Universidade Estadual de Londrina

NAYARA RAMPAZZO MORELLI

ENVOLVIMENTO DO ESTRESSE OXIDATIVO E/OU NITROSATIVO EM INDIVÍDUOS COM TRANSTORNOS DE HUMOR E NOS COMPONENTES DA SÍNDROME METABÓLICA

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Tese de Doutorado apresentada ao Programa de Pós-Graduação em Ciências da Saúde da Universidade Estadual de Londrina, como requisito parcial para obtenção do título de doutora em Ciências da Saúde.

Orientador: Prof. Dr. Décio Sabbatini Barbosa

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“Um dia, quando olhares para trás, verás que os dias mais belos foram aqueles em que lutaste”.

Sigmund Freud

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RESUMO

Os transtornos de humor, tais como o Transtorno Depressivo Maior (TDM) e o Transtorno Afetivo Bipolar (TAB) – classificado em TAB tipos I e II – compõem um grupo de doenças psiquiátricas que podem afetar ao mesmo tempo as emoções, a energia e a motivação. Indivíduos com TDM e TAB apresentam um risco maior para Síndrome Metabólica (SM), um conjunto de fatores de risco que incluem obesidade abdominal, dislipidemia, hipertensão e resistência à insulina. O estresse oxidativo (EO) e nitrosativo (EN), definido como um desequilíbrio entre defesas antioxidantes e espécies reativas de oxigênio (ERO) e nitrogênio (ERN), tem sido estudado nos transtornos de humor e na SM. Por esse motivo, o objetivo do presente estudo foi avaliar o envolvimento de diferentes biomarcadores de EO/EN com os transtornos de humor e demonstrar a influência da SM, quando presente nestes transtornos, sobre o EO. Trata-se de um estudo transversal que contou com a participação de 191 indivíduos entre controles e pacientes com transtornos de humor (TDM, TAB I e TAB II). Os pacientes foram recrutados no Ambulatório de Psiquiatria do Hospital Universitário da Universidade Estadual de Londrina (UEL) e os controles entre estudantes e funcionários da mesma universidade. Todos os participantes foram submetidos a uma avaliação clínica para obter os diagnósticos necessários. Foi realizada avaliação antropométrica e da pressão sanguínea. As amostras de sangue foram coletadas após 12 horas de jejum para a avaliação dos parâmetros bioquímicos e dos biomarcadores de EO/EN. Além disso, foram propostos diferentes índices de EO/EN. O estudo resultou em dois trabalhos (artigos). No primeiro trabalho, foi observado um aumento nos níveis de superóxido dismutase (SOD) e em todos os índices de EO/EN em pacientes depressivos. Os níveis de produtos avançados de oxidação proteica (AOPP) foram mais altos em pacientes com TAB I enquanto alguns índices de EO/EN foram mais baixos em pacientes com TAB II. No segundo trabalho, os níveis de AOPP foram associados com os níveis de triglicerídeos (TG) em pacientes com transtornos de humor e SM. AOPP parece ser o principal biomarcador de EO envolvido com a SM no TAB, principalmente no TAB I. Correlações entre parâmetros da SM e os níveis de capacidade antioxidante total (TRAP/AU) e SOD foram observadas no TAB II e TDM. Os níveis de glicose foram associados com os níveis de malondialdeído (MDA) em pacientes com transtornos de humor e SM. Os resultados do presente estudo sugerem que o TDM, TAB I e TAB II podem apresentar características específicas no que diz respeito ao EO/EN e, por isso, mais estudos são necessários para avaliar a aplicabilidade dos biomarcadores de EO/EN.

Palavras-chave: transtorno depressivo; transtorno bipolar; síndrome metabólica; estresse oxidativo; estresse nitrosativo.

MORELLI, Nayara Rampazzo. **Involvement of oxidative and/or nitrosative stress in individuals with mood disorders and metabolic syndrome components**. 2020. 77 p. Thesis (Doctorate in Health Sciences) – State University of Londrina, Londrina, 2020.

ABSTRACT

Mood disorders, such as Major Depressive Disorder (MDD) and Bipolar Disorder (BD) – classified in BD types I and II – make up a group of psychiatric illnesses that can affect emotions, energy and anxiety at the same time. Subjects with MDD and BD are at increased risk for Metabolic Syndrome (MetS), a set of risk factors that include abdominal obesity, dyslipidemia, hypertension and insulin resistance. Oxidative (OS) and nitrosative (NS) stress, defined as an imbalance between antioxidant defenses and reactive oxygen (ROS) and nitrogen (RNS) species, has been studied in mood disorders and MetS. For this reason, the aim of the present study was to assess the involvement of different OS/NS biomarkers with mood disorders and to demonstrate the influence of MetS, when present in these disorders, on OS. This is a cross-sectional study with the participation of 191 subjects between controls and patients with mood disorders (MDD, BD I and BD II). The patients were recruited at the Psychiatry Outpatient Clinic from the University Hospital of the State University of Londrina (UEL) and the controls between students and staff of the same university. All participants underwent a clinical assessment to obtain the necessary diagnoses. Anthropometric and blood pressure assessments were performed. Blood samples were collected after 12 hours of fasting to evaluate biochemical parameters and OS/NS biomarkers. In addition, different OS/NS indexes have been proposed. The study resulted in two works (papers). In the first one, an increase in superoxide dismutase (SOD) levels and in all OS/NS indexes was observed in depressed patients. Advanced protein oxidation products (AOPP) levels were higher in patients with BD I while some OS/NS indexes were lower in patients with BD II. In the second one, AOPP levels were associated with triglycerides (TG) levels in patients with mood disorders and MetS. AOPP seems to be the main OS biomarker involved with MetS in BD, mainly in BD I. Correlations between parameters of MetS and the levels of total antioxidant capacity (TRAP/UA) and SOD were observed in BD II and MDD. Glucose levels were associated with malondialdehyde (MDA) levels in patients with mood disorders and MetS. The results of the present study suggest that MDD, BD I and BD II may have specific characteristics with regard to OS/NS and, therefore, further studies are needed to assess the applicability of OS/NS biomarkers.

Keywords: depressive disorder; bipolar disorder; metabolic syndrome; oxidative stress; nitrosative stress.

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1 INTRODUÇÃO

1.1 Transtornos de Humor

Os transtornos de humor, tais como o Transtorno Depressivo Maior (TDM) e o Transtorno Afetivo Bipolar (TAB), compõem um grupo de doenças psiquiátricas que podem afetar ao mesmo tempo as emoções, a energia e a motivação (RAKOFSKY; RAPAPORT, 2018).

O TDM caracteriza-se por episódios depressivos, enquanto indivíduos com TAB apresentam oscilações no humor, com episódios de mania, hipomania e depressão, alternados ou conjuntamente. A prevalência ao longo da vida para TDM é de 16%, sendo maior em mulheres do que em homens e com início em média aos 32 anos de idade. Para o TAB, incluindo os indivíduos que apresentam espectro do transtorno bipolar, a prevalência ao longo da vida fica próximo de 5%. O TAB é ainda classificado em tipos I e II de acordo com o curso da doença. O TAB I afeta igualmente homens e mulheres e tem início em média aos 18,2 anos de idade, enquanto o TAB II é mais comum em mulheres e seu início ocorre em média aos 20,3 anos de idade (GRANDE *et al*., 2016; RAKOFSKY; RAPAPORT, 2018).

Comorbidades psiquiátricas são frequentemente observadas no TDM e no TAB. Em indivíduos com TDM, cerca de 60% apresentam transtornos de ansiedade e 24% transtornos por uso de substâncias. No TAB, aproximadamente 75% dos indivíduos apresentam transtornos de ansiedade, 42,3% transtornos por uso de substâncias e 60% transtornos de personalidade (RAKOFSKY; RAPAPORT, 2018).

Os sintomas centrais do TDM compreendem humor depressivo, como redução da motivação ou falta de esperança, falta de interesse por atividades prazerosas, como comer e interações sociais, falta de energia, irritabilidade, dificuldade de concentração, perturbação do sono, apetite e cognição e tendência para suicídio (YANG *et al*., 2015). Como os critérios para um episódio depressivo maior no TAB são os mesmos utilizados para o TDM, algumas diferenças na sintomatologia são importantes para distinguir os episódios entre os dois transtornos de humor (GRANDE *et al*., 2016; RAKOFSKY; RAPAPORT, 2018).

No TAB, durante os episódios depressivos são observados redução da energia, tristeza, retraimento social, sono excessivo e baixa autoestima. Sintomas psicóticos, que estão relacionados com a mudança de percepção da realidade (por exemplo, alucinação e delírio), são mais característicos dos indivíduos bipolares e podem ocorrer durante os episódios depressivos, embora sejam mais comuns na mania. Além disso, os indivíduos bipolares normalmente apresentam episódios mais frequentes, de menor duração e com um início e fim abrupto (GRANDE *et al*., 2016; SCHRIMPF; AGGARWAL; LAURIELLO, 2018; VIETA *et al*., 2018).

Episódios maníacos ou hipomaníacos são considerados um estado de elevação do humor e agitação motora com diferentes tempos de duração e severidade. Os episódios maníacos são caracterizados por hiperatividade, aumento da autoestima, grandiosidade, necessidade reduzida de sono, comportamento e humor expansivo e sintomas psicóticos. Os episódios hipomaníacos são uma forma mais leve e mais curta da mania. Embora ambos os episódios possam prejudicar o convívio social dos indivíduos, as crises de hipomania normalmente não trazem um comprometimento tão grave, enquanto a mania pode levar até mesmo à hospitalização (GRANDE *et al*., 2016; VIETA *et al*., 2018).

O diagnóstico de TAB I é conferido a indivíduos que tiveram episódios maníacos, enquanto o diagnóstico de TAB II é direcionado para aqueles que tiveram episódios hipomaníacos e pelo menos um episódio de depressão maior, mas nunca apresentaram mania (RAKOFSKY; RAPAPORT, 2018) (Figura 1). O TAB I parece apresentar uma evolução mais tortuosa e um prognóstico mais grave devido à severidade dos episódios, porém, no TAB II a qualidade de vida dos indivíduos é mais prejudicada por apresentarem frequência maior dos episódios, taxas elevadas de comorbidades psiquiátricas e comportamento suicida recorrente (GRANDE *et al*., 2016).

