induction therapy in ulcerative colitis. World J Gastroenterol. 2015;21:1880-6.
17. Landy J, Wahed M, Peake STC, Hussein M, Ng SC, Lindsay JO, Hart AL. Oral tacrolimus s maintenance therapy for refractory ulcerative colitis – an analysis of outcomes in two London tertiary centers. J Crohn Colitis. 2013;7:e516-e521
18. Lichtiger S, Present DH, Kornbluth A, Gelernt I, Bauer J, Galler G, Michelassi M, Hanauer S. Cyclosporine in severe ulcerative colitis refractory to steroid therapy. N Engl J Med. 1994;330:1841-5.
19. McSharry K, Dalzell AM, Leiper K, El-Matary W. Systematic review: the role of tacrolimus in the management of Crohn's disease. Aliment Pharmacol Ther. 2011;34:1282-94.
20. Minami N, Yoshino T, Matsuura M, Koshikawa Y, Yamada S, Toyonaga T, Madian A, Honzawa Y, Nakase H. Tacrolimus or infliximab for severe ulcerative colitis: short-term and long-term data from a retrospective observational study. BMJ Open Gastro. 2015;2:e000021.
21. Miyoshi J, Matsuoka K, Inoue N, Hisamatsu T, Ichikawa R, Yajima T, Okamoto S, Naganuma M, Sato T, Kanai T, Ogata H, Iwao Y, Hibi T. Mucosal healing with oral tacrolimus is associated with medium-nd long-term prognosis in steroid-refractory/dependent ulcerative colitis patients. J Crohn Colitis. 2013;7:e609-e614.
22. Ng SC, Arebi N, Kamm MA. Medium-term results of oral tacrolimus treatmentin refractory inflammatory bowel disease. Inflamm Bowel Dis. 2007;3:129-34.
23. Nuki Y, Esaki M, Asano K, Maehata Y, Umeno J, Moriyama T, Nakamura S, Matsumoto T, Kitazono T. Comparison of the therapeutic efficacy and safety between tacrolimus and infliximab for moderate-to severe ulcerative colitis: a single center experience. Scand J Gastroenterol 2016;51:700-5.
24. Ogata H, Kato J, Hirai F, Hida N, Matsui T, Matsumoto T, Koyanagi K, Hibi T. Double-blind, placebo controlled trial of oral tacrolimus (FK506) in the management og hospitalized patients with steroid-refractory ulcerative colitis. Inflamm Bowel Dis. 2012;18:803-8.
25. Ogata H, Matsui T, Nakamura M, Lida M, Takazoe M, Suzuki Y, Hibi T. A randomised dose finding study of oral tacrolimus (FK506) therapy in refractory ulcerative colitis. Gut. 2006;55:1255-62.
26. Pineton de Chambrun G, Peyrin-Biroulet L, Lémann M, Colombel JF. Clinical implications of mucosal healing for the management of IBD. Nat Rev Gastroenterol Hepatol. 2010;7:15–29.
27. Roda G, Jharap B, Neeraj N, Colombel JF. Loss of Response to Anti-TNFs: Definition, Epidemiology, and Management. Clin Transl Gastroenterol. 2016;7:e135.
28. Schmidt KJ, Herrlinger KR, Emmrich J, Barthel D, Koc H, Lehnert H, Stange EF, Fellermann K, Buning J. Short-term efficacy of tacrolimus in steroid-refractory ulcerative colitis – experience in 130 patients. Aliment Pharmacol Ther. 2013;37:129-136.
29. Schoenfeld P, Cook D, Hamilton F, Laine L, Morgan D, Peterson W. An evidence-based approach to gastroenterology therapy. Gastroenterology. 1998; 114:1318-25.
30. Thin LW, Murray K, Lawrance IC. Oral tacrolimus for the treatment of refractory inflammatory bowel diseasein the biologic era. Inflamm Bowel Dis. 2013;19:1490-8.
31. Webster A, Woodroffe RC, Taylor RS. Tacrolimus versus cyclosporin as primary immunosupresion for kidney transplants recipients. CDS Review. 2005;4:CD003961.
32. Yamamoto S, Nakase H, Mikami S, Inoue S, Yoshino T, Takeda Y, et al. Long-term effect of tacrolimus therapy in patients with refractory ulcerative colitis. Aliment Pharmacol Ther. 2008;28:589-97.
33. Yamamoto T, Shimoyama T, Umegae S, Matsumoto K. Tacrolimus vs anti-tumor necrosis factor agents formoderately to severely active ulcerative colitis: a retrospective observational study. Aliment Pharmacol Ther. 2016;43:705-16.

