UNIVERSIDADE FEEVALE

MESTRADO ACADÊMICO EM TOXICOLOGIA E ANÁLISES TOXICOLÓGICAS

Avaliação do estresse oxidativo, níveis de vitamina B12 e cognição em pacientes que fazem uso prolongado de omeprazol

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Linha de Pesquisa: Toxicologia Humana e Análises Toxicológicas

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Novo Hamburgo, julho de 2019

LARISSA SELBACH DRIES

AVALIAÇÃO DO ESTRESSE OXIDATIVO, NÍVEIS DE VITAMINA B12 E COGNIÇÃO EM PACIENTES QUE FAZEM USO PROLONGADO DE OMEPRAZOL

Dissertação apresentada para obtenção do GRAU DE MESTRE em Toxicologia e Análises Toxicológicas pela Universidade Feevale.

Orientadora: Prof.ª Dr.ª Magda Susana Perassolo

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LARISSA SELBACH DRIES

Dissertação intitulada “Avaliação do estresse oxidativo, níveis de vitamina B12 e cognição em pacientes que fazem uso prolongado de omeprazol” apresentada ao Programa de Pós-Graduação em Toxicologia e Análises Toxicológicas, da Universidade Feevale, como requisito necessário para obtenção do grau de mestre.

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Novo Hamburgo, julho de 2019.

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Agradeço aos meus pais pela vida,
Ao meu noivo pelo café,
Ao meu irmão, o abrigo e parceria
E a Deus por me manter de pé.

A minha orientadora, agradeço a orientação
e o constante incentivo.
Aos amigos, por estenderem a mão
e aos familiares pelo ouvido.

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pois olhando pra eles quis ser maior enquanto ainda vivo
e agradeço aos pequenos
pelo mesmo motivo.

RESUMO

O omeprazol é um dos medicamentos mais utilizados da História e, apesar de ser considerado um fármaco seguro quando utilizado da forma correta, vem sendo estudada uma possível ligação do seu uso prolongado com risco aumentado de prejuízo cognitivo. O estresse oxidativo e a deficiência de vitamina B12 são dois fatores cujo envolvimento neste processo é investigado. Dessa forma, o objetivo do presente trabalho foi avaliar a relação entre diferenças de desempenho cognitivo, o estresse oxidativo e os níveis de vitamina B12 em usuários de omeprazol por no mínimo 6 meses. Para isto, foi realizado um estudo caso-controle com 44 usuários (81,8 % de mulheres, idade de 66 ± 8,7 anos) e 35 não usuários de omeprazol (88,6 % de mulheres, idade de 62 ± 8,7 anos), cuja cognição foi avaliada através da aplicação de testes e baterias de avaliação da atenção, memória e funções executivas, além da dosagem em plasma dos níveis de vitamina B12 através de imunoensaio quimioluminescente e o estresse oxidativo foi avaliado através dos biomarcadores superóxido dismutase, catalase, glutationa peroxidase, malondialdeído e poder antioxidante total. Foi possível verificar um aumento significativo no poder antioxidante total (grupo OU= 1690,29 µM± 441,05 e grupo NOU=1307,66 ± 616,09; p=0,002) e uma diminuição nos níveis de glutationa peroxidase no grupo de usuários de omeprazol (grupo OU= 0,534 (0,27 – 10,63) e grupo NOU=71,86 (14,36 – 173,1); p=0,006), assim como uma performance cognitiva inferior, com prejuízos na cognição automatizada, atencional e nas funções executivas, o que sugere uma possível ligação destes achados com o uso prolongado de omeprazol.

Palavras-chave: Omeprazol. Estresse oxidativo. Vitamina B12. Prejuízo cognitivo. Inibidores da bomba de prótons.

ABSTRACT

Omeprazole is one of the most used drugs in History and, although it is considered safe when used correctly, a possible connection between its long-term use and greater risk for cognitive damage has been investigated. Oxidative stress and vitamin B12 deficiency are indicated as potentially involved in this process. Thus, the aim of the present study was to evaluate the connection between an inferior cognitive ability, oxidative stress and vitamin B12 levels in omeprazole users under treatment for longer than 6 months. For that reason, a case-control study was developed with 44 omeprazole users (81.8 % female, 66 ± 8.7 years old) and 35 nonusers (88.6 % female, 62 ± 8.7 years old), whose cognitive ability was assessed through batteries and tests approaching attention, memory and executive functions, in addition to vitamin B12 dosage using a chemiluminescent immunoassay and oxidative stress analysis, based on the evaluation of the biomarkers malondialdehyde, enzymatic activity of extracellular superoxide dismutase, glutathione peroxidase, catalase and the ferric reducing antioxidant power in plasma. It was possible to verify a statistically significant increase of the ferric reducing antioxidant power (OU group = 1690.29 µM± 441.05 and NOU group=1307.66 ± 616.09; p value=0.002) and a decrease on glutathione peroxidase levels on the omeprazole users group (OU group = 0.534 (0.27 – 10.63) and NOU group=71.86 (14.36 – 173.1); p value=0.006), as well as an inferior cognitive performance, with impairments on executive functions, automatic and attentional processing, suggesting a potential connection of these findings with omeprazol long-term use.

Key words: Omeprazole. Oxidative stress. Vitamin B12. Cognition assessment. Cognitive damage. Proton pump inhibitors.

LISTA DE ABREVIATURAS E SIGLAS

AA	Atenção Alternada (*Alternating Attention*)
AC	Atenção Concentrada (*Concentrated Attention*)
AD	Atenção Dividida (*Divided Attention*)
BMI	*Body Mass Index*
BPA	Bateria Psicológica da Atenção (*Psychological Battery for Attention Assessment*)
DBP	*Diastolic Blood Pressure*
DM	Diabetes Mellitus
FDT	Teste dos Cinco Dígitos (*Five Digit Test*)
FE	Função Executiva (*Executive Function*)
FVF	Fluência Verbal Fonêmica (*Phonemic Verbal Fluency*)
FVL	Fluência Verbal Livre (*Free Verbal Fluency*)
FVS	Fluência Verbal Semântica (*Semantic Verbal Fluency*)
GPx	Glutationa peroxidase (*Glutathione peroxidase*)
HPLC-DAD	*Diode Array Detector and a Fluorescence Detector*
IBP	Inibidor da Bomba Protônica
MDA	Malondialdeído *(Malondialdehyde)*
NCD	*Neurocognitive Disorder*
NBT	N*itrotetrazolium Blue Method*
NEUPSILIN	Instrumento de Avaliação Neuropsicológica Breve (*Brief Neuropsychological Assessment Battery*)
NOU	Não-usuários de omeprazol (*Omeprazole nonusers*)
OU	Usuários de omeprazol (*Omeprazole users*)
PPI	*Proton Pump Inhibitor*
RAVLT	Teste de Aprendizagem Auditivo-Verbal de Rey (*Rey Auditory Verbal Learning Test*)
ROS	Espécies Reativas de Oxigênio (*Reactive Oxygen Species*)
SAH	*Systemic Arterial Hypertension*
SBP	*Systolic Blood Pressure*
SOD	Superóxido dismutase *(Superoxide dismutase)*
SPSS	*Statistical Package for the Social Sciences*
TCLE	Termo de Consentimento Livre Esclarecido (*Consent Form*)

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SUMÁRIO

1 APRESENTAÇÃO GERAL

O presente trabalho tem como objetivo geral relacionar a habilidade cognitiva, o estresse oxidativo e os níveis de vitamina B12 em pacientes que fazem uso de omeprazol por mais de 6 meses. Este trabalho inicia com uma introdução geral revisando brevemente a bibliografia relacionada a pesquisa desenvolvida, seguida da apresentação de um capítulo composto do artigo científico que será encaminhado para publicação, conforme segue:

> CAPÍTULO 1: Artigo que será encaminhado para publicação na revista *Journal of Psychopharmacology* intitulado "*Cognition, oxidative stress and vitamin B12 levels evaluation on patients under long-term Omeprazole use*".

Complementando o artigo que será submetido, durante a realização desta pesquisa os seguintes trabalhos foram apresentados em eventos científicos:

- "Avaliação da qualidade de vida em usuários de fluoxetina de Novo Hamburgo", apresentado na forma de seminário estendido no Seminário de Pós – Graduação – Inovamundi, realizado em outubro de 2017.
- "Avaliação do estresse oxidativo em pacientes portadores de doenças crônicas e usuários de omeprazol", apresentado na forma de seminário estendido no Seminário de Pós – Graduação – Inovamundi, realizado em outubro de 2018.