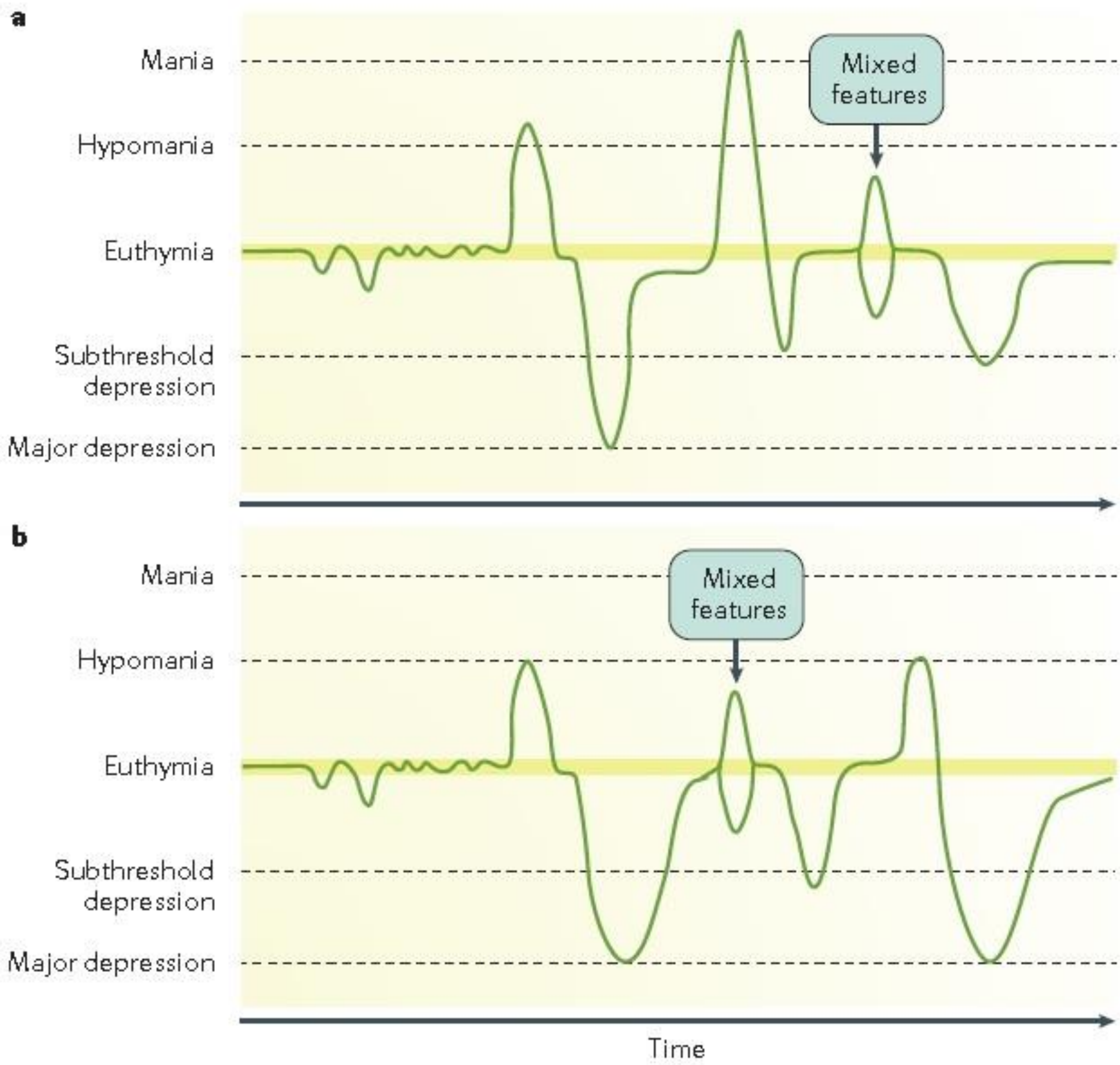

Figura 1: Classificação do Transtorno Afetivo Bipolar (TAB) em tipos I e II de acordo com o curso da doença. TAB I **(a)**; TAB II **(b)** (adaptado de VIETA *et al*., 2018).

Os transtornos psiquiátricos representam grande parte dos suicídios e tentativas suicidas, com números pelo menos dez vezes maiores que na população em geral (BACHMANN, 2018). O risco para suicídio parece ser maior durante os primeiros meses após o diagnóstico dos transtornos psiquiátricos. Além disso, existe um alto risco na primeira semana após a alta por internação psiquiátrica e entre indivíduos que apresentaram automutilação (BOLTON; GUNNELL; TURECKI, 2015).

A depressão é a principal causa de mortes por suicídio em todo o mundo. Os suicídios decorrentes da depressão ocorrem com mais frequência em idosos que na grande maioria vivenciam sintomas psicóticos. No TAB, os suicídios e tentativas suicidas coincidem com o primeiro episódio depressivo na juventude (BACHMANN, 2018). A presença de comorbidades como os transtornos de ansiedade e por uso de substâncias são fatores fortemente associados ao suicídio e tentativa suicida nesses transtornos de humor (GRANDE *et al*., 2016; HAWTON *et al*., 2013).

Transtornos por uso de substâncias como o álcool e drogas ilícitas, o uso indevido de medicação e o tabagismo, coexistem com os transtornos psiquiátricos. Um transtorno pode influenciar o outro diretamente, por exemplo, o consumo excessivo de álcool por indivíduos com dependência pode levar à depressão. Da mesma forma, essa comorbidade pode ocorrer indiretamente, como a dependência resultante do uso contínuo de substâncias para automedicação ou para aliviar o sofrimento causado pelos transtornos psiquiátricos (KLEIN, 2016; LAI *et al*., 2015).

Sintomas depressivos são fatores importantes relacionados com o início, a manutenção e a cessação do tabagismo, assim como a dependência de nicotina está associada com a suscetibilidade para depressão (KUTLU; PARIKH; GOULD, 2015). Os transtornos por uso de substâncias são comuns em indivíduos com TAB apresentando maior prevalência em comparação com o TDM (GOLD *et al*., 2018).

Anteriormente, acreditava-se que os transtornos de humor eram o resultado de um desequilíbrio nos sistemas de neurotransmissores monoaminérgicos, incluindo a serotonina, a noradrenalina e, particularmente no TAB, a dopamina. Existem evidências de que esses sistemas provavelmente desempenham um papel nos transtornos de humor, entretanto nenhuma disfunção exclusiva foi identificada até o momento (GRANDE *et al*., 2016).

O tratamento com antidepressivos envolve os sistemas de neurotransmissores monoaminérgicos. Os antidepressivos tricíclicos foram a primeira classe de medicamento utilizada no tratamento da depressão. Seu mecanismo de ação ocorre através da inibição da recaptação de serotonina e/ou noradrenalina. Outros agentes foram desenvolvidos para serem inibidores mais seletivos e reduzir os efeitos colaterais que tornam os antidepressivos tricíclicos pouco tolerados. Atualmente, a primeira linha de tratamento para a depressão inclui os inibidores seletivos da recaptação de serotonina (ISRS), como a fluoxetina, e os inibidores da recaptação de serotonina-noradrenalina (IRSN), como a venlafaxina (BLOCK; NEMEROFF, 2014; HARMER; DUMAN; COWEN, 2017).

Os inibidores da monoamina oxidase (MAO) atuam bloqueando a degradação enzimática da serotonina, noradrenalina e dopamina. Tanto os ISRS e IRSN como os inibidores da MAO resultam no aumento dos níveis desses neurotransmissores na fenda sináptica. Os antidepressivos atípicos também atuam nos neurotransmissores monoaminérgicos, receptores e transportadores, como a bupropiona, que tem o uso associado ao tratamento para a cessação do tabagismo (BLOCK; NEMEROFF, 2014).

No paciente com TAB as estratégias terapêuticas, principalmente no que diz respeito ao tratamento a longo prazo, serão diferentes de acordo com a polaridade predominante. O tratamento farmacológico normalmente consiste no uso de um estabilizador de humor (por exemplo, valproato) isolado ou em combinação com um antipsicótico ou antidepressivo. Embora o alvo principal dos antipsicóticos sejam os receptores dopaminérgicos D2, os antipsicóticos atípicos, como a quetiapina, também apresentam como alvo os receptores serotoninérgicos (5-HT) (BLOCK; NEMEROFF, 2014; GRANDE *et al*., 2016).

O lítio é uma das drogas mais frequentemente prescritas para o tratamento do TAB, devido suas propriedades como estabilizador de humor. No entanto, seu mecanismo de ação ainda não foi totalmente esclarecido. As propriedades do lítio como estabilizador de humor podem ser explicadas pela sua capacidade de reduzir a liberação de noradrenalina e dopamina e de poder, transitoriamente, aumentar a liberação de serotonina. O lítio apresenta efeito tanto nas fases agudas como na manutenção dos episódios maníacos e depressivos. Ele atua na prevenção de novos episódios e é a única droga eficaz contra o suicídio. Contudo, o lítio possui alta toxicidade, de forma que o seu uso terapêutico deve ser monitorado constantemente (BAIRD-GUNNING *et al*., 2017; WON; KIM, 2017; METHANEETHORN, 2018).

1.2 SÍNDROME METABÓLICA

A Síndrome Metabólica (SM) é um conjunto de fatores de risco que incluem obesidade abdominal, dislipidemia, hipertensão e resistência à insulina, aumentando o risco para o desenvolvimento de *diabetes mellitus* tipo 2, doença arterial coronariana e acidente vascular encefálico (ZAFAR *et al*., 2018). Um estudo de meta-análise encontrou que indivíduos com transtornos psiquiátricos, incluindo o TDM e o TAB, apresentam um risco maior para SM quando comparados com a população em geral (VANCAMPFORT *et al*., 2015).