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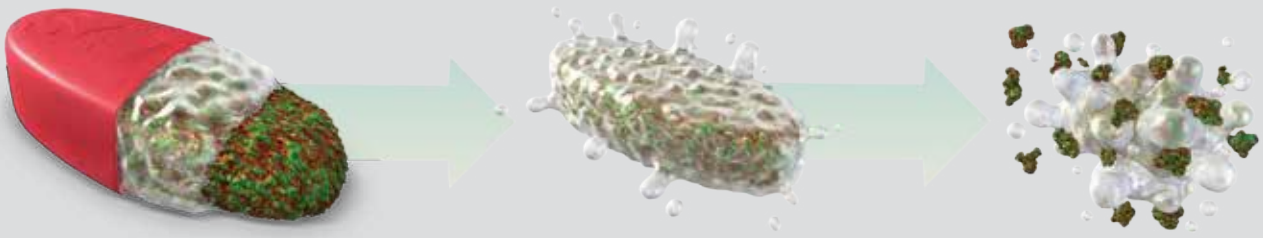

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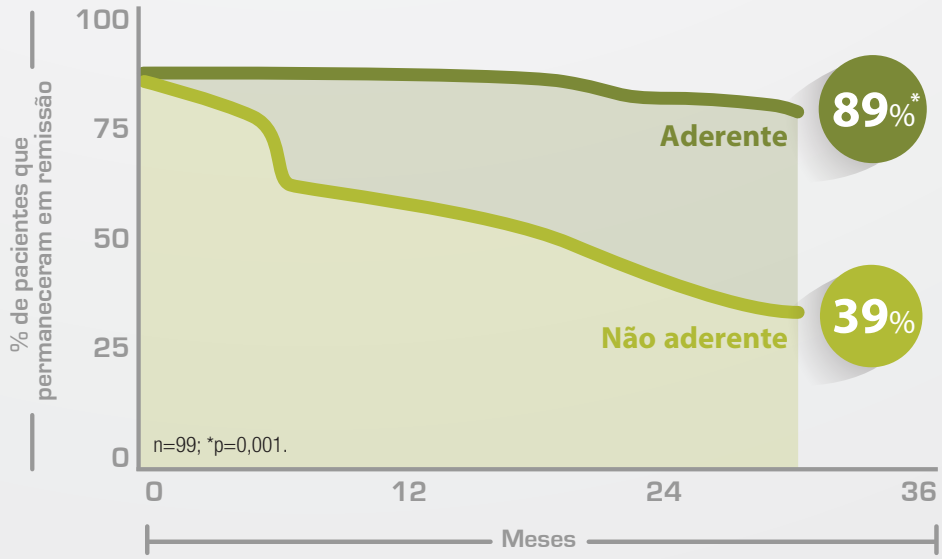

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*** 2,4 a 4,8 g/dia.

Contraindicação: Mesacol® MMX® não é recomendado em casos de hipersensibilidade aos salicilatos. **Interação Medicamentosa:** a administração da mesalazina pode potencializar a toxicidade do metotrexato.