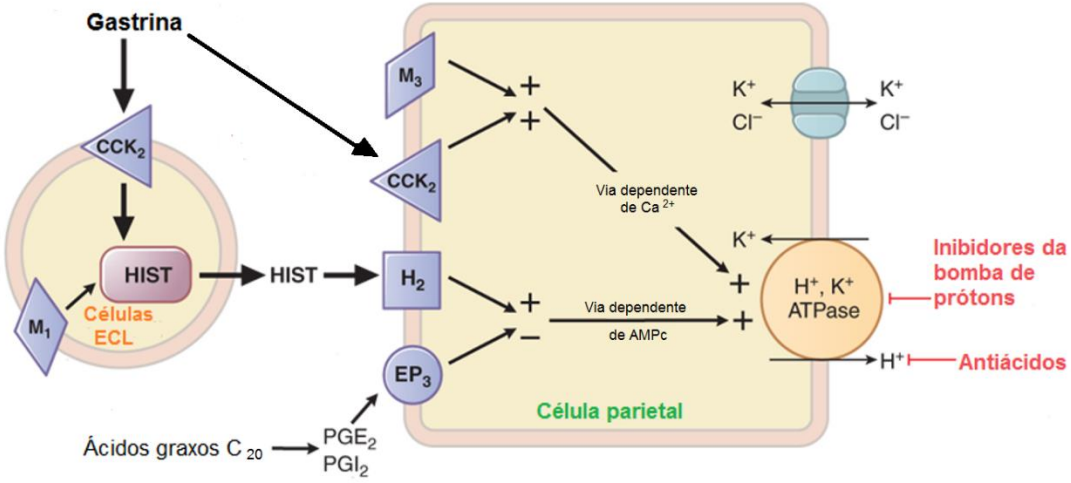
2 INTRODUÇÃO GERAL

A secreção de ácido gástrico no estômago é um processo contínuo e complexo que envolve múltiplos fatores centrais e periféricos. Apesar de sua propriedade cáustica, em geral o ácido gástrico não provoca lesões nem sintomas, devido aos mecanismos de defesa intrínsecos, porém, em situações de distúrbios, o ácido gástrico constitui fator patogênico. (BRUNTON, 2012). Um destes mecanismos de defesa é a barreira ao refluxo de conteúdo gástrico para o esôfago. Em caso de falha desta barreira, pode surgir a doença do refluxo gastroesofágico (DRGE) (HOROWITZ et al., 2007). Outro mecanismo é a "defesa mucosa", que se refere a inúmeros fatores que coletivamente protegem o estômago. A ruptura dessa defesa favorece o surgimento de lesões teciduais, que são a principal característica da úlcera péptica (MÉGRAUD, 1993).

Os inibidores da bomba de prótons são a classe de medicamentos mais amplamente utilizada para o tratamento de distúrbios gastrointestinais relacionados à secreção de ácido gástrico (STRAND, KIM e PEURA, 2017). A alta prevalência destes distúrbios associada a facilidade de acesso e a alta eficácia desta classe de medicamentos favorecem o seu alto consumo, que frequentemente é usado de forma prolongada e não é precedido de indicação médica (HEIDELBAUGH, GOLDBERG e INADOMI, 2009).

Por este motivo, nos últimos anos, um maior número de estudos vem sendo realizado para avaliar a segurança dos IBPs à longo prazo, principalmente relacionada ao seu efeito supressor gástrico. O omeprazol, após absorção na circulação sanguínea, difunde-se nas células parietais do estômago e acumula-se nos canalículos secretores ácidos, onde é ativado e em seguida, liga-se a bomba de prótons, responsável pela secreção gástrica, inativando-a irreversivelmente. (Figura 1). A secreção de ácidos só volta ao normal após a síntese de novas moléculas da bomba, promovendo, assim, uma supressão prolongada da secreção ácida (BRUNTON, CHABNER e KNOLLMANN, 2012).

Figura 1 - Mecanismo de ação do omeprazol



Fonte: BRUNTON, CHABNER e KNOLLMANN, 2012.

Mesmo sendo considerado seguro no tratamento à curto prazo, o omeprazol pode promover efeitos adversos de baixa complexidade, como dor de cabeça, diarreia e constipação. Porém, seu uso pode estar ligado a efeitos de maior complexidade relacionados à inibição gástrica, como por exemplo pneumonia, complicações cardiovasculares e baixa absorção de nutrientes (CORSONELLO et al., 2018), sendo um deles a vitamina B12.

A associação da deficiência de vitamina B12 com o uso prolongado de omeprazol baseia-se amplamente em relatos de caso e estudos observacionais, dispondo de poucas evidências clínicas e científicas para comprovação desta ligação (QORRAJ-BYTYQI et al., 2018)

A deficiência de vitamina B12 impacta os sistemas hematopoiético e nervoso, impedindo as células de completarem seu processo de maturação, o que leva à lise das células. Por conta deste processo, pode ocorrer anemia megaloblástica e lesões no sistema nervoso, sendo que, neste último caso, manifestações clínicas de mau humor, perda de memória, confusão, delírios e alucinações podem estar presentes, motivo pelo qual é considerada um dos possíveis mecanismos que estabelece uma ligação entre o uso de IBPs e o maior risco de desempenho cognitivo prejudicado, apesar de ainda não ter sido completamente determinada (LAM et al, 2013),

Habilidades cognitivas prejudicadas são fator importante na caracterização do transtorno neurocognitivo maior (demência), condição que, devido à deterioração cognitiva, impede que seu portador tenha autonomia em

sua rotina (HAENISCH et al., 2015). Suas causas podem ser múltiplas e não estão completamente elucidadas, porém, uma das hipóteses estudadas é a sua ligação com baixos níveis séricos de vitamina B12 (MOORE et al. 2012).

Um estudo de coorte desenvolvido na Alemanha que avaliou dados de 73.679 participantes com idade igual ou superior a 75 anos, encontrou evidências de que pacientes em tratamento com omeprazol, pantoprazol e esomeprazol tiveram um aumento significativo no risco de surgimento de demência em comparação com pacientes que não recebiam esta classe de medicamentos (GOMM et al., 2016).

Embora a patogênese dos transtornos neurocognitivos seja complexa, sabe-se que o estresse oxidativo tem função chave nos estágios iniciais deste transtorno, por este motivo ele tem sido avaliado como um possível biomarcador para detecção precoce de transtornos neurocognitivos (GARCÍA-BLANCO et al., 2017).

Além disso, as desordens cognitivas têm sido fortemente ligadas aos danos causados pelo estresse oxidativo, condição em que as vias de defesa antioxidante (como a superóxido dismutase, a catalase e a glutationa peroxidase) falham em proteger o organismo de alterações da sinalização neuronal, neuroinflamação e ativação de mecanismos de morte celular, resultando em degeneração neuronal e perda de memória (KALYANARAMAN, 2013; POPA-WAGNER et al., 2013).

Lagemaat, Groot e Heuvel (2019) realizaram uma revisão sistemática para investigar evidências da ligação dos níveis de vitamina B12 com os parâmetros de estresse oxidativo e, apesar de ter encontrado resultados que apontam uma possível ligação da deficiência desta vitamina com a diminuição da defesa antioxidante do organismo, salienta a baixa confiabilidade destes achados, tendo em vista a ausência de estudos controlados randomizados e prospectivos em humanos avaliando tais parâmetros.

A maior suscetibilidade do cérebro aos danos do estresse oxidativo pode ser explicada pela alta metabolização de oxigênio no cérebro e pela alta quantidade de ácidos graxos poliinsaturados facilmente peroxidáveis. O dano às proteínas e a menor atividade das enzimas protetoras contribuem para a desmielinização e para o dano axonal, que podem representar a base do prejuízo cognitivo (DI PENTA et al., 2013).

Até o presente momento, não foram encontrados trabalhos que abordassem a associação entre os níveis de vitamina B12, o estresse oxidativo e a perda de habilidade cognitiva em pacientes que fazem uso de omeprazol, sendo, desta forma, importante avaliar a relação destes parâmetros com o uso deste fármaco e seu efeito sobre a habilidade cognitiva, a fim de identificar um possível desencadeador do transtorno neurocognitivo e impedir sua progressão em decorrência do uso de omeprazol.

3 OBJETIVOS

A seguir, os objetivos estabelecidos para o estudo realizado:

3.1 OBJETIVO GERAL

O objetivo deste trabalho foi relacionar a habilidade cognitiva, o estresse oxidativo e os níveis de vitamina B12 em pacientes que fazem uso de omeprazol por mais de 6 meses.

3.2 OBJETIVOS ESPECÍFICOS

Os objetivos específicos que conduziram este trabalho foram:

- Avaliar a presença de diferenças de desempenho cognitivo de usuários e não usuários de omeprazol;
- Verificar se há alterações nos níveis de vitamina B12 entre estes mesmos grupos;
- Analisar a relação entre os parâmetros de estresse oxidativo com o uso de omeprazol;
- Verificar a relação entre a cognição e os parâmetros de estresse oxidativo (níveis de catalase, glutationa peroxidase, malondialdeído, atividade de superóxido dismutase e poder antioxidante total) das amostras de cada grupo;
- Avaliar a influência das características clínicas e sociodemográficas e tratamentos diversos utilizados sobre todos os parâmetros analisados em ambos os grupos.