A prevalência global da SM pode ser estimada em cerca de um quarto da população mundial (SAKLAYEN, 2018). De acordo com a meta-análise de Vancampfort *et al*. (2015), a prevalência de SM em indivíduos com transtornos psiquiátricos é de 32,6%. Entre indivíduos com transtornos de humor, a prevalência de SM é maior no TAB (28,4%) em comparação com o TDM (20,2%) (SILAROVA *et al*., 2015). Um dos possíveis motivos para a relação entre a SM e os transtornos psiquiátricos, é o estilo de vida inadequado comumente observado nestes indivíduos, como tabagismo, consumo de álcool, sedentarismo e hábitos alimentares não saudáveis (PENNINX; LANGE, 2018).

As estimativas de prevalência variam de acordo com os critérios utilizados para o diagnóstico da SM (SAKLAYEN, 2018). Os critérios para o diagnóstico da SM foram estabelecidos por diferentes grupos como *World Health Organization* (WHO) em 1998, *European Group for Study of Insulin Resistance* (EGIR) em 1999, *National Cholesterol Education Program* (NCEP) *Adult Treatment Panel III* (ATP III) em 2001, *American Association of Clinical Endocrinologists* (AACE) em 2003 e *International Diabetes Federation* (IDF) em 2005 (ZAFAR *et al*., 2018).

Os critérios definidos pelo WHO, EGIR e AACE determinaram como requisito para o diagnóstico da SM a presença de resistência à insulina. O IDF, por sua vez, exigiu a presença de obesidade abdominal (medida pela circunferência da cintura), considerando a sua forte relação com a resistência à insulina, e especificou os pontos de corte de acordo com a nacionalidade ou etnia. O NCEP ATP III, modificado em 2005, apesar de não utilizar nenhum critério isolado para o diagnóstico da SM, reconhece a obesidade abdominal como um importante fator de risco subjacente à SM (ALBERTI; ZIMMET; SHAW, 2006; GRUNDY *et al*., 2005).

Os fatores de risco incluídos nos critérios estabelecidos pelo NCEP ATP III (GRUNDY *et al*., 2005) e IDF (ALBERTI; ZIMMET; SHAW, 2006) são circunferência da cintura aumentada, níveis reduzidos de lipoproteínas de alta densidade (*High-Density Lipoprotein*, HDL) e níveis aumentados de triglicerídeos (TG), glicose e pressão sanguínea, descritos abaixo:

a. Circunferência da cintura:
   i. NCEP ATP III: ≥ 102 cm para homens e ≥ 88 cm para mulheres;
   ii. IDF: Origem europeia: ≥ 94 cm para homens e ≥ 80 cm para mulheres; Sul-Asiáticos e Chineses: ≥ 90 cm para homens e ≥ 80 cm para mulheres; Japoneses: ≥ 85 cm para homens e 90 cm para mulheres;
b. TG ≥ 150 mg/dL;
c. HDL < 40 mg/dL para homens e < 50 mg/dL para mulheres;

d. Pressão arterial sistólica (PAS) ≥ 130 mmHg ou pressão arterial diastólica (PAD) ≥ 85 mmHg e/ou uso de anti-hipertensivos;
e. Glicose em jejum ≥ 100 mg/dL e/ou uso de medicamento hipoglicemiante.

De acordo com o NCEP ATP III, para o diagnóstico da SM os indivíduos devem apresentar pelo menos 3 dos 5 fatores de risco, enquanto pelo IDF deve-se apresentar circunferência da cintura aumentada com mais 2 fatores de risco (ALBERTI; ZIMMET; SHAW, 2006; GRUNDY *et al*., 2005).

A fisiopatologia da SM envolve a combinação de diversas vias de interação, alterações genéticas e fatores ambientais (estilo de vida) (ZAFAR *et al*., 2018). A resistência à insulina, por exemplo, está relacionada com uma predisposição genética, entretanto, o aumento da obesidade abdominal contribui para este processo. A gordura visceral como principal componente da obesidade abdominal, está associada a liberação de ácidos graxos livres (AGL) que podem se acumular no fígado e músculo, predispondo ainda mais a resistência à insulina. Essas características, em conjunto com a hipertensão e dislipidemia, predispõe o indivíduo a riscos pró-trombóticos e pró-inflamatórios (SHERLING; PERUMAREDDI; HENNEKENS, 2017).

1.3 Estresse Oxidativo e Nitrosativo

Atualmente o diagnóstico para TDM e TAB é baseado principalmente em informações obtidas através de relatos dos próprios pacientes e aplicação de uma entrevista clínica estruturada, trazendo algumas limitações que podem resultar em um diagnóstico errado e prejudicar a escolha do tratamento. Por essa razão, potenciais biomarcadores como os de estresse oxidativo (EO) e nitrosativo (EN) têm sido avaliados para auxiliar no diagnóstico clínico desses transtornos de humor (LOPRESTI *et al*., 2014; SCOLA; ANDREAZZA, 2014).

Os biomarcadores de EO/EN também estão associados com a SM. As características da SM estão envolvidas com a desregulação da atividade dos sistemas responsáveis pela produção de espécies reativas de oxigênio (ERO) e nitrogênio (ERN) (VONA *et al*., 2019).

ERO/ERN são produzidas durante o metabolismo celular e, em pequenas concentrações, essas espécies reativas são utilizadas pelas células em processos fisiológicos. Para neutralizar os efeitos das ERO/ERN, o organismo é composto por um sistema antioxidante. No entanto, se a produção de ERO/ERN for exacerbada, pode causar danos a lipídeos, proteínas e DNA. O desequilíbrio entre ERO/ERN e antioxidantes, favorecendo a ação das espécies reativas, é definido como EO/EN (BIRBEN *et al*., 2012; FRAUNBERGER *et al*., 2016).

Entre as principais ERO estão o ânion superóxido ($O_2^{\bullet-}$), o radical hidroxila ($OH^{\bullet}$) e o peróxido de hidrogênio (H_2O_2). O $O_2^{\bullet-}$ pode reagir com o óxido nítrico (NO) e gerar peroxinitrito ($ONOO^-$), uma ERN altamente reativa. O sistema antioxidante pode ser dividido em antioxidantes enzimáticos e não enzimáticos, responsáveis por impedir os efeitos nocivos das espécies reativas. A superóxido dismutase (SOD), catalase (CAT) e glutationa peroxidase (GPx) são exemplos de enzimas antioxidantes. Os antioxidantes não enzimáticos incluem diferentes compostos, como vitaminas (por exemplo, vitaminas C e E), ácido úrico (AU) e glutationa reduzida (GSH) (BIRBEN *et al*., 2012; FRAUNBERGER *et al*., 2016).

O $O_2^{\bullet-}$ é produzido a partir da reação de um elétron com uma molécula de oxigênio e a sua produção ocorre principalmente na mitocôndria, através da cadeia transportadora de elétrons (Figura 2). Outros complexos enzimáticos, como a NADPH oxidase presente em leucócitos polimorfonucleares, monócitos e macrófagos, também é responsável pela produção de $O_2^{\bullet-}$ (*burst*) após o processo de fagocitose para ação antimicrobiana (BIRBEN *et al*., 2012).

$O_2^{\bullet-}$ é convertido em H_2O_2 pela SOD e, em seguida, o H_2O_2 pode ser convertido em água e oxigênio pela CAT ou somente em água pela GPx, que recebe um elétron da GSH para poder desempenhar a sua função. Esse mecanismo converte a GSH em glutationa oxidada (GSSG) que, por sua vez, recebe um elétron da enzima glutationa redutase (GR) e é convertida novamente em GSH (FRAUNBERGER *et al*., 2016). A atividade da GSH é conferida pelo grupamento sulfidrila (SH), presente no aminoácido cisteína de sua estrutura e que possui potencial de redução (POOLE, 2015).

O *Total Radical-Trapping Antioxidant Parameter* (TRAP) é um método amplamente utilizado para avaliar a capacidade antioxidante e, aparentemente, representa uma estimativa mais confiável do que a avaliação de cada antioxidante isoladamente. A capacidade antioxidante é resultado da interação entre diferentes compostos, além de interações metabólicas sistêmicas, que podem incluir antioxidantes ainda não conhecidos ou que não são facilmente quantificados. Dessa forma, a medida do TRAP fornece informações sobre o status antioxidante geral de um indivíduo (CHANG *et al*., 2015).

Na presença de metais de transição o H_2O_2 pode reagir, por exemplo, com o ferro (Fe^{2+}) através da reação de Fenton, e gerar $OH^{\bullet}$, considerada a ERO mais reativa. O $OH^{\bullet}$ pode iniciar a reação em cadeia de peroxidação lipídica sequestrando um elétron dos ácidos graxos poliinsaturados, prejudicando a integridade das membranas celulares e levando a formação de produtos como os hidroperóxidos lipídicos (LOOH) e o malondialdeído (MDA). O H_2O_2 também pode reagir com íons cloro, via mieloperoxidases (MPO) em neutrófilos ativados, e formar o ácido hipocloroso (HOCl) que é altamente oxidativo. O HOCl é responsável pela oxidação da albumina que resulta na formação dos produtos avançados de oxidação proteica (*Advanced Oxidation Protein Products*, AOPP) (BIRBEN *et al*., 2012; FRAUNBERGER *et al*., 2016; OU *et al*., 2017).

A óxido nítrico sintase endotelial (eNOS) e neuronal (nNOS) são enzimas constitutivas que produzem baixas quantidades de NO sob condições normais. Particularmente, o NO produzido pela eNOS representa um fator protetor do endotélio. Dentre as suas funções, está o controle do tônus vascular. Entretanto, em condições associadas à inflamação e ao EO, ocorre um aumento na expressão da óxido nítrico sintase induzível (iNOS), responsável pela produção de NO em grandes quantidades e por longos períodos, facilitando a geração de $ONOO^-$ (FÖRSTERMANN; XIA; LI, 2017; MARÍN; MOYA; MÁÑEZ, 2019).