Mesacol® MMX® - mesalazina. USO ORAL. USO ADULTO ACIMA DE 18 ANOS. Apresentações e composição: Comprimidos revestidos de liberação prolongada, com 1,2 g de mesalazina cada. Embalagens com 10 e 30 unidades. **Indicações:** anti-inflamatório de ação local no tratamento da colite ulcerativa ativa leve a moderada, na fase aguda (indução da remissão) e na manutenção da remissão. **Contraindicações:** hipersensibilidade aos salicilatos (que inclui o ácido acetilsalicílico), à mesalazina, à sulfassalazina ou a qualquer dos componentes da fórmula; pacientes com insuficiência hepática e/ou renal graves; pacientes com úlcera gástrica e duodenal ativa; pacientes com tendência elevada a sangramento. **Este medicamento é contraindicado para menores de 18 anos. Precauções e advertências:** As mesmas precauções e advertências relacionadas com o uso de preparados contendo mesalazina ou pró-drogas de mesalazina devem ser consideradas para Mesacol® MMX®. Assim como todos os salicilatos, a mesalazina deve ser utilizada com cautela em pacientes com história de úlcera gástrica ou duodenal, por pacientes asmáticos (em razão das reações de hipersensibilidade), com disfunção renal ou hepática (leve a moderada), ou com história de miocardite ou pericardite. Ainda não está estabelecida a segurança do produto em crianças. Gravidez e lactação: Mesacol® MMX® está classificado na categoria B de risco de fármacos destinados ao uso em grávidas, devendo ser usado com cautela durante a gravidez e somente quando os benefícios para a mãe forem superiores aos riscos potenciais ao feto. Pacientes pediátricos: Mesacol® MMX® não é recomendado para pacientes menores de 18 anos. Pacientes idosos: O impacto potencial sobre o uso seguro da mesalazina na população idosa deve ser avaliado na prática clínica. Pacientes com insuficiência renal: a mesalazina deve ser administrada com precaução em pacientes com disfunção renal leve a moderada. Seu uso é contraindicado para pacientes com insuficiência renal grave. Pacientes com insuficiência hepática: a mesalazina deve ser administrada com precaução em pacientes com insuficiência hepática leve a moderada. Seu uso é contraindicado para pacientes com insuficiência hepática grave. Dirigir e operar máquinas: É improvável que o uso deste medicamento tenha qualquer efeito sobre a capacidade de dirigir veículos ou de operar máquinas. **Interações medicamentosas:** Não foram observadas interações relevantes clinicamente entre a mesalazina com amoxicilina, ciprofloxacino XR, metronidazol ou sulfametoxazol. O uso concomitante da mesalazina com agentes sabidamente nefrotóxicos, inclusive com os anti-inflamatórios não hormonais (AINHs – como aspirina, ibuprofeno, diclofenaco, etc.) e azatioprina pode aumentar o risco de reações renais; o potencial para discrasias sanguíneas da azatioprina e da 6-mercaptopurina pode aumentar; a ação hipoglicemiante das sulfonilureias pode ser intensificada; a atividade anticoagulante dos derivados cumarínicos (varfarina) pode ser reduzida; a toxicidade do metotrexato pode ser potencializada; o efeito uricosúrico da probenecida e da sulfimpirazona pode diminuir, assim como a ação diurética da furosemida e da espironolactona e a ação tuberculostática da rifampicina. Em tese, a administração concomitante de anticoagulantes orais deve ser feita com cautela. Substâncias como a lactulose, que diminuem o pH do cólon, podem reduzir a liberação da mesalazina dos comprimidos revestidos de Mesacol® MMX®. **Reações adversas:** diarreia, náusea, cefaleia, dor abdominal, hipersensibilidade como urticária e prurido, erupção cutânea e eczema. **Posologia e modo de usar:** Mesacol® MMX® destina-se a uso exclusivo por via oral. Para o tratamento da colite ulcerativa leve a moderada, a dose usual para adultos acima de 18 anos é de 2.400 mg a 4.800 mg (dois a quatro comprimidos) ao dia, administrada em dose única, de preferência sempre na mesma hora de cada dia, acompanhada de uma refeição. Caso o paciente esteja tomando a dose mais elevada (4.800 mg/dia), ele deve ser reavaliado após oito semanas de tratamento. Não apresentando mais sintomas, pode-se prescrever uma dose diária de 2.400 mg (dois comprimidos) para manutenção da remissão. A duração recomendada é de oito semanas consecutivas, salvo critério médico diferente. **Este medicamento não deve ser partido, mastigado ou dissolvido.** MS - 1.0639. 0248. **MEDICAMENTO SOB PRESCRIÇÃO.** AO PERSISTIREM OS SINTOMAS, O MÉDICO DEVERÁ SER CONSULTADO. MEMX_0414_0614_VPS.