4 CAPÍTULO 1

Original Article STM

Cognition, oxidative stress and vitamin B12 levels evaluation on patients under long-term omeprazole use

Larissa S Dries, Rochelli Haefliger, Bruna S Seibert, Caroline DO Cardoso and Magda S Perassolo

Abstract

Background: Omeprazole is broadly used in digestive disorders treatments and, although considered safe when used correctly, a possible connection between its long-term use and risk of cognitive damage has been investigated. Oxidative stress and vitamin B12 deficiency are indicated as potentially involved in this process.

Aim: To evaluate the differences concerning cognitive performance, oxidative stress and vitamin B12 levels in omeprazole users under treatment for longer than 6 months.

Method: A case-control study was developed with 44 omeprazole users (81.8 % female, 66 ± 8.7 years old) and 35 nonusers (88.6 % female, 62 ± 8.7 years old), whose cognitive ability was assessed through tests approaching attention, memory and executive functions, in addition to vitamin B12 dosage using a chemiluminescent immunoassay and oxidative stress analysis, based on the evaluation of malondialdehyde, enzymatic activity of extracellular superoxide

dismutase, glutathione peroxidase, catalase and the ferric reducing antioxidant power in plasma.

Results: A significant increase of the ferric reducing antioxidant power (OU group = 1690.29 µM± 441.05 and NOU group=1307.66 ± 616.09; p value=0.002) and a decrease on glutathione peroxidase levels (OU group = 0.534 (0.27 – 10.63) and NOU group=71.86 (14.36 – 173.1); p value=0.006) were found on omeprazole users group, as well as differences on cognitive performance, with impairments on executive functions, automatic and attentional processing.

Conclusions: Findings suggest a potential connection between oxidative stress and impairment on cognitive ability of omeprazole long-term users.

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Keywords

Omeprazole, cognitive impairment, oxidative stress, vitamin B12, PPIs, psychological evaluation, neurocognitive damage.

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Introduction

Proton pump inhibitors (PPIs) are the most effective acid-suppressors and are the main choice for the treatment of disorders from the digestive tract, such as gastro esophageal reflux disease and peptic ulcer (Katz, Gerson and Vela,

2013). In the typical dosages, this class of drugs decreases from 80 to 95% the daily acid production and it is constituted by 6 inhibitors commercially available for clinical use: omeprazole, esomeprazole, lansoprazole, dexlansoprazole, rabeprazole and pantoprazole (Strand, Kim and Peura, 2017).

Omeprazole was the first PPI released in the market in 1989 and soon became one of the most used drugs in History (Devault and Talley, 2009). After being acid-activated, it irreversibly inactivates the proton pump, responsible for acid secretion. The acid secretion is only reestablished after the synthesis of new proton pump molecules, which allows the long-term suppression (Brunton, Chabner and Knollmann, 2012). Even being considered a safe drug for short-term treatments use, for less than 12 weeks (Welage and Berardi, 2000), adverse events may occur. The low complexity events more commonly reported are headache, constipation and diarrhea. There is also a range of events of higher complexity, usually linked to the acid inhibition, as is the case of pneumonia, cardio-vascular complications and reduced absorption of nutrients (Corsonello et al., 2018), for example, magnesium, iron, calcium and vitamin B12, that need the gastric acid to be absorbed (Khatib et al., 2002; O'Connell et al., 2005; Epstein, Mcgrath and Law, 2006; Abraham, 2012).

Indeed essential for a diverse range of processes in the human body, vitamin B12, also known as cobalamin, is a nutrient that comes exclusively from exogenous sources, present in animal origin products (Lam et al, 2013). Therefore, its deficiency may impair hematopoietic and nervous system, affecting cells maturation process, which leads to cell lyses. On account to that, pathologic condition may appear, such as megaloblastic anemia and lesions on the nervous system, which in this last case may be clinically manifested by memory loss,

confusion, delirium, hallucinations or even psychosis (Brunton, Chabner E Knollmann, 2012). For that reason, it has been investigated as a possible mechanism between the long-term PPI treatment and a greater risk for the patient to present cognitive impairment (Lam et al., 2013).

The connection between the long-term use of omeprazole and a possible cognitive damage has also been investigated, but the path of this connection isn't clear yet. A cohort study that evaluated 73.679 individuals that were 75 years old or more, found evidences that patients undergoing treatment with omeprazole, pantoprazole and esomeprazole had a significant higher risk of incident dementia, presenting cognitive impairment, in comparison to individuals that weren't under treatment with PPIs (Gomm et al., 2016).

Cognitive impairment can be an early evidence for neurocognitive disorders (NCDs), which causes can be multiple and remain unclear. However, among the many possible triggers, a potential connection between this inferior cognitive performance and the use of PPIs has been investigated (Arteaga-Vásquez et al., 2017).

A study from 2015 evaluated 700 patients under regular treatment with lansoprazole/omeprazole and detected a higher risk for these patients to develop a neurocognitive disorder and Alzheimer's Disease in comparison to PPI nonusers (Haenisch et al., 2015).

Cognitive disorders have also been strongly connected to damages caused by oxidative stress, which can lead to neuronal degeneration and memory loss due to altered neural signaling, neuroinflammation and activation of mechanisms of cell death (Popa-Wagner et al., 2013).

Although the pathogenesis of neurocognitive disorders is complex, it is known that oxidative stress performs a key role on its early stages, that is why it has been evaluated as a possible biomarker for early detection of neurocognitive impairments (García-Blanco et al., 2017).

A study developed to verify oxidative stress in patients diagnosed with Alzheimer, it was observed a significant increase of protein oxidation (protein carbonyls), lipid peroxidation (4-hydroxynonenal) and protein nitration (3-nitrotyrosine), which are oxidative stress markers, and a strong association of this increase to the changes on synaptic proteins levels (Scheff, Ansari and Mufson, 2016).

Oxidative stress is an essential factor in the aging process, as well as on the neurodegenerative disorders. Superoxide dismutase, catalase, and glutathione peroxidase are some of the enzymes responsible for the antioxidant defense (Kalyanaraman, 2013).

Recently, considering the connection between oxidative stress and vitamin B12 with cognitive disorders, Solomon (2015) verified that there was a higher incidence of vitamin B12 deficiency in patients with neurocognitive disorders that have the oxidative stress as biomarker.

To this moment, only few studies have already approached the link between omeprazole use and oxidative stress. To verify the connection between omeprazole use and oxidative stress in animal models, it was observed impairment of vascular redox biology as a result of increased reactive oxygen species, which may trigger cell damage and death as part of the oxidative process. (Pinheiro et al., 2016).

Until the present moment, no further studies were found approaching the association between vitamin B12 levels, oxidative stress parameters and cognitive impairment in omeprazole users, fact that points a relevant matter to be evaluated, in order to identify o possible early trigger for neurocognitive disorders and prevent its progression as a result of long-term omeprazole use.

A proper identification of cognitive damage causes may help prevent neurocognitive disorders evolution and its early detection may reduce the negative impact it has on patients and their families' lives and on the health care system.

Therefore, the aim of the present work was to evaluate the connection between differences on cognitive performances, oxidative stress and vitamin B12 levels in omeprazole users under treatment for longer than 6 months.

Materials and methods

Sample and study design

The sample studied was formed by convenience, consisting of subjects assisted by the health services provided by Feevale University, from Novo Hamburgo in southern Brazil. A case-control study was developed and subjects able to participate were divided into omeprazole users group (OU) and nonusers group (NOU). On the day scheduled, participants signed the consent form, attended the interview, went through blood collection and answered the cognitive tests. Clinical characteristics (weight, height, body mass index and smoking habits) were evaluated through the application of a structured questionnaire, besides social demographic history and education time. They were selected based on their age (≥40 years old) and similar treatment and comorbidities profile.

To be able to participate of the studied group, subjects had to be under continuous treatment with omeprazole for longer than 6 months, without interruptions. To compose the NOU group, subjects couldn't be under treatment with omeprazole for at least one year. For both groups, the exclusion criteria were the treatment with any other proton pump inhibitor for at least a year prior to the participation on the study, history of drug or alcohol abuse, diagnosed neurological disabilities and vision impairment.

Blood collection

To perform vitamin B12 dosage and oxidative stress analysis, samples of blood of 20mL were collected and distributed in three EDTA tubes and in one heparin tube. The schedule for blood collection was the same for all participants (around 2 p.m.) and, as it didn't represent an interference on the variables evaluated, it was not required fasting before the collection. This material was stored in thermal boxes for less than one hour for transportation and then processed. One EDTA tube was collected by the clinical analysis laboratory from Feevale University for the blood count and the other EDTA and heparin tubes were centrifuged under 2500 rpm for 10 minutes, and then aliquots of plasma were transferred to eppendorf tubes for vitamin B12 dosage, superoxide dismutase (SOD), glutathione peroxidase (GPx), ferric reducing antioxidant power (FRAP), malondialdehyde analysis. These samples were properly identified and stored in a refrigerator under -80ºC until its use within a period of two months. The heparinized tube was processed for catalase analysis immediately after blood collection.