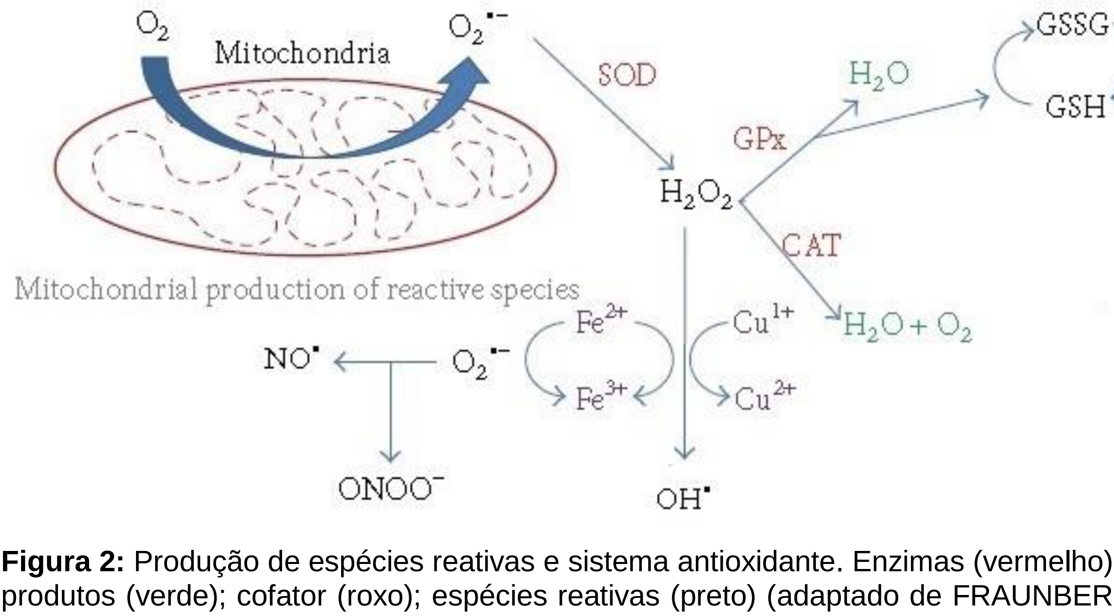

Figura 2: Produção de espécies reativas e sistema antioxidante. Enzimas (vermelho); outros produtos (verde); cofator (roxo); espécies reativas (preto) (adaptado de FRAUNBERGER *et al*., 2016).

Existem evidências de que o TDM está associado à inflamação e ativação de células imunes, que costumam ser acompanhadas por um aumento na produção de ERO/ERN (LEONARD; MAES, 2012). Em indivíduos com TAB, a disfunção mitocondrial tem sido associada ao EO. A redução na expressão de algumas subunidades do complexo I na mitocôndria sugere uma propensão desses indivíduos para desregulação da cadeia transportadora de elétrons, que pode aumentar a produção de ERO (SCOLA; ANDREAZZA, 2014). Além disso, existe uma relação entre a disfunção mitocondrial e a inflamação no TAB. A produção de ERO pela mitocôndria parece estar envolvida com a ativação do *nod-like receptor pyrin domain-containing 3* (NLRP3) e formação do inflamassoma NLRP3, responsável por estimular a liberação de citocinas inflamatórias (KIM; CHEN; ANDREAZZA, 2015).

O EO/EN está envolvido com a SM através de vários mecanismos. Em condições de hiperglicemia, por exemplo, um fluxo maior de glicose passa pela via glicolítica resultando em grandes quantidades de NADH, que quando acumulados podem sobrecarregar a mitocôndria e, consequentemente, levar ao aumento na produção de ERO (YAN, 2014). O EO também está relacionado com a disfunção endotelial observada na SM e a liberação de AGL desempenha um importante papel neste processo. AGL podem estimular a produção de ERO, principalmente pela ativação da NADPH oxidase, e induzir a inflamação, contribuindo para redução na biodisponibilidade de NO no endotélio (GHOSH *et al.*, 2017).

2 JUSTIFICATIVA

O estudo do EO/EN como um fator subjacente envolvido na patogênese de diversas doenças tem emergido em meio à comunidade científica e tem despertado cada vez mais o interesse em encontrar um biomarcador que represente a doença estudada e possa auxiliar no seu diagnóstico. O TDM e o TAB são grandes exemplos, uma vez que estes ainda dependem exclusivamente de uma avaliação clínica para o seu diagnóstico. A realização de um diagnóstico adequado de TDM e TAB é importante para que estratégias terapêuticas eficazes sejam estabelecidas e os prejuízos decorrentes destes transtornos sejam minimizados. Deve-se levar em consideração também a presença de comorbidades, como transtornos por uso de substâncias e SM, que prejudicam ainda mais a qualidade de vida dos indivíduos com transtornos de humor. Sendo assim, a avaliação de biomarcadores de EO/EN pode colaborar com o entendimento dos mecanismos associados a esses transtornos de humor, assim como a relação que existe entre eles e algumas comorbidades.

3 OBJETIVOS

3.1 Objetivo Geral

Avaliar o envolvimento de diferentes biomarcadores de EO/EN com os transtornos de humor e demonstrar a influência da SM, quando presente nestes transtornos, sobre o EO.

3.2 Objetivos Específicos

a. Avaliar os níveis dos biomarcadores de EO/EN em indivíduos com TDM, TAB I e TAB II;
b. Avaliar se os biomarcadores de EO/EN podem apresentar diferenças entre os diagnósticos de TDM, TAB I e TAB II;
c. Avaliar os níveis dos biomarcadores de EO em indivíduos com TDM, TAB I e TAB II de acordo com o diagnóstico de SM;
d. Avaliar a associação entre biomarcadores de EO e parâmetros da SM em indivíduos com TDM, TAB I e TAB II.

4 METODOLOGIA

4.1 Delineamento do Estudo

Trata-se de um estudo transversal. No total, participaram do estudo 191 indivíduos (amostra de conveniência) entre controles e pacientes com transtornos de humor, incluindo TDM, TAB I e TAB II. O estudo apresentou dois segmentos. O primeiro segmento contou com a participação de 54 controles e 105 pacientes com transtornos de humor. O grupo de pacientes foi dividido de acordo com o diagnóstico de TDM (n = 37), TAB I (n = 45) e TAB II (n = 23). No segundo segmento, controles e pacientes foram divididos de acordo com o diagnóstico de SM, sendo controles com SM (n = 20), controles sem SM (n = 56), transtornos de humor com SM (n = 38) e transtornos de humor sem SM (n = 71). Em seguida, todos os pacientes foram divididos de acordo com o diagnóstico de TDM (n = 41), TAB I (n = 39) e TAB II (n = 29) e subdivididos novamente pela presença de SM.

4.2 Aspectos Éticos

O estudo foi aprovado pelo Comitê de Ética e Pesquisa Envolvendo Seres Humanos da Universidade Estadual de Londrina (UEL) CAAE 34935814.2.0000.5231 e suas atualizações. O Termo de Consentimento Livre e Esclarecido (TCLE) foi obtido de todos os indivíduos que participaram do estudo (Apêndice A).

4.3 População de Estudo

Os pacientes com transtornos de humor (TDM, TAB I e TAB II) foram recrutados no Ambulatório de Psiquiatria do Ambulatório de Especialidades do Hospital Universitário (AEHU) da UEL. Os controles foram recrutados entre estudantes e funcionários da UEL.

Os participantes eram de ambos os sexos (masculino e feminino) e tinham entre 20 e 68 anos de idade. Os critérios de exclusão para pacientes e controles foram: doenças infecciosas, como hepatite B e C e HIV; doenças neurodegenerativas, como doença de Alzheimer e doença de Parkinson; doenças neuroimunológicas, como esclerose múltipla; doença pulmonar obstrutiva crônica; doença renal crônica; câncer;

doenças autoimunes, como *diabetes mellitus* tipo 1, artrite reumatoide e lúpus eritematoso sistêmico; diagnóstico para outros transtornos psiquiátricos, como autismo, esquizofrenia, transtorno esquizo-afetivo e síndromes psico-orgânicas; e gravidez. Além disso, também foram excluídos controles e pacientes em tratamento com medicamento anti-inflamatório não esteroidal, interferon, glicocorticoides, suplementos a base de ervas, antioxidantes e ômega-3 nas 4 semanas que antecederam o estudo.

4.4 Avaliação Antropométrica e da Pressão Sanguínea

O peso corporal foi medido em quilogramas (kg) utilizando uma balança eletrônica (Filizola™, São Paulo, SP, Brasil) e a altura foi medida em metros (m) utilizando um estadiômetro acoplado à balança. Peso e altura foram utilizados para o cálculo do Índice de Massa Corporal (IMC) de acordo com a seguinte fórmula: peso dividido pela altura ao quadrado (kg/m^2) (MADDEN; SMITH, 2016). A circunferência da cintura, representada pela distância média entre a última costela e a crista ilíaca, foi medida em centímetros (cm) utilizando uma fita métrica com os indivíduos na horizontal (MADDEN; SMITH, 2016). A pressão arterial foi aferida com os indivíduos em repouso utilizando um esfigmomanômetro e o resultado expresso em milímetros de mercúrio (mm/Hg) (VISCHER; BURKARD, 2017).

4.5 Avaliação Clínica e Diagnósticos

A avaliação clínica foi realizada por um médico psiquiatra. Todos os participantes responderam a um questionário semiestruturado contendo dados sócio demográficos, como etnia, anos de educação e estado civil. Dados quanto ao uso de qualquer tipo de medicação também foram informados pelos participantes. Além disso, os pacientes com transtornos de humor informaram dados clínicos, como número de episódios depressivos, hipomaníacos e maníacos anteriores.