Referências bibliográficas: 1) Kamm MA, *et al.* Once-daily, high-concentration MMX mesalamine in active ulcerative colitis. Gastroenterology. 2007;132(1):66-75. **2)** Brunner M, *et al.* Gastrointestinal transit and 5-ASA release from a new mesalazine extended-release formulation. Aliment Pharmacol Ther. 2003;17(3):395-402. **3)** Prantera C, *et al.* A new oral delivery system for 5-ASA: preliminary clinical findings for MMX. Inflamm Bowel Dis. 2005;11(5):421-7. **4)** Tenjarla S, *et al.* Release of 5-aminosalicylate from an MMX mesalamine tablet during transit through a simulated gastrointestinal tract system. Adv Ther. 2007;24(4):826-40. **5)** Mesacol® MMX® [Bula]. São Paulo: Takeda Pharma Ltda.**6)** Mesacol® [Bula]. São Paulo: Takeda Pharma Ltda. **7)** Osterman MT, et al. Reformulation of an aminosalicylate: an example of the importance of pill burden on medical compliance rates. Methods Find Exp Clin Pharmacol. 2009;31(1):41-6. **8)** Moraes AC, *et al.* Análise de custo-utilidade da mesalazina MMX em comparação à mesalazina convencional no tratamento da retocolite ulcerativa leve ou moderada sob a perspectiva do Sistema Público de Saúde Brasileiro. JBES.2012;4(3):436-43.

Material destinado exclusivamente à classe médica.

Material produzido em fevereiro/2017.
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- Elimina rapidamente o sangramento na doença hemorroidária, induzindo a vasoconstrição da área tratada.[2,4]
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PARA O CUIDADO DA REGIÃO ANAL[1]

HIGIENIZAÇÃO ⟷ TRATAMENTO

Uso diário

Aplicar 2 ou 3 vezes ao dia[2]

O 1º SABONETE ÍNTIMO ESPECÍFICO PARA HIGIENIZAÇÃO E CUIDADO DA REGIÃO ANAL

ALÍVIO RÁPIDO E EFICAZ* NO TRATAMENTO DAS AFECÇÕES ANORRETAIS[3-5]

*Elimina rapidamente o sangramento induzindo a vasoconstrição da área tratada[2,3]. Favorecendo a regeneração dos tecidos lesados[2].

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Referências bibliográficas:
1) Neiger A. [Pathogenesis, clinical aspects and conservative therapy of hemorrhoids]. Schweiz Med Wochenschr. 1980;110(38):1387-90. **2)** Proctyl® [Bula]. São Paulo: Takeda Pharma Ltda. **3)** Espinosa DJ. Revisión analítica de estudios multicentricos con policresuleno en patologia hemorroidal. Acta Gastroenterol Latinoam. 2000; 30(3): 177-86. **4)** Arnold K, et al. Multicenter clinical study of a new non-steroid preparation in proctology. Arzneimittel-Forschung Drug Res.1976;26(1):95-9. **5)** Seppänen J, Mattinen P, Salo H. Experience of the topical use of Faktu ointment in the treatment of various conditions and postoperative wounds in the enorectal region. Diagnosis. 1979; 4: 449-52.

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