Vitamin B12 dosage

For the quantitative determination of vitamin B12 levels, 500 µL of plasma sample previously prepared were homogenized, added to a vessel and went through chemiluminescent immunoassay, in which the amount of analyte in the sample is determined from a stored, multi-point calibration curve and results are expressed in pg/mL.

Oxidative stress analysis

Oxidative stress was evaluated on plasma samples, based on the analysis of malondialdehyde (MDA), enzymatic activity of extracellular superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase and the ferric reducing antioxidant power (FRAP).

The enzymatic activity of SOD was evaluated using a commercially available kit (#19160, SigmaAldrich, Steinheim, Germany), based on the indirect method of nitrotetrazolium blue (NBT). This assay used xanthine and xanthine oxidase to generate superoxide radicals which react with 2- (4-iodophenyl) -3- (4-nitrophenol) -5-p-phenyltetrazoliumchloride to produce a compound that absorbs light at 450 nm. The inhibition of chromogen production is proportional to SOD activity in the sample. The reading was performed on microplates spectrophotometer and the results were expressed as U/L.

MDA was evaluated according to the method described by Antunes et al (2008), in which the dosage of this biomarker initiates with an alkaline hydrolysis of 200 µL of plasma, using 1.5 M NaOH with 60° C dry bath incubation for 30 minutes, in order to release protein fraction, which was subsequently precipitated by the addition of 15% HClO4. The sample was then centrifuged at 4°C for 10

minutes at 12000 rpm. To 250 µL of the supernatant, 25 µL of the DNPH derivative was added and incubated at room temperature, protected from light for 30 minutes. The chromatographic run was performed with 50 µL of the prepared sample, in HPLC-DAD in a Shimadzu Class VP high performance liquid chromatograph with a Lichrospher Merck RP-18 cc (250 x 4 mm, di 5 µm) column, the mobile phase being constituted of acetic acid 0.2% (w / v): acetonitrile (62:38), with a flow rate of 1 mL/min, and controlled at 310 nm.

The method used to evaluate catalase was described by Aebi (1984). Tubes containing blood (heparinized ones) were centrifuged at 2500 rpm for 10 minutes. Plasma and leukocytes were discarded. Next, the red cells were washed with 0.9% NaCl solution 3 times. An aliquot of 1 mL of erythrocytes was transferred to another tube, adding 4 mL of water (diluted 1). To 20 µl of diluent 1, 9980 µl of a phosphate buffer solution pH 7.0 were added (diluted 2). The spectrophotometer used was a Varian, Dig Varian Cary model (50 NSEL03127475) at 240 nm reading at 0 and 15 s. For each sample, blank tube was prepared. The blank tube was composed of 0.5 mL buffer + 1 mL diluted 2, and the sample tube was composed of 0.5 mL of a solution of 30 mM hydrogen peroxide + 1 mL diluted 2. The results were expressed as seconds (s) corrected by hemoglobin of patients, Hemoglobin was evaluated in the blood count performed by the clinical analysis laboratory from Feevale University.

GPx analysis was performed by the method described by Pleban; Munyani and Beachum (1982). The working solution was prepared with: 50 mmol/l Tris buffer at pH 7.6, containing 1 mmol Na2EDTA per liter, 2 mmol reduced glutathione, 0.2 mmol NADPH, 4 mmol sodium azide and 1000 U of glutathione reductase. The mixture was incubated for 5 minutes at 37ºC. To determine

enzymatic activity in plasma, 50 µL of undiluted plasma was added to 950 µL of the working solution. The activity of GPx was expressed in plasma U/L. After a period of 30 seconds, the decrease in absorbance is linear with time. 10 µL of H2O2 8.8 mmol/L was added to the start of the reaction, followed by the decrease of the NADPH at 340 nm absorbance for 3 minutes. The blank tube was prepared, and instead of plasma, water was added. The results were used in this equation: K = [2.3 / (t2 - t1)] x log [GSH at t1 (1 min) / GSH at t2 (3 min)] where t1 = reading at time 1; t2 = reading after three minutes and GSH = reduced glutathione (FARAJI, KANG and VALENTINE, 1987).

For the evaluation of FRAP, plasma from subjects was added to FRAP (10 mM TPTZ - 2,4,6-tripyridyl-s-triazine in 40 mM HCl, 300 mM acetate buffer pH 3.6 ; 20 mM FeCl 3 6H 2 O), which at low pH and with the presence of antioxidants reduces to an intense blue coloration, which was monitored by measuring the change in absorption at 593 nm. The ferric reducing ability was calculated using ascorbic acid and a solution of ferrous sulfate as standards (Benzie and Strain, 1996).

Cognitive evaluation

Cognition was evaluated through the application of 6 tests and batteries in order to cover up all the different aspects involved in human cognition (Table 1).

Table 1. Tests and Batteries applied for the cognition assessment of omeprazole users and nonusers groups.

Test	What it evaluates	Score
Psychological Battery (BPA)	Concentrated, divided and alternating attention	Right answers (120 target stimulus total) minus errors and omissions
Rey Auditory Verbal Learning Test (RAVLT)	Short-term and long-term episodic memory, learning and retention of information	Learning rate (A1 to A5), proactive interference rate (B1/A1), retroactive interference rate (A6/A5), forgetting speed
Brief Neuropsychological Assessment Battery (NEUPSILIN)	Working memory	28 (total amount of words to recall) and 5 (total groups of words to recall)
Verbal Fluency Test (FVS)	Initiation, inhibition, verbal planning, strategy choice, memory, lexical-semantic language	Amount of right words pronounced minus errors
Five Digit Test (FDT)	Attentional interference, inhibitory control and processing speed	Time and errors
Hayling Test	Executive functions (initiation, inhibition and processing speed)	Time, total of right answers and total of errors.

References: Psychological Battery (Rueda, 2013). Rey Auditory Verbal Learning Test (Rey, 1964, adapted in Brazil by Malloy-Diniz et al., 2007). Brief Neuropsychological Assessment Battery Neupsilin (Fonseca, De Salles and Parente, 2008). Verbal Fluency Test (Joanette, Ska and Côté, 2004, adapted to Portuguese by Fonseca, De Salles and Parente, 2008). Five Digit Test (Sedó, De Paula and MALLOY-DINIZ, 2007, adapted in Brazil by De Paula and Malloy-Diniz, 2013). Hayling Test (Burgess and Shallice, 1997, adapted in Brazil by Fonseca, Prando and Zimmermann, 2010).

The Psychological Battery for Attention Assessment (BPA) uses tests composed from several abstract stimulations to evaluate concentrated, divided and alternating attention (Rueda, 2013).

Rey Auditory Verbal Learning Test (RAVLT) consists of a list of 15 nouns (list A) read out loud for the subject with a pause of 1 second between words, 5 consecutive times (A1 to A5). Each attempt is followed by a test of an immediate recall. After the fifth attempt, an interference list, also composed by 15 nouns (list B), is read for the subject and also followed by its recall. Next, it is requested to the subject to recall the words from list A without its repetition. After A 20-minute interval, a delayed recall of list A is required. Through this test, it is possible to assess the learning rate throughout the attempts (A1 to A5), the rate of proactive interference (B1/A1), which is the ability of the subject to resist to distractors, the retroactive interference rate (A6/A5), that evaluates the interference of a new content on the learning process of a previews learned matter, and the forgetting speed, which refers to the vulnerability over time of a learned content (Rey, 1964, adapted in Brazil By Malloy-Diniz et al., 2007).

The subtest Auditory span words in sentence of the Brief Neuropsychological Assessment Battery (NEUPSILIN) (Fonseca, De Salles and Parente, 2008) is based on the memory and recall of the last words of sentences after reading sets of two, three, four and five sentences.

In the first part of the Verbal Fluency Test (FVS) (Joanette, Ska and Côté, 2004, adapted to Portuguese by Fonseca, De Salles and Parente, 2008) is requested to the subject to say any words, except for first names and numbers, for two minutes and thirty seconds with eyes closed. On the phonological evaluation, the subject will say words starting with the letter p for two minutes

and, for the semantic evaluation, also for two minutes, subjects must say names of clothes/dressing items.

The Five Digit Test (FDT) is a four steps instrument, based on reading numbers from one to five, counting from one to five, ignoring an automatic processing routine (reading) to a controlled one (counting) when counting conflictive items and avoid reading them and, finally, switching from the mental task of counting to the mental task of reading the number (Sedó, De Paula and Malloy-Diniz, 2007, adapted in Brazil by de Paula And Malloy-Diniz, 2013).

The Hayling Test is a task of completing sentences where, in part A, subject must choose words connected to the meaning of the sentence and, in part B, sentences must be completed with unconnected words (Burgess and Shallice, 1997, adapted in Brazil by Fonseca, Prando and Zimmermann, 2010).