Para o diagnóstico de TDM, TAB I e TAB II foi utilizada uma versão brasileira validada do *Structured Clinical Interview for DSM-IV Axis I Disorders* (SCID-I) de acordo com os critérios de diagnóstico do *Diagnostic and Statistical Manual of Mental Disorders, 4th Edition, Text Revision* (DSM-IV-TR) (AMERICAN PSYCHIATRIC ASSOCIATION, 2000; DEL-BEN *et al*., 2001).

A versão brasileira da *17-item Hamilton Depression Rating Scale* (HAM-D) (MORENO; MORENO, 1998) e da *Young Mania Rating Scale* (YMRS) (VILELA *et al*., 2005) foram utilizadas, respectivamente, para avaliar a severidade da depressão e a severidade dos sintomas de mania.

Para avaliar o abuso de substâncias, a versão brasileira do *Alcohol, Smoking and Substance Involvement Screening Test* (ASSIST) desenvolvido pela WHO (HENRIQUE *et al*., 2004; WHO ASSIST WORKING GROUP, 2002) foi utilizada. O diagnóstico de dependência da nicotina foi realizado utilizando a versão brasileira validada da *Fagerström Test for Nicotine Dependence* (de MENESES-GAYA *et al*., 2009; HEATHERTON *et al*., 1991).

Para o diagnóstico da SM foram utilizados os critérios do IDF (ALBERTI; ZIMMET; SHAW, 2006) no primeiro segmento e do NCEP ATP III (GRUNDY *et al*., 2005) no segundo segmento.

4.6. Coleta de Sangue

As amostras de sangue de todos os participantes foram coletadas após 12 horas de jejum utilizando tubos com vácuo (Vacutainer®) contendo EDTA, fluoreto e sem anticoagulante, destinados para a avaliação dos parâmetros bioquímicos e dos biomarcadores de EO/EN.

4.7 Avaliação dos Parâmetros Bioquímicos

Os níveis de glicose, colesterol total (CT), HDL, TG, AU e proteínas totais foram analisados por um analisador bioquímico automático (Dimension AR, Siemens, Berlin, Germany). Os níveis de lipoproteínas de baixa densidade (*Low-Density Lipoprotein*, LDL) foram calculados pela fórmula de Friedewald: CT - (HDL + TG/5) (FRIEDEWALD; LEVY; FREDRICKSON, 1972). Os níveis de insulina foram analisados por metodologia automatizada (Architect, Abbot Laboratories, Abbot Park, IL, USA). Os níveis de glicose, CT, HDL, LDL e TG foram expressos em mg/dL enquanto os níveis de insulina foram expressos em µU/mL.

O índice de resistência à insulina foi calculado através do *Homeostasis Model Assessment for Insulin Resistance* (HOMA-IR) utilizando a fórmula: insulina em jejum (µU/mL) x glicose em jejum (mmol/L)/22,5 (HAFFNER; MIETTINEN; STERN, 1997).

Os índices de Castelli I e II, utilizados para avaliar risco aterogênico, foram calculados respectivamente pela razão entre CT/HDL e LDL/HDL (MILLÁN *et al*., 2009).

4.8 Avaliação dos Biomarcadores de EO/EN

Para as análises de EO/EN, as amostras de sangue foram centrifugadas por 10 minutos a 1000 x g em centrífuga (EVLAB®, Londrina, PR, Brasil) e as porções do sangue, como plasma, soro e hemácias, foram aliquotadas e armazenadas em freezer a -80ºC até a realização dos testes.

4.8.1 Determinação de LOOH

A avaliação dos níveis de LOOH foi realizada por quimiluminescência (QL) iniciada por *tert*-butil de acordo com a adaptação da técnica descrita por Gonzalez Flecha, Llesuy e Boveris (1991). Esta técnica baseia-se na reação entre o *tert*-butil e os LOOH presentes na amostra (soro) que resulta na emissão de fótons. O experimento foi realizado em um luminômetro Glomax® 20/20 (Promega, Madson, WI, USA), ao abrigo da luz e com a temperatura da sala a 30º C. As leituras obtidas foram utilizadas para calcular a área sob a curva no *software* Origin. Os resultados foram expressos em unidades relativas de luz (URL).

4.8.2 Determinação de MDA

A quantificação de MDA foi realizada segundo a técnica descrita por Bastos *et al*. (2012). Esta técnica detecta os níveis de MDA na amostra (soro) através da complexação com duas moléculas do ácido tiobarbitúrico (TBA), utilizando cromatografia líquida de alta eficiência (*High Performance Liquid Chromatography*, HPLC). Para a realização da análise do aduto TBA-MDA-TBA foi utilizado um sistema de CLAE Alliance e2695 (Waters™, Barueri, SP, Brasil) com uma coluna Eclipse XDB-C18 4,6mm x 250mm 5µm (Agilent, Santa Clara, CA, USA). A fase móvel consiste em 65% de tampão fosfato (50 nM pH 7,0) e 35% de metanol grau HPLC com quociente de vazão de 1 mL/min e temperatura a 30ºC. A leitura foi feita no comprimento de onda de 532 nm. Para análise dos dados foi utilizada curva de calibração preparada com soluções de MDA em diferentes concentrações (faixa entre 0,05 e 2,00 µM), comparando medidas de área de pico das amostras com as concentrações conhecidas da curva. As concentrações de MDA foram corrigidas pelos níveis de proteínas totais e os resultados expressos em μM/mg proteínas.

4.8.3 Determinação de AOPP

Para a quantificação de AOPP foi utilizado o método descrito por Hanasand *et al*. (2012). Este teste quantifica os níveis de proteínas oxidadas na amostra (soro) por espectrofotometria. A leitura da reação foi feita em um leitor de microplaca modelo EnSpire (Perkin Elmer®, Waltham, MA, USA) no comprimento de onda de 340 nm. Para o cálculo de concentração das amostras foi utilizado o fator obtido pela curva de calibração com cloramina T. As concentrações de AOPP foram expressas em μM equivalente de cloramina T.

4.8.4 Determinação de Metabólitos do NO (NOx)

A quantificação de NOx foi realizada através da técnica descrita por Navarro-González, García-Benayas e Arenas (1998). O método baseia-se na redução de nitrato a nitrito, mediada por reações de óxido-redução ocorridas entre o nitrato presente na amostra (soro) e o sistema cádmio-cobre dos reagentes, com posterior diazotação e detecção colorimétrica do azocomposto formado pela adição do reagente de Griess. A leitura da reação foi feita em um leitor de microplaca modelo EnSpire (Perkin Elmer®, Waltham, MA, USA) no comprimento de onda de 550 nm. Para o cálculo de concentração das amostras foi utilizado o fator obtido pela curva de calibração com nitrito de sódio ($NaNO_2$). As concentrações de NOx foram expressas em μM equivalente de $NaNO_2$.

4.8.5 Determinação do TRAP

O TRAP avalia a capacidade antioxidante total da amostra (soro), usando como comparativo o trolox (análogo da vitamina E), por QL a partir de uma adaptação do método descrito por Repetto *et al*. (1996). A metodologia é baseada na geração de radicais peroxila ($RO_2^{\bullet}$), por decomposição térmica, a uma velocidade controlada, do azoiniciador dicloridrato de 2,2’-azobis-(2-metilpropanoamidina) (ABAP). Este experimento foi conduzido em um leitor de microplaca modelo Victor X-3 (Perkin Elmer®, Waltham, MA, USA) em um modo de contagem não coincidente e uma faixa de resposta entre 300 a 620 nm com controle de temperatura a 30°C. O AU representa cerca de 50% da capacidade antioxidante total (NDREPEPA, 2018), por esse motivo, os níveis de TRAP foram normalizados pelos níveis de AU (TRAP/AU) e os resultados expressos em µM trolox/mg AU.

4.8.6 Determinação do Grupamento SH

O grupamento SH, que apresenta ação antioxidante, foi avaliado segundo o método descrito previamente por Hu (1994) e adaptado para microplaca por Taylan e Resmi (2010). O método baseia-se na detecção colorimétrica da reação do ácido 5,5-ditiobis 2-nitrobenzóico (DTNB) com o grupamento SH de proteínas e compostos não proteicos na amostra (soro). A leitura da reação foi feita em um leitor de microplaca modelo EnSpire (Perkin Elmer®, Waltham, MA, USA) no comprimento de onda de 412 nm. Para o cálculo de concentração das amostras foi utilizado o fator obtido pela curva de calibração com GSH. As concentrações de SH foram expressas em µM equivalente de GSH.

4.8.7 Determinação da SOD

A atividade da enzima antioxidante SOD foi determinada nas hemácias através do método do pirogalol descrito por Marklund e Marklund (1974). Este método baseia-se na inibição que esta enzima promove na auto oxidação do pirogalol em solução aquosa. A quantidade de SOD que foi capaz de inibir 50% da oxidação do pirogalol foi definida como uma unidade de atividade enzimática. A leitura da reação foi feita em um leitor de microplaca modelo EnSpire (Perkin Elmer®, Waltham, MA, USA) no comprimento de onda de 420 nm e temperatura a 37ºC. As concentrações de SOD foram expressas em U/mg hemoglobina (Hb).

4.8.8 Determinação da CAT

A atividade da enzima antioxidante CAT foi determinada nas hemácias através da medida do decaimento na concentração de H_2O_2 e geração de oxigênio, utilizando a técnica descrita por Aebi (1984). A leitura da reação foi feita em um leitor de microplaca modelo EnSpire (Perkin Elmer®, Waltham, MA, USA) no comprimento de onda de 240 nm e temperatura a 25ºC. As concentrações de CAT foram expressas em U/mg Hb.

4.9 Índices de EO/EN

Para o primeiro segmento, os resultados de SOD, LOOH, CAT, NOx, MDA e AOPP foram transformados em z valor e computados para a elaboração de cinco índices compostos que refletem diferentes vias de EO/EN. Os índices foram elaborados da seguinte forma:

a. zLOOH+SOD computado como z valor LOOH (zLOOH) + zSOD: reflete a produção de ERO levando ao processo de peroxidação lipídica;
b. zLOOH+SOD-CAT computado como zLOOH + zSOD - zCAT: reflete a produção de ERO levando a peroxidação lipídica e contabilizando o efeito protetor da CAT;
c. zLOOH+SOD+NOx computado como zLOOH + zSOD + zNOx: reflete o estresse nitro-oxidativo, com a produção de ERO levando a peroxidação lipídica em conjunto com a produção de NOx;
d. zLOOH+SOD+NOx+MDA computado como zLOOH + zSOD + zNOx + zMDA: reflete o estresse nitro-oxidativo e formação de aldeídos;
e. zLOOH+SOD+NOx+AOPP computado como zLOOH + zSOD + zNOx + zAOPP: reflete o estresse nitro-oxidativo e oxidação de proteínas.

4.10 Análise Estatística

Teste de normalidade de Kolmogorov-Smirnov foi aplicado para analisar a distribuição das variáveis e o teste de Levene para avaliar a homogeneidade. O teste qui-quadrado (análise de tabelas de contingência) foi aplicado para analisar variáveis categóricas enquanto a análise de variância (ANOVA) com post hoc de Tukey e o teste de Kruskal-Wallis com post hoc de Dunn foram utilizados para analisar variáveis contínuas. Análise univariada e multivariada de modelo linear generalizado (GLM), regressão multivariada, regressão logística, correlação de Pearson, correlação de

Spearman e curva ROC foram aplicados quando apropriado. Os *softwares* SPSS Statistics, MedCalc, GraphPad InStat e Statistica foram utilizados para a análise dos dados. A significância estatística foi estabelecida em $p < 0.05$.

5 RESULTADOS E DISCUSSÃO

Artigo 1: Major differences in neurooxidative and neuronitrosative stress pathways between major depressive disorder and types I and II bipolar disorder.

Artigo 2: Metabolic syndrome components might be associated with the oxidative stress alterations in patients with mood disorders.

5.1 ARTIGO 1

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Major Differences in Neurooxidative and Neuronitrosative Stress Pathways Between Major Depressive Disorder and Types I and II Bipolar Disorder

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Abstract

Accumulating evidence indicates that oxidative and nitrosative stress (O&NS) pathways play a key role in the pathophysiology of bipolar disorder (BD) and major depressive disorder (MDD). However, only a handful of studies have directly compared alterations in O&NS pathways among patients with MDD and BD types I (BPI) and BPII. Thus, the current study compared superoxide dismutase (SOD1), lipid hydroperoxides (LOOH), catalase, nitric oxide metabolites (NOx), malondialdehyde (MDA), and advanced oxidation protein products (AOPP) between mood disorder patients in a clinically remitted state. To this end 45, 23, and 37 participants with BPI, BPII, and MDD, respectively, as well as 54 healthy controls (HCs) were recruited. Z-unit weighted composite scores were computed as indices of reactive oxygen species (ROS) production and nitro-oxidative stress driving lipid or protein oxidation. SOD1, NOx, and MDA were significantly higher in MDD than in the other three groups. AOPP was significantly higher in BPI than in HCs and BPII patients. BPII patients showed lower SOD1 compared to all other groups. Furthermore, MDD was characterized by increased indices of ROS and lipid hydroperoxide production compared to BPI and BPII groups. Indices of nitro-oxidative stress coupled with aldehyde production or protein oxidation were significantly different among the three patient groups (BDII > BDI > MDD). Finally, depressive symptom scores were significantly associated with higher LOOH and AOPP levels. In conclusion, depression is accompanied by increased ROS production, which is insufficiently dampened by catalase activity, thereby increasing nitro-oxidative damage to lipids and aldehyde production. Increased protein oxidation with formation of AOPP appeared to be hallmark of MDD and BPI. In addition, patients with BPII may have protection against the damaging effects of ROS including lipid peroxidation and aldehyde formation. This study suggests that biomarkers related to O&NS could aid in the differentiation of MDD, BPI, and BPII.

Keywords Depression · Bipolar disorder · Oxidative and nitrosative stress · Immune · Inflammation

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Introduction

Research on the pathophysiology of major depressive disorder (MDD) and bipolar disorder (BD) has highlighted that aberrations in neurooxidative, neuronitrosative stress, and neuroimmune pathways play a key pathophysiological role in these disorders [1 7]. Chronic immune activation and inflammatory processes, as observed in mood disorders, are frequently accompanied by elevated levels of reactive oxygen (ROS) and nitrogen (RNS) species, including superoxide, peroxides, nitric oxide (NO), and peroxynitrite, while increased oxidative and nitrosative damage to lipids and proteins may cause immune activation [3, 5]. There are also data that point that activated neurooxidative, neuronitrosative, and neuroimmune pathways are interrelated phenomena in both major depression and bipolar disorder [3, 5, 8].

Both depression and BD are associated with lowered lipid-associated antioxidant defenses including lowered activity of lecithin cholesterol acyltransferase (LCAT), lower levels of high-density lipoprotein (HDL) cholesterol, vitamin E, coenzyme Q10 and paraoxonase 1 and glutathione peroxidase activities [9 17]. This specific reduction in lipid-targeted antioxidant defenses may contribute to increased ROS levels and oxidative damage to lipid membranes (lipid peroxidation) including to polyunsaturated fatty acids [18 20]. Lipid hydroperoxide chain reactions eventually cause the formation of reactive aldehydes, the end-product of lipid peroxidation, as indicated by increased levels of malondialdehyde (MDA) or thiobarbituric acid reactive substances (TBARS) and increased autoimmune responses (IgG- or IgM-mediated) directed against oxidatively formed neoepitopes, including azelaic acid and MDA, oxidized low-density lipoprotein cholesterol, and anchorage molecules [21 27]. Signs of lipid peroxidation coupled with reactive aldehyde production as measured with plasma TBARS or MDA are now among the most frequently reported biomarkers for depression and BD [21, 26 34]. Recent meta-analyses also report elevated TBARS and MDA concentrations in depression [35, 36] and BD [37, 38]. In both mood disorders, increased TBARS seem to be associated with severity of illness, suicidal behaviors, and the number of manic and/or depressive episodes in the year prior to the assay of MDA [8]. No significant differences could be detected in MDA levels and associated immune-inflammatory biomarkers among patients in acute phases of depression versus BD [8, 39]. Therefore, it remains unclear whether specific aspects of the nitro-oxidative pathways ranging from ROS production to lipid peroxidation could differ between individuals with depression and BD. These abnormalities may include changes in superoxide dismutase (SOD) activity, lipid hydroperoxide levels (LOOH), catalase activity, increased ROS coupled with RNS, or lipid peroxidation with aldehyde formation.

Major depression is accompanied not only by increased ROS and lipid peroxidation but also by oxidative damage to proteins as indicated by elevated levels of advanced oxidation protein products (AOPPs) [40, 41]. AOPPs are formed via increased ROS and peroxynitrite production coupled with increased myeloperoxidase activity and hypochlorous acid production [42]. Depression is also characterized by increased inducible nitric oxide (NO) synthase (iNOS) activity and NO production, which eventually may lead to nitrosative stress and hypernitrosylation [2, 3, 43, 44]. In addition, evidence suggests that NO production is elevated in euthymic patients with BD compared to controls [45]. Therefore, major affective disorders are now conceptualized as neurooxidative, neuronitrosative, and neuroimmune disorders, which are characterized by nitro-oxidative and nitrosative stress (O&NS)-induced neurotoxic responses leading to aberrations in neuroprotection, neuronal functions, neurogenesis, synaptic plasticity, neurotransmitter signaling, and receptor expression [3, 4, 46]. Potential differences between depression and BD on specific aspects of O&NS pathways remain under-explored.

Therefore, the aim of the present study was to examine levels of SOD, LOOH, catalase, NO metabolites (NOx), MDA, and AOPP among clinically stable patients with depression, type I BD (BDI), and BPII, as well as healthy controls (HCs). The a priori hypothesis was that these mood disorders are accompanied by activated O&NS pathways and that are no significant differences between depression and BD would emerge.

Subjects and Methods

Participants

In this cross-sectional study, we included 54 HCs and 105 patients with mood disorders, namely 37 patients with MDD, 45 with BPI, and 23 with BPII. All participants were Brazilian of both gender and aged 20 to 63 years old. All participants with mood disorders were outpatients admitted to the Psychiatry outpatient clinics at the University Hospital of the Universidade Estadual de Londrina (UEL), Parana, Brazil. They were all in remission or partial remission, and the index episode in BD patients was not of (hypo)manic polarity. The HC sample was derived from the same catchment area. The following exclusion criteria were applied for patients and controls: (a) pregnant women; (b) subjects with medical illness affecting immune functions, including hepatitis B and C virus infection, HIV infection, neuroimmune and neurodegenerative disorders (e.g., Alzheimer's disease, multiple sclerosis, Parkinson's disease), chronic obstructive pulmonary disease, chronic kidney disease, cancers, autoimmune diseases such as rheumatoid arthritis, type 1 diabetes, and systemic lupus erythematosus; (c) subjects with other axis-1 diagnoses according to DSM-IV-TR criteria, including schizophrenia, schizo-affective disorder, autism, psycho-organic syndromes; and (d) subjects who were treated with nonsteroidal anti-inflammatory drugs, interferon, glucocorticoids, antioxidants, herbal supplements and omega-3 polyunsaturated fatty acids during the past 4 weeks prior to study enrollment. Some patients with MDD and BD were currently treated with antidepressants ($n = 44$), atypical antipsychotics ($n = 32$), lithium ($n = 26$), and other mood stabilizers ($n = 33$) including carbamazepine and valproic acid. All participants provided written informed consent to take part in the current study, whose experimental procedures were previously approved by the Research Ethics Committee at UEL (protocol number CAAE 34935814.2.0000.5231).