Statistical analyses

Statistical analyses were performed using the program SPSS 25.0 (SPSS, Chicago, IL). Normality of data was tested with Shapiro-Wilk test and comparisons among groups were checked with t-Student test (parametric) and Mann–Whitney U test (non-parametric) or Pearson Chi-Square, according to the data. Spearman correlation was used to evaluate the connection between variables (cognitive assessment and education time). Multiple linear regression models were carried out to test the association of oxidative stress parameters (dependent variable) and factors with possible biological relevance (pathologies and drugs in use) were used like independents variables. All independent variables selected were added in a block in a single step. Different oxidative stress parameters were included as dependent variables in each model, one at

a time. Data was expressed as median (percentile 25 and percentile 75) or mean ± standard deviation (SD). P values < 0.05 were considered statistically significant and effect size was calculated using an online calculator and results under 0.2 were considered small, 0.5 medium and 0.8 a large effect size.

Ethic aspects

Participants who were able to participate, signed two copies of the consent form (TCLE), one for the participant and the other one to be kept by the research. This study is part of an institutional project called “Assessment of neurotoxicity related to omeprazole administration” approved by the Research Ethics Committee from Feevale University under registration number 80153617.0.0000.5348. All the material generated by the project (questionnaires, forms, consent forms) will remain filed for 5 years after the conclusion of the study. After this period the material will be shredded.

Results

Characterization

Initially, 85 patients were selected. From those, a total of 06 were excluded due to treatment with other PPIs. Thus, 44 subjects for the OU group and 35 for the NOU group attended the interview and went through blood collection. On the next stage, 14 subjects were excluded of the cognitive assessment: 5 subjects were excluded due to cerebrovascular accident, 4 because of vision impairment and 5 subjects couldn’t attend the evaluation on the days scheduled. Thus, 35 subjects for the OU group and 30 for the NOU group had their cognitive ability evaluated.

Both groups were composed predominantly by women, with average age of 66 years old for the OU group and 62 years old for the NOU. Groups also presented similar profiles concerning weight, height, BMI and average blood pressure. All the participants presented hemoglobin within the normal range. Comorbidities profile of the OU group pointed systemic arterial hypertension as the most prevalent pathology, followed by dyslipidemia, osteoporosis/osteopenia, depression and diabetes mellitus. Systemic arterial hypertension was also the most present pathology in the NOU group, followed by arthritis/osteoarthritis, osteoporosis/osteopenia, depression and dyslipidemia. In the studied group, all subjects administered daily the same dosage of omeprazole (20mg). The OU group most common drugs were simvastatin, hydrochlorothiazide and losartan, while the NOU group most frequent drugs were simvastatin, losartan and glucosamine and chondroitin (Table 2).

Table 2. General characteristics of omeprazole users and nonusers groups.

Characteristics	Omeprazole use (n = 44)	No omeprazole use (n = 35)
Gender		
Female	36 (81.8%)	31 (88.6%)
Male	8 (18.2%)	4 (11.4%)
Age (years)	66 ± 8.7	62 ± 8.7
Weight (Kg)	73.93 ± 11.7	72.32 ± 15.2
Height (m)	1.62 ± 0.1	1.62 ± 0.1

BMI (Kg/m^2)	28.3 ± 4.9	27.6 ± 4.1
Systemic blood pressure (mmHg)		
SBP	123 ± 13.6	120 ± 7.6
DBP	76 ± 15.5	75 ± 13.5
Smoking habit		
No	39 (88.6%)	34 (97.1%)
Yes	5 (11.4%)	1 (2.9%)
Pathologies		
SAH	36 (81.8%)	14 (40%)
Dyslipidemia	26 (59.1%)	7 (20%)
Osteoporosis/Osteopenia	16 (36.7%)	9 (25.7%)
Depression	11 (25%)	8 (22.8%)
DM	11 (25%)	4 (11.4%)
Hypothyroidism	10 (22.7%)	2 (5.7%)
Arthritis / Osteoarthritis	10 (22.7%)	10 (28.6%)
Drugs		
Simvastatin	21 (47.7%)	13 (37.1%)
Hydrochlorothiazide	13 (29.5%)	6 (17.1%)
Losartan	13 (29.5%)	11 (31.4%)
Levothyroxine	10 (22.7%)	2 (5.7%)
Metformin	9 (20.5%)	4 (11.4%)
Fluoxetine	9 (20.5%)	5 (14.3%)

Glucosamine+chondroitin	8 (18.2%)	9 (25.7%)
Sodium alendronate	7 (15.9%)	7 (20%)
Anlodipine	7 (15.9%)	4 (11.4%)

BMS: body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure; SAH: systemic arterial hypertension; DM: diabetes mellitus.

The duration of the treatment with omeprazole among users was from 1 to 20 years, with over 40% (18 individuals) of long-term treatments above 10 years of daily use and 31.8% (8 individuals) from 4 to 8 years of continuous use.

Vitamin B12 levels

Levels of vitamin B12 were measured and the OU group presented an average of 386 pg/mL (± 256.06 pg/mL) of vitamin B12, while the NOU group had an average of 397.55 pg/mL (± 192.82 pg/mL). There was no significant difference between the groups (p=0.866) and results found were within the considered normal range reference for vitamin B12 (180-914 pg/mL). To obtain further information related to the vitamin B12 levels, volunteers were also questioned during the interview, about their eating habits, specifically about frequency of ingestion of animal origin foods, which constitute the main source of vitamin B12. The great majority of the groups reported the daily ingestion of some kind of animal source food (97.7% of the OU group and 91.4% of the NOU group).

Oxidative stress parameters evaluation

Analyzing oxidative stress parameters, two of the biomarkers presented statistically significant differences between omeprazole users and nonusers groups: glutathione peroxidase and FRAP (Table 3).

Table 3. Oxidative stress parameters evaluation in omeprazole users and nonusers groups.

Biomarkers	Omeprazole use (n = 43)	No omeprazole use (n = 35)	p
SOD (U/L)	850 (550 – 1650)	875 (400 – 1550)	0.842
MDA (µM)	1.80 ± 0.614	1.80 ± 0.624	0.985
Catalase (K/s)	0.82 (0.24 – 4.24)	0.78 (0.32 – 4.38)	0.699
GPx (U/L)	0.534 (0.27 – 10.63)	71.86 (14.36 – 173.1)	0.006*
FRAP (µM)	1690.29 ± 441.05	1307.66 ± 616.09	0.002*

Statistical method: t-Student test. Results for catalase, SOD e GPx are expressed with median (P25 – P75) and FRAP and MDA as mean (standard deviation).

SOD: superoxide dismutase; MDA: malondialdehyde FRAP: Ferric reducing antioxidant power; GPx: glutathione peroxidase.

As mentioned before, some pathologies and drugs are known to influence oxidative stress parameters levels, that is why a multivariate analysis was held based on these variables in order to evaluate their influence on FRAP and glutathione peroxidase levels. As potential confounding factors, the pathologies evaluated were arterial hypertension, dyslipidemia, diabetes and depression and the drugs evaluated were simvastatin, metformin, fluoxetine and anti-inflammatories. It was found that levels of FRAP are not statistically influenced by these variables, as shown in Table 4. On the other hand, when analyzing the influence of these variables over the glutathione peroxidase levels, it was observed a correlation between its levels with systemic arterial hypertension.

Table 4. Multiple linear regression analysis of oxidative stress parameters in omeprazole users and nonusers groups.

Dependent variables	FRAP		GPx	
R^2 (*p*)	0.337 (<0.001)		0.390 (<0.001)	
Independent variables	P	Beta value	P	Beta value
SAH	0.332	- 0.125	0.015*	- 0.348
Dyslipidemia	0.713	0.050	0.123	0.214
Diabetes	0.931	- 0.011	0.347	0.123
Depression	0.126	0.181	0.926	0.011
Use of simvastatin	0.843	0.025	0.583	0.072
Use of fluoxetine	0.184	0.159	0.767	- 0.037
Use of metformin	0.789	- 0.031	0.732	- 0.214
Use of anti-inflammatories	0.692	0.045	0.078	0.042

Statistical method: Spearman correlation. SAH: systemic arterial hypertension; FRAP: Ferric reducing antioxidant power; GPx: glutathione peroxidase.

Cognitive ability evaluation

From the 6 tests applied to evaluate cognition, 5 presented results with statistically significant differences between the OU and NOU group, as shown in Table 5.

Table 5. Results from the cognition evaluation of omeprazole users and nonusers.