Methods

The clinical diagnoses of MDD, BPI, and BPII were made by a research psychiatrist using the validated Brazilian Portuguese version of the structured clinical interview for DSM-IV interview (SCID) axis I [47] in accordance with *Diagnostic and Statistical Manual of Mental Disorders, 4th Edition, Text Revision* (DSM-IV-TR) diagnostic criteria [48]. Moreover, all participants completed a semi-structured interview comprising socio-demographic data (self-perceived ethnicity, years of education, marital status) and clinical data (number of previous depressive, hypomanic, and manic episodes). We used the 17-item Hamilton Depression Rating Scale (HAM-D), translated and adapted for use with Brazilian individuals [49], to measure severity of depression, while severity of manic symptoms was scored employing the Brazilian Portuguese version of the Young Mania Rating Scale (YMRS) [50]. The Alcohol, Smoking and Substance Involvement Screening Test (ASSIST) was employed to assess substance misuse, namely use of alcohol and hypnotics. This rating scale was developed by the World Health Organization [51] and translated into Brazilian Portuguese by Henrique et al. [52]. The diagnosis of nicotine dependence was made with the Fagerstrom Nicotine Dependence Scale [53], which has been previously validated for use in Brazilian samples [54]. We used two cutoff values yielding three groups, namely 0 1, no nicotine dependence; 2 5, mild dependence; and ≥ 6, severe dependence.

A diagnosis of metabolic syndrome (MetS) was made according to the International Diabetes Federation criteria [55], namely presence of three out of the following criteria: (a) abdominal obesity (waist circumference ≥ 90 cm for men and ≥ 80 cm for women in South Asian and South Americans and ≥ 94.0 cm for men and ≥ 80.0 cm for women in Caucasians); (b) low HDL cholesterol (< 40 mg/dL in men and < 50 mg/dL in women) or use of hypolipidemic drugs; (c) hypertriglyceridemia (triglycerides > 150 mg/dL) or use of hypolipidemic agent; (d) increased fasting glucose (> 100 mg/dL) or use of oral antidiabetic medications; and (e) increased average blood pressure (130/85 mmHg) or currently taking antihypertensive medication. We measured the body mass index (BMI) according to the following formula: weight (in kg) divided by square of height (in m^2).

Assays

Peripheral fasting (12 h) blood was sampled at 8 a.m. the same day as the diagnosis was made and clinical data were collected. We measured the activities of superoxide dismutase (SOD1) and catalase and the concentrations of lipid hydroperoxides (LOOH), NO metabolites (NOx), malondialdehyde (MDA) and advanced oxidation protein products (AOPP). SOD activity in erythrocytes was determined using the pyrogallol method described by Marklund and Marklund [56]. This technique is based on the inhibition of pyrogallol self-oxidation by SOD in aqueous solution. The assay was conducted in a spectrophotometer Helios α, Thermo Spectronic (Waltham, MA, USA) at 420 nm and 37 °C. During 5 min, variation in optical density (OD) was recorded every minute. The level of SOD that inhibited 50% of the pyrogallol oxidation was defined as one unit of enzymatic activity. The results were expressed U/mg of hemoglobin (Hb). Lipid hydroperoxides (LOOH) are assayed by chemiluminescence (CL-LOOH) [57, 58]. This method uses the compound tert-butyl hydroperoxide to start a lipid chain reaction that can be detected by photon emission during the formation of lipid hydroperoxides. Readings were performed in a Glomax luminometer (TD 20/20 Turner Designers, USA) over 1 h at one reading per second. Results are expressed as relative units of light. Measurement of catalase activity was estimated through the difference between the initial reading and the reading conducted 30 s after the addition of 200 mM H_2O_2 30% at 240 nm in a microplate reader (model EnSpire, Perkin Elmer, USA) with the temperature maintained at 25 °C. The catalase values are expressed as U/mg Hb. NO metabolite (NOx) levels were assessed indirectly by determining the plasma nitrite concentration using an adaptation of the technique described Navarro-Gonzalvez et al. [59]. This method is based on the reduction of the nitrate present in the sample to nitrite by oxidation-reduction reactions mediated by the system cadmium-copper reagent. Thereafter, Griess reagent was added to induce diazotization, forming a colored complex and subsequent detection at 540 nm. The quantification of NOx was made in a microplate reader Asys Expert Plus, Biochrom (Holliston, MA, USA). The nitric oxide concentration was expressed in μM. MDA levels were measured through complexation with two molecules of thiobarbituric acid (TBA) using MDA estimation through high performance liquid chromatography (HPLC Alliance e2695, Waters', Barueri, SP, Brasil) [60]. Experimental conditions included the use of a column Eclipse XDB-C18 (Agilent, USA), mobile phase consisting of 65% phosphate buffer (50 nM pH 7.0) and 35% HPLC-grade methanol, flow rate of 1.0 mL/min, temperature of 30 °C, and wavelength of 532 nm. MDA concentration in the samples was quantified based on a calibration curve and are expressed in mmol of MDA/mg proteins. AOPP was quantified using the method described by Hanasand et al. [61] in a microplate reader, Perkin Elmer, model EnSpire (Waltham, MA, EUA), at a wavelength of 340 nm. AOPP concentration was expressed in μM of equivalent chloramine T.

Figure 1 shows the pathway from superoxide formation to lipid peroxidation and the generation of aldehydes. In order to examine this pathway and the pathway to AOPP formation, we computed five composite scores reflecting different O&NS concepts.

1. zLOOH+SOD1 computed as z value LOOH (zLOOH) + zSOD. Increased superoxide induces SOD thereby catalyzing superoxide radicals into peroxides [62]. Lipid hydroperoxides are mainly derived from cholesterol, unsaturated phospholipids, and glycolipids and are intermediates of peroxidative reactions, which may be induced by hydroxyl radicals, peroxides, peroxyl radicals, and peroxynitrite [63]. As such, the sum of zLOOH + zSOD reflects ROS (peroxide + hydroxyl) production leading to lipid peroxidation.
2. zLOOH+SOD-CAT computed as zLOOH + zSOD − zCAT. Catalase catalyzes the decomposition of peroxides into oxygen and water, and therefore, this composite score reflects the formation of ROS (peroxide + hydroxyl) production leading to lipid peroxidation taking into account the protective effects of catalase.
3. zLOOH+SOD+NOx computed as zLOOH + zSOD + zNOx. This score reflects ROS (peroxide + hydroxyl) production leading to lipid peroxidation coupled with the production of NO and is therefore an index of nitro-oxidative stress and the potential to generate peroxynitrite and lipid hydroperxides [63, 64].
4. zLOOH+SOD+NOx+MDA computed as zLOOH + zSOD + zNOx + zMDA reflects the pathway from ROS production to nitro-oxidative stress and lipid peroxidation leading to increased production of reactive aldehydes with long-lasting detrimental consequences [3].

zLOOH+SOD+NOx+AOPP computed as zLOOH + zSOD + zNOx + zAOPP. This composite score reflects increased nitro-oxidative stress leading to protein oxidation (AOPP) via increased ROS and peroxynitrite production coupled with increased myeloperoxidase activity and hypochlorous acid production [42].

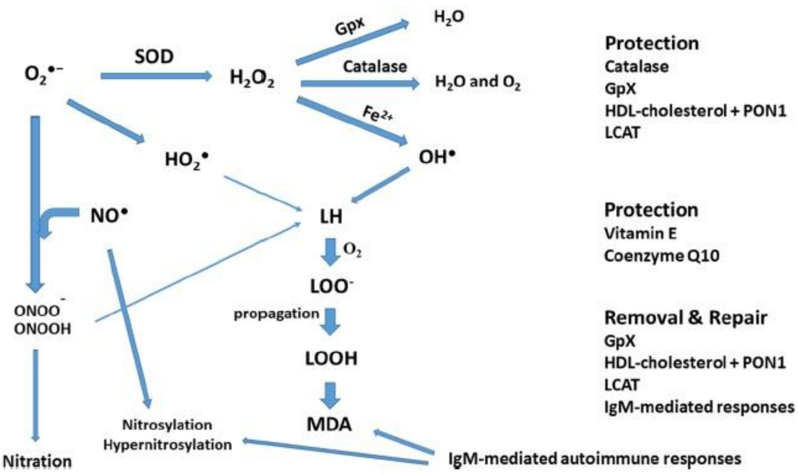

Fig. 1 Increased superoxide ($O_2^{\bullet}$) is accompanied by increased superoxide dismutase (SOD), which catalyzes superoxide radicals into peroxides. Catalase and glutathione peroxidase (Gpx) catalyze the decomposition of peroxides into oxygen and water. The Fenton reaction (iron and hydrogen peroxides) may generate superoxide-dependent hydroxyl radicals ($OH^{\bullet}$) which together with perhydroxyl radicals ($HO_2^{\bullet}$) and peroxynitrite (ONOO) attack lipid membranes especially polyunsaturated fatty acids (PUFAs). The generated fatty acid radicals react with oxygen and form lipid peroxy (LOO) radicals which react with other lipids or PUFAs producing lipid hydroperoxides (LOOH), thereby damaging the lipid membranes and causing formation of aldehydes, including malondialdehyde (MDA), which are the end-product of lipid peroxidation. Different antioxidant defenses may protect against lipid peroxidation, including Gpx, catalase, high-density lipoprotein (HDL) cholesterol, paraoxonase 1 (PON1) and L-lecithin/cholesterol acyltransferase (LCAT), and cell membrane-associated vitamin E and coenzyme Q10. Removal (excision) and repair of the lipid peroxidation lesions may protect against lipid peroxidation-induced cell apoptosis or necrosis, including Gpx, HDL cholesterol, LCAT, and natural IgM-mediated autoimmune responses directed to MDA. Increased levels of nitric oxide (NO) react with superoxide to form peroxynitrous acid (ONOOH) and peroxynitrite, which consequently may contribute to lipid peroxidation and cause oxidation and nitration of proteins. Increased NO production may lead to nitrosylation or hypernitrosylation of proteins. An IgM-mediated autoimmune response may be mounted against neoepitopes, including NO (nitroso)- and NO_2 (nitro)-adducts and MDA

Statistical Analyses

Differences in scale variables between diagnostic groups were assessed using analyses of variance (ANOVAs), while differences in nominal variables between diagnostic groups were assessed using analyses of contingency tables (χ^2 tests). Multivariate GLM analysis with the six O&NS biomarkers or the five z-unit composite scores as dependent variables were used to assess the effects of diagnosis (primary explanatory variable), while adjusting for age, sex, BMI, years of education, and nicotine dependence. Tests for between-subject effects were used to delineate the effects of the primary explanatory variable on the separate biomarkers and composite scores. Model-derived estimated marginal means were computed, and post hoc analyses were used to assess the differences between the diagnostic categories. Linear multiple regression analyses were employed to delineate the associations between one dependent variable (the biomarkers) and a set of explanatory variables. Binary logistic regression analysis was used to delineate the most significant predictors of diagnostic groups. Nagelkerke values are used as effect estimate, and odds ratios and 95% confidence intervals are computed. Results of multiple comparisons were p-corrected for false discovery rate according to Benjamini and Hochberg [65]. We used the IBM SPSS Windows version 22 and Statistica 8 to analyze all data. Statistical significance was set at 0.05, two-tailed.