Test	Omeprazole use (n=35)	No omeprazole use (n =30)	p	Cohen's d	*r*
ATTENTION ASSESSMENT					
Psychological Battery					
Concentrated attention					
BPA AC Right answers	61.76 ± 18.85	79.97 ± 20.57	0.143	-1.246	-0.529**
BPA AC Errors	0.50 ± 1.135	0.77 ± 2.012	0.189	-0.165	-0.082
BPA AC Omissions	2.41 ± 2.463	2.93 ± 4.884	0.714	-0.134	-0.067
Divided attention					
BPA AD Right answers	46.85 ± 14.521	58.13 ± 20.574	0.013*	-0.633	-0.301**
BPA AD Errors	2.59 ± 8.068	3.57 ± 10.241	0.222	-0.106	-0.053
BPA AD Omissions	17.88 ± 15.338	15.83 ± 14.331	0.371	0.138	0.068
Alternating attention					
BPA AA Right answers	49.88 ± 18.018	57.80 ± 21.581	<0.001*	-0.398	-0.195
BPA AA Errors	1.29 ± 2.769	0.80 ± 1.349	0.758	0.224	0.111

BPA AA Omissions	6.32 ± 5.284	6.30 ± 5.730	0.786	0.003	0.001
MEMORY ASSESSMENT					
Rey Auditory Verbal Learning Test					
REY-A1A5 Total	36.31 ± 7.020	35.93 ± 8.963	0.623	0.047	0.023
REY-A6	5.14 ± 2.415	6.53 ± 2.991	0.042*	-0.511	-0.247
REY-A7	5.37 ± 2.713	6.33 ± 2.578	0.373	-0.362	-0.178
Brief Neuropsychological Assessment Battery (NEUPSILIN)					
NEUPSILIN span total	17.94 ± 4.767	18.07 ± 5.982	0.564	-0.024	-0.012
NEUPSILIN span major	1.56 ± 0.860	1.87 ± 1.074	0.097	-0.318	-0.157
EXECUTIVE FUNCTION ASSESSMENT					
Verbal Fluency Test (FVS)					
FVL Total	36.43 ± 14.914	40.80 ± 15.185	0.274	-0.290	-0.143
FVL Errors	0.54 ± 1.172	0.63 ± 1.245	0.680	-0.074	-0.037
FVF1	6.59 ± 2.743	8.87 ± 2.825	0.02*	-0.818	-0.378**
FVF2	3.26 ± 2.274	4.80 ± 2.355	0.01*	-0.665	-0.315**

FVF3	2.62 ± 2.229	3.23 ± 2.128	0.183	-0.279	-0.138
FVF4	2.15 ± 2.217	3.03 ± 2.266	0.245	-0.392	-0.192
FVF Total	14.62 ± 7.816	19.93 ± 7.488	0.007*	-0.693	-0.327**
FVF Errors	0.21 ± 0.538	0.53 ± 0.776	0.087	-0.479	-0.233**
VFS1	8.41 ± 2.488	10.50 ± 2.610	0.002*	-0.819	-0.379**
VFS2	4.59 ± 2.500	5.47 ± 2.417	0.148	-0.357	-0.176
VFS3	2.88 ± 2.071	3.73 ± 1.639	0.119	-0.455	-0.221
VFS4	1.91 ± 1.658	2.70 ± 1.932	0.120	-0.438	-0.214
VFS Total	17.79 ± 6.149	22.40 ± 6.032	0.004*	-0.756	-0.353**
VFS Errors	0.15 ± 0.558	0.27 ± 0.640	0.498	-0.199	-0.099
Five Digit Test					
FDT Reading Time	52.97 ± 20.34	46.13 ± 22.73	0.075	0.317	0.156
FDT Reading Errors	0.91 ± 4.895	0	0.346	0.262	0.130

FDT Choosing Time	107.73 ± 71.97	80.41 ± 29.92	0.046*	0.495	0.240
FDT Choosing Errors	4.34 ± 8.489	1.80 ± 2.369	0.244	0.407	0.199
FDT Counting Time	60.55 ± 19.46	49.05 ± 14.12	0.008*	0.676	0.320**
FDT Counting Errors	1.40 ± 7.093	0.10 ± 0.305	0.191	0.258	0.128
FDT Shifting Time	142.21 ± 54.005	126.9 ± 62.24	0.918	0.262	0.130
FDT Shifting Errors	5.97 ± 6.797	4.23 ± 5.519	0.579	0.281	0.139
Hayling Test					
Hayling Time A	29.24 ± 20.59	23.4 ± 17.18	0.132	0.307	0.152
Hayling Errors A	0.80 ± 0.964	0.43 ± 0.728	0.074	0.433	0.211
Hayling Time B	80.94 ± 39.91	76.7 ± 49.72	0.182	0.094	0.046
Hayling Errors B/15	5.03 ± 3.303	3.50 ± 3.214	0.066	0.469	0.228
Hayling Errors B/45	15.06 ± 9.876	10.50 ± 9.641	0.068	0.467	0.227
Hayling Time B-A1	50.1 ± 30.49	53.29 ± 43.62	0.453	-0.084	-0.042
Hayling Time B/A2	3.14 ± 1.19	3.97 ± 2.99	0.426	-0.364	-0.179

Hayling CB11	10.94 ± 8.643	15.27 ± 7.404	0.036*	-0.538	-0.259
Hayling CB12	9.69 ± 6.471	13.80 ± 8.298	0.032*	-0.552	-0.266
Hayling CB13	13.11 ± 9.380	15.80 ± 6.316	0.152	-0.336	-0.165
Hayling CB14	8.89 ± 8.145	13.13 ± 7.964	0.038*	-0.526	-0.254

Statistical method: t-Student test. Results are expressed with mean (standard deviation). BPA AC: Psychological battery assessment on concentrated attention; BPA AD: Psychological battery assessment on divided attention; BPA AA: Psychological battery assessment on alternating attention. FVF: Phonemic verbal fluency. VFS: Semantic verbal fluency. BPA: Psychological battery for attention assessment; FDT: Five Digit Test.

On the Psychological Battery, the OU group presented statistically inferior outcomes concerning divided and alternating attention when compared to the NOU group. The same was found on the Rey Auditory Verbal Learning Test, that is related to short term episodic memory. For the executive function assessment, the OU group recalled a shorter amount of words that the NOU group (Verbal Fluency Test (FVS)), besides taking a longer time to fulfil the activities (Five Digit Test) and weren't able to complete sentences as correctly as the NOU group (Hayling Test).

Due to its relevance to the cognitive ability assessment, the variable education time was also evaluated in order to verify if groups sustained a similar pattern. The OU group had an average of 5.8 (± 2.3) years of education time, while the NOU group presented the average of 8.4 (± 3.3) years, a statistically significant difference was found between groups (p=0.025).

Therefore, a second analysis was held to evaluate the correlation between education time and the results of the cognitive evaluation of the OU group and

the NOU group. No correlation was found in the OU group, showing the education time didn't interfere significantly on the omeprazole users' performance. In the NOU group, the education time showed correlation with some of the results of the cognitive assessment. However, when crossing these results with the performance differences between the OU and NOU groups, it only overlapped with two tests results with statistically significant findings: the Verbal Fluency Test (FVS) and the Five Digit Test, both part of the executive functions assessment. The other performances with statistically significant differences were not influenced by the education time of each group.

The relation of pathologies and drugs was also tested and didn't present any statistical correlation to the results found.

When evaluating the cognitive performance correlation with oxidative stress parameters analyzed, all oxidative stress parameters presented inverse correlation with several cognitive results (Table 6).

Table 6. Correlation of oxidative stress parameters and cognitive evaluation performance of omeprazole users

Cognitive test	SOD	FRAP	GPX	MDA	CAT
	R / p	R / p	R / p	R / p	R / p
ATTENTION ASSESSMENT					
Psychological Battery					
BPAAC	0.561 / 0.002	-	-	-	-
omissions					
MEMORY ASSESSMENT					
Rey Auditory Verbal Learning Test					
REYA3	-0.534 / 0.003	-	-	-	-
REYA4	-0.417 / 0.024	-	-	-	-
REYA6	-	-0.05 / 0.002	-	-	-
REYA7	-	-	-0.43 / 0.019	-	-
REYB1	-0.390 / 0.037	-	-	-	-
REYA1A5	-0.405 / 0.029	-	-	-	-
Brief Neuropsychological Assessment Battery (NEUPSILIN)					
NEUSPAN	-	-	-	0.348 / 0.044	-
Total					
EXECUTIVE FUNCTION ASSESSMENT					
Verbal Fluency Test (FVS)					
FVL Total	-0.576 / 0.001	-	-	-	-
FVL2	-0.587 / 0.001	-	-	-	-
FVL3	-0.411 / 0.0027	-	-	-	-
FVL4	-0.662 / <0.001	-	-	-	-
FVL5	-0.562 / 0.002	-	-0.383 / 0.040	-	-
FVF4	-	-	-0.470 / 0.012	-	-
SFV3	-0.620 / <0.001	-	-	-	-
SFV 4	-0.378 / 0.047	-	-	-	-
SFV	-	-0.393 / 0.024	-	-	-
Errors					
SFV Total	-0.540 / 0.003	-	-	-	-
The Five Digit Test					

FDT Reading Time	-	-	-	0.376 / 0.026	-
FDT Choosing Time	-	-	-	0.377 / 0.026	-
FDT Alternating Time	-	-	-	-	-0.348/0.04
Hayling Test					
HAYCB3	-	-	-	-	-
HAYCB4	-	-	-0.376 / 0.044	-	-
HAYCB8	-0.509 / 0.005	-	-	-	-
HAYCB9	-	0.414 / 0.015	-	-	-

Statistical method: Spearman correlation. BPA AC: Psychological battery assessment on concentrated attention; FVL: Free verbal fluency. FVF: Phonemic verbal fluency. VFS: Semantic verbal fluency. BPA: Psychological battery for attention assessment; FDT: Five Digit Test.

Discussion

The aim of the present study was to evaluate the correlation between oxidative stress parameters, cognitive impairment and vitamin B12 levels in long-term omeprazole users.

Omeprazole users and nonusers groups presented similar characteristics regarding gender, age and smoking habits, therefore these variables did not interfere on the results found when evaluating the cognitive performance and the oxidative stress parameters. Levels of vitamin B12 remained on the same range among omeprazole users and nonusers, even though some previous studies indicated a connection between levels of vitamin B12 with an inferior cognitive performance, as observed in a study that evaluated patients from a memory clinic setting (Pereira, Do Couto and Mendonça, 2006). On the other hand, recent studies show poor evidence of the connection of vitamin B12 levels in plasma

and omeprazole use, as seen in a prospective study that evaluated 200 omeprazole, esomeprazole, lansoprazole and pantoprazole users for 12 months, which did not result in clinically significant vitamin B12 deficiency (Qorraj-Bytyqi et al., 2018). Low levels of vitamin B12 in plasma are only one of the possible factors involved in the cognitive damage process and even though the findings of this study are normal, that does not exclude the possibility of an early cognitive impairment.

Although there were differences on the comorbidities and treatments profile, when investigated, these factors did not affect the results obtained on the cognitive ability assessment. Their connection with the oxidative stress parameters was also checked and no relation was found, except for a relation between GPx levels and systemic arterial hypertension, an evidence already established in a previous case-control study, where low levels of glutathione peroxidase were observed in hypertensive patients compared to controls (Muda et al., 2003).

On the evaluation of oxidative stress parameters, the levels of ferric reducing antioxidant power (FRAP) and glutathione peroxidase (GPx) showed statistically significant difference when comparing OU and NOU groups. As previously mentioned, up to the present moment, there are only few studies available verifying the connection between proton pump inhibitors use with oxidative stress parameters.

Shivanna et al. (2015) verified the effects of omeprazole in newborn mice treated daily for 14 days. Through lung tissue analysis, there was pulmonary vascular injury and increased levels of malondialdehyde. However, Agnihotri et al. (2007) and Chanchal et al. (2016) also evaluating mice, observed an up

regulation of SOD and catalase activity and decreased levels of MDA, suggesting omeprazole prevents inflammatory damage and increases endogenous anti-oxidant defense system.

Besides using animal models exposed to omeprazole for a short-term period, these studies did not evaluate FRAP and GPx and, for these reasons, the outcomes observed in these studies relating omeprazole use and oxidative stress parameters are not suitable for comparison to the findings from the present study.

The increased levels of FRAP with the low levels of GPx may suggest compensatory anti-oxidant response of the organism to an overproduction of reactive oxygen species, which are known to be involved in several neurocognitive disorders, such as Alzheimer's and Parkinson's Disease (Rahman, 2007). No previous studies evaluating the connection between oxidative stress parameters and omeprazole in humans were found.

The assessment of the connection between oxidative stress parameters and the cognitive evaluation results also pointed an unprecedented outcome: the statistically significant correlation between these two variables. GPx, FRAP, catalase and SOD activity were inversely correlated to the cognitive performance, while malondialdehyde presented a positive correlation. These results may indicate a higher level of reactive oxygen species (ROS) among subjects with inferior cognitive assessment performances. ROS are known to be neurotoxic, as can freely pass through the plasma membrane and accumulate in the brain (LI et al. 2013), causing mutations and cell death and resulting in neurodegenerative disease.

Besides that, it is possible to observe that SOD activity presented these correlations in all three domains assessed (attention, episodic memory and

executive functions), FRAP, GPx and MDA correlations were related to episodic memory and executive functions and, lastly, catalase correlated to an executive function assessment.

Regarding the cognitive ability assessment, even with the statistically significant influence of the education time in two of the test scores, overall the OU group performance was inferior in several domains evaluated, with a statistically significant difference and medium to large effect size in comparison to the NOU group. On the attention assessment, the omeprazole users group had greater difficulty to maintain attention in face of more than one focal matter. Besides that, episodic memory evaluation showed omeprazole users had lower scores when compared to nonusers, specifically on short-term episodic memory.

The greater difficulty of omeprazole users was reinforced by the executive functions assessment, where this group failed to provide more adequate answers and avoid wrong ones, which relates to the ability of manipulating the information, also known as work memory.

Therefore, observing the cognitive outcome, it is possible to verify that the assessments with statistically significant difference suggest omeprazole users show impairment on the automatic processing, besides attention, inhibitory control loss and episodic memory.

These results are in accordance to the ones found by other studies, which evaluated the impact of proton pump inhibitors use over the cognitive ability of subjects. Akter and collaborators (2015) evaluated 60 healthy subjects with average age of 24 years old, divided into six groups. Each of the groups received a 7-day treatment with a different PPI (omeprazole, rabeprazole, esomeprazole, pantoprazole e lansoprazole) and a control group. Cognitive ability was evaluated

prior and after treatment. In all the tests performed, at least in 3 groups there were statistically significant differences on the cognitive results found. Differently from the present research, the referred study focused on young healthy subjects under short-term exposure to PPIs, which raises the interest about its influence over older subjects. However, conducting studies evaluating specific criteria in an older population is a challenging mission, since, as age progresses, subjects tend to develop comorbidities and conditions that may influence outcomes through other pathways, specifically pointing to oxidative stress parameters and cognitive ability assessment, which are the focus of the present study.

Regarding neurocognitive disorders, studies have been verifying strong associations between cognitive impairment to oxidative stress damage, through mechanisms that involve neuronal degeneration, alterations of neuronal signaling and neuroinflammation (Popa-Wagner et al., 2013).

Even the analyses performed being based on groups with similar profiles, results presented may have suffered influences from comorbidities, treatments and education time differences. Besides that, participants didn't go through psychological screening tests to evaluate previous history of psychological disorders and the sample studied was limited and data concerning eating habits and life style were obtained through subjects' report only.

In conclusion, based on the results found in the present study concerning oxidative stress parameters and cognitive outcome, it is suggested a potential connection between omeprazole use, oxidative stress and cognitive impairment.

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Artigo encaminhado para publicação na revista *Journal of Psychopharmacology*

Declaration of Conflicting Interests

The authors declare that there is no conflict of interest.

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5 CONSIDERAÇÕES FINAIS

O alto consumo de omeprazol é fator preocupante devido às possíveis complicações relacionadas ao seu uso, abrangendo o presente estudo especificamente o prejuízo da habilidade cognitiva, que pode ser um indicativo de propensão do indivíduo ao surgimento de transtornos neurocognitivos, em que o paciente pode se tornar completamente dependente e sem autonomia para atividades cotidianas.

Na caracterização destes transtornos, já há a identificação da presença de estresse oxidativo, principal fator no processo de envelhecimento do organismo, responsável por danos ao DNA e envolvido na patogênese de desordens neurodegenerativas. Apesar desta ligação ser bem estabelecida, não foi encontrada na literatura estudos prévios avaliando a associação entre o estresse oxidativo e o déficit cognitivo em usuários de omeprazol. Portanto, este é o primeiro estudo a investigar a correlação entre estes fatores.

O presente estudo encontrou resultados que indicam uma potencial ligação entre o estresse oxidativo e o prejuízo cognitivo em pacientes que fazem uso prolongado de omeprazol, com interferências no desempenho de suas funções executivas, memória e atenção. Tendo em vista a continuidade da exploração deste tema, como perspectiva para o futuro deste assunto, faz-se relevante analisar o efeito do omeprazol de forma isolada, tanto sobre os parâmetros de estresse oxidativo à longo prazo, quanto aos seus potenciais efeitos diretamente em tecido neurológico. Além disso quanto à influência do uso de omeprazol sobre o desempenho cognitivo, poderia se realizar um estudo de acompanhamento com pacientes que iniciam o tratamento com o omeprazol, realizando inclusive avaliação cognitiva prévia ao tratamento, como indicativo mais confiável quanto à caracterização de prejuízo cognitivo.

A identificação das causas de problemas cognitivos pode colaborar com a prevenção do transtorno neurocognitivo maior (demência) e sua descoberta precoce pode impedir sua evolução, reduzindo assim o impacto negativo que causa na vida de seus portadores. Além disso, sua associação ao uso de omeprazol possibilita uma maior conscientização quantos aos eventos adversos

relacionados a este fármaco, cuja extensão ainda é subestimada, tendo em visto sua ampla utilização.

6 REFERÊNCIAS

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APÊNDICE A – Características Gerais Do Paciente

CARACTERÍSTICAS GERAIS DO PACIENTE

Nome:_____Data:__/___/_____

Data de nascimento: ___/___/_____ Idade: _____ anos Sexo: ( ) M ( ) F

Escolaridade: ( ) analfabeto ( ) fundamental incompleto ( ) fundamental completo ( ) médio incompleto ( ) médio completo ( ) superior incompleto ( ) superior completo ( ) pós-graduação

Qual a sua renda mensal familiar?_____

Tabagismo ( ) sim ( ) não Tempo__ Quantidade___ Ex - fumante há___ anos/meses

Alcoolismo ( ) sim ( ) não Tempo __ Quantidade___ Ex - alcoólatra há__ anos/meses

Peso _____kg Altura _____ IMC _____ Pressão Arterial _____

Com que frequência consome peixes, carnes, ovos, queijo e leite durante a semana?

_____

_____

Faz uso de vitaminas/antioxidantes? ( )sim ( )não

Se utiliza, qual(is)_____

Demais patologias:

Patologia	Tempo diagnóstico

Uso de medicamentos:

Número	Medicamento	Dose	Indicação	Tempo de uso
1				
2				
3				
4				
5				

ANEXO A – Termo De Consentimento Livre E Esclarecido (TCLE)

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO (TCLE)

Você está sendo convidado a participar do projeto institucional intitulado: Avaliação da neurotoxicidade associada ao uso de omeprazol, sob responsabilidade da professora Dra. Magda Susana Perassolo. Os objetivos deste estudo são avaliar a neurotoxicidade relacionada ao uso crônico de omeprazol.

Sua participação nesta pesquisa será voluntária e consistirá em realizar uma coleta de sangue e responder aos questionários de avaliação das características gerais dos pacientes e uso do omeprazol (composto por 15 questões), atividade física (composto por 8 questões) e os testes de avaliação cognitiva (Escala Wechsler de Inteligência para Adultos; Teste de Atenção Dividida; Teste de Atenção Sustentada; Teste Hayling; Teste de trilhas; Bateria Montreal de Avaliação da Comunicação e Teste de Aprendizagem Auditivo-Verbal de Rey). O tempo médio de aplicação de todos os questionários é de 60 min.

Os riscos e/ou desconfortos relacionados a sua participação são o desconforto da picada para retirada do sangue, sendo que os procedimentos realizados não envolvem qualquer risco de vida para os pacientes.

O pesquisador responsável e as instituições e/ou organizações (nomear patrocinadores, instituições e organizações coparticipantes) envolvidas nas diferentes fases da pesquisa proporcionarão assistência imediata e integral aos participantes da pesquisa no que se refere às possíveis complicações e danos decorrentes. Os participantes da pesquisa que vierem a sofrer qualquer tipo de dano resultante de sua participação na pesquisa, previsto ou não neste documento, têm direito à indenização, por parte do pesquisador, do patrocinador e das instituições envolvidas nas diferentes fases da pesquisa.

A sua participação nesta pesquisa estará contribuindo para esclarecer o papel do omeprazol na deficiência da vitamina B12, no processo de estresse oxidativo e na perda de função cognitiva possivelmente causada pelo uso deste medicamento.

Garantimos o sigilo de seus dados de identificação primando pela privacidade e por seu anonimato. Manteremos em arquivo, sob nossa guarda,

por 5 anos, todos os dados e documentos da pesquisa. Após transcorrido esse período, os mesmos serão destruídos. Os dados obtidos a partir desta pesquisa não serão usados para outros fins além dos previstos neste documento.

Você tem a liberdade de optar pela participação na pesquisa e retirar o consentimento a qualquer momento, sem a necessidade de comunicar-se com o(s) pesquisador(es).

Este Termo de Consentimento Livre e Esclarecido será rubricado em todas as folhas e assinado em duas vias, permanecendo uma com você e a outra deverá retornar ao pesquisador. Abaixo, você tem acesso ao telefone e endereço eletrônico institucional do pesquisador responsável, podendo esclarecer suas dúvidas sobre o projeto a qualquer momento no decorrer da pesquisa.

Nome do pesquisador responsável: Magda Susana Perassolo
Telefone institucional do pesquisador responsável: 35868800 – ramal 8938
E-mail institucional do pesquisador responsável: magdaperassolo@feevale.br

_____
Assinatura do pesquisador responsável

Local e data: _____, _____ de _____20_____.

Declaro que li o TCLE: concordo com o que me foi exposto e aceito participar da pesquisa proposta.

_____
Assinatura do participante da pesquisa

APROVADO PELO CEP/FEEVALE – TELEFONE: (51) 3586-8800
Ramal 9000

ANEXO B – Parecer Consubstancial Do CEP

UNIVERSIDADE FEEVALE
COMITÊ DE ÉTICA EM PESQUISA - CEP

UNIVERSIDADE FEEVALE/ASSOCIAÇÃO PRÓ-ENSINO SUPERIOR EM NOVO

Plataforma Brasil

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Avaliação da neurotoxicidade associada ao uso de omeprazol
Pesquisador: MAGDA SUSANA PERASSOLO
Área Temática:
Versão: 2
CAAE: 80153617.0.0000.5348
Instituição Proponente: ASSOCIACAO PRO ENSINO SUPERIOR EM NOVO HAMBURGO
Patrocinador Principal: ASSOCIACAO PRO ENSINO SUPERIOR EM NOVO HAMBURGO

DADOS DO PARECER

Número do Parecer: 2.455.741

Apresentação do Projeto:
De acordo

Objetivo da Pesquisa:
De acordo

Avaliação dos Riscos e Benefícios:
De acordo.

Comentários e Considerações sobre a Pesquisa:
De acordo.

Considerações sobre os Termos de apresentação obrigatória:
De acordo.

Recomendações:
Não Há

Conclusões ou Pendências e Lista de Inadequações:
De acordo.

Considerações Finais a critério do CEP:
Em conformidade com a Resolução nº 466 de 12 de dezembro de 2012, do Conselho Nacional de Saúde, e com as normas internas do Comitê de Ética em Pesquisa da Universidade Feevale, todos os documentos necessários à análise do projeto acima referido por este Comitê foram

Endereço: RS 239, nº 2755
Bairro: Vila Nova **CEP:** 93.525-075
UF: RS **Município:** NOVO HAMBURGO
Telefone: (51)3586-8800 **Fax:** (51)3586-8800 **E-mail:** raniell@feevale.br

UNIVERSIDADE FEEVALE

UNIVERSIDADE FEEVALE COMITÊ DE ÉTICA EM PESQUISA - CEP

UNIVERSIDADE FEEVALE/ASSOCIAÇÃO PRÓ-ENSINO SUPERIOR EM NOVO

Plataforma Brasil

Continuação do Parecer: 2.455.741

apresentados.

Este projeto preserva os aspectos éticos dos sujeitos da pesquisa, sendo, portanto, aprovado pelo Comitê de Ética em Pesquisa da Universidade Feevale.

Reiteramos que o Comitê de Ética em Pesquisa da Instituição encontra-se à sua disposição para equacionar eventuais dúvidas e/ou esclarecimentos que se fizerem necessários.

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_1026714.pdf	12/12/2017 14:27:08		Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE.doc	12/12/2017 14:26:47	MAGDA SUSANA PERASSOLO	Aceito
Outros	formulario.pdf	20/11/2017 17:33:44	MAGDA SUSANA PERASSOLO	Aceito
Declaração de Pesquisadores	declaracaopesquisador1.pdf	20/11/2017 17:33:11	MAGDA SUSANA PERASSOLO	Aceito
Folha de Rosto	folhaderostoassinada.pdf	20/11/2017 17:32:54	MAGDA SUSANA PERASSOLO	Aceito
Outros	CARACTERISTICAS.docx	06/11/2017 11:10:16	MAGDA SUSANA PERASSOLO	Aceito
Outros	WHOQOL.docx	06/11/2017 11:09:44	MAGDA SUSANA PERASSOLO	Aceito
Projeto Detalhado / Brochura Investigador	formulario.doc	06/11/2017 11:07:52	MAGDA SUSANA PERASSOLO	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

Endereço: RS 239, nº 2755
Bairro: Vila Nova **CEP:** 93.525-075
UF: RS **Município:** NOVO HAMBURGO
Telefone: (51)3586-8800 **Fax:** (51)3586-8800 **E-mail:** raniell@feevale.br

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Plataforma Brasil

Continuação do Parecer: 2.455.741

NOVO HAMBURGO, 22 de Dezembro de 2017

Assinado por:
Ranieli Gehlen Zapelini
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