Results

Descriptive Statistics

Table 1 shows the socio-demographic and clinical data in normal volunteers and patients with mood disorders. There were no significant differences in age, sex, marital status, BMI, MetS, and Fagerstrom score between the two groups. After p-correction for false discovery rate, there were no significant differences in education ($p = 0.085$), ethnicity ($p = 0.113$), and YMRS ($p = 0.115$) between both samples. The HAM-D score was higher in patients with mood disorders as compared with controls (p-correction: $p = 0.017$). There were no significant differences in the raw O&NS biomarkers between both groups (unadjusted for confounders such as age, sex, education, BMI, nicotine dependence). The correlation matrix among the six biomarkers shows that (without p-correction) there are significant correlations between SOD1 and MDA ($r = 0.310$, $p < 0.001$, $n = 136$) and between LOOH and AOPP ($r = 0.352$, $p < 0.001$, $n = 137$). All other correlation coefficients were non-significant.

Differences Between the Four Study Groups

Figure 1 shows the z-transformed values of the six O&NS biomarkers in the four study groups. Table 2, regression #1, shows the results of multivariate GLM analysis with the six biomarkers as dependent variables and diagnosis as primary explanatory variable, while adjusting for age, sex, education, nicotine dependence, and BMI. We found a significant effect of diagnosis on the six biomarkers (for sex and education: see below). Tests for between-subject effects showed significant effects of diagnosis on SOD1 and AOPP levels. Table 3 shows the model-generated estimated marginal mean values after adjusting for the confounders. Post hoc analyses showed that SOD1 was significantly higher in MDD patients as compared with controls and BPII patients, while those with BPI showed an intermediate position. AOPP levels were significantly higher in BPI than in controls and BPII patients, while MDD patients occupied an intermediate position.

Figures 2 and 3 show the z-transformed values of the O&NS biomarkers and the five composite scores in the four study groups, respectively. Table 2, regression #2, shows the results of a multivariate GLM analysis with the five composite scores as dependent variables and diagnostic groups as primary explanatory variables, while adjusting for age, sex, education, nicotine dependence, and BMI. We found a significant effect of diagnostic groups on the five composite scores. Tests for between-subject effects showed significant effects on all scores. Table 3 shows that the five scores were significantly higher in major depression than in controls (except LOOH+SOD-CAT) and patients with BPII. Moreover, zLOOH+SOD, zLOOH+SOD+NOx, and zLOOH+SOD+NOx+MDA were significantly higher in major depression than in BPI. zLOOH+SOD and zLOOH+SOD+NOx+AOPP were significantly higher in BPI than BPII, while LOOH+SOD+NOx+MDA was significantly lower in BPI than in controls.

Effects of the HAM-D Score

In order to adjust the effects of diagnosis for severity of illness, we have entered the HAM-D score in regression #2, Table 2. The results of this analysis (Table 2, regression #3) show that diagnosis and HAM-D were both significant. Nevertheless, the HAM-D was only associated with zLOOH+SOD+NOx+AOPP, while the effects of diagnosis on all five composite scores remained significant. After adding the YMRS score in regression #2, Table 2, no significant effects of YMRS on the composite scores was found ($F = 0.93$, $df = 5/113$, $p = 0.462$).

We have also examined the differences among the three diagnostic groups after entering the dichotomized HAM-D score (cutoff value < 7 versus ≥ 7) as a second factor, thereby adjusting for the remitted versus the non-remitted state (while also adjusting for sex, age, education, nicotine dependence,

Table 1 Socio-demographic, clinical, and biomarker data of patients with mood disorders (MOOD) and healthy controls (HCs)

Variables	HC ($n = 54$)	MOOD ($n = 105$)	F/X^2	*df*	*p*
Age (years)	43.6 (11.7)	42.7 (10.8)	0.28	1/157	0.598
Sex (M/F)	44/23	30/7	5.09	2	0.078
Education (years)	12.5 (5.8)	10.3 (4.7)	6.76	1/157	0.010
Single/separated-widowed /married	15/10/ 29	22/26/57	1.33	2	0.514
Caucasian/other	34/20	84/21	5.41	1	0.020
Body mass index (kg/m^2)	26.3 (4.9)	26.6 (5.0)	0.09	1/149	0.092
Metabolic syndrome (no/yes)	33/20	66/39	0.00	1	0.942
Fagerstrom score	2.8 (3.3)	3.1 (3.4)	0.56	1/157	0.454
Fagerstrom_3 groups[a]	30/7/17	53/18/34	0.58	2	0.749
HAM-D [q25 q75]	2.6 (3.4) [0 4]	9.9 (6.5) [4 13]	58.95	1/157	< 0.001
YMRS	0.8 (1.7)	1.7 (2.5)	4.99	1/157	0.027
Number depressive episodes		5.1 (4.6)			
Number manic episodes		4.4 (6.2)			
SOD1 (U/mg Hb)	91.4 (40.8)	101.0 (41.7)	1.67	1/134	0.199
LOOH (RLU × 10^6)[b]	1552 (1011)	1640 (1149)	0.27	1/135	0.607
CAT (U/mg Hb)	57.1 (14.9)	62.0 (14.4)	3.56	1/133	0.061
NOx (μM)[b]	6.1 (3.2)	6.9 (3.8)	1.34	1/136	0.249
MDA (mmol/mg of protein)[b]	64.3 (22.1)	65.6 (22.3)	0.12	1/136	0.731
AOPP (μM)[b]	71.6 (44.6)	85.5 (43.3)	2.72	1/136	0.076

All results are shown as mean (± SD)

F results of analyses of variance, X^2 results of analyses of contingency tables, *HAM-D* Hamilton Depression rating Scale score, *Q25 and q75* 25% and 75% quartile values, *SOD* superoxide dismutase, LOOH lipid hydroperoxides, *CAT* catalase, *NOx* nitric oxide metabolites, *MDA* malondialdehyde, *AOPP* advanced oxidation protein products

[a] Three groups using cutoff values 2 and 6 (thus group 1, 0 1; group 2, 3 5; group 3, > 6)

[b] These data are processed in Ln transformation

and BMI). Multivariate GLM analysis #4, Table 2, shows a significant effect of diagnosis, but not of the dichotomized HAM-D scores. Figure 4 shows the residualized composite scores after regression on age, sex, BMI, education, nicotine dependence, and the dichotomized HAM-D values in the three mood disorders groups. zLOOH+SOD and zLOOH+SOD-CAT were significantly higher in major depression that in BPI and BPII, while there were no differences between both BD subtypes ($p = 0.066$ and $p = 0.166$, respectively). zLOOH+SOD+NOx, zLOOH+SOD+NOx+MDA, and zLOOH+SOD+NOx+AOPP were significantly different between the three groups and increased from BPII to BPI to major depression.

In multivariate GLM analysis #5, Table 2, we examined the differences between the three mood disorder groups in subjects with a HAM-D score < 11 (14 BP1, 13 BP2, and 22 depressed patients). Also, this analysis showed a significant effect of diagnostic groups with an effect size of 0.306 and univariate effects on all five scores. Figure 5 shows the unadjusted mean values of the composite scores in the three study groups with HAM-D values < 11. zLOOH+SOD, zLOOH+SOD-CAT, zLOOH+SOD+NOx, and zLOOH+SOD+NOx+AOPP were significantly higher in depression and BPI than BPII patients, while there were no significant differences between depressed and BPI patients. zLOOH+SOD+NOx+MDA was significantly different between the three groups and increased from BPII to PBI to depression.

Effects of Confounding Variables

LOOH ($F = 5.46$, $df = 1/118$, $p = 0.021$; partial eta squared = 0.044) and AOPP ($F = 23.52$, $df = 1/118$, $p < 0.001$, partial eta squared = 0.166) were significantly higher in males than females. All five composite scores were significantly higher in men than in women. Education was significantly and inversely associated with SOD1 ($F = 6.91$, $df = 1/118$, $p = 0.010$, partial eta squared = 0.055), MDA ($F = 8.90$, $df = 1/118$, $p = 0.003$, partial eta squared = 0.070), zLOOH+SOD ($F = 4.71$, $df = 1/118$, $p = 0.032$, partial eta squared = 0.038), zLOOH+SOD-CAT ($F = 5.83$, $df = 1/118$, $p = 0.017$, partial eta squared = 0.047), and zLOOH+SOD+NOx+MDA ($F = 5.65$, $df = 1/118$, $p = 0.019$, partial eta squared = 0.046). There were no significant effects of age, nicotine dependence, and BMI on the biomarkers.

In order to examine possible effects of other confounders on the associations between diagnostic groups and the five

Table 2 Results of multivariate general linear model (GLM) analysis with six oxidative and nitrosative stress biomarkers as dependent variables and diagnostic groups, namely controls (HC), bipolar 1 (BPI), bipolar 2 (BPII), and major depression (MDD) as primary explanatory variables, while adjusting for age, sex, body mass index (BMI), nicotine dependence (ND), and education

Type test	Dependent variables	Explanatory variables	*F*	*df*	*p*	Partial eta squared
Multivariate ≠ 1	SOD1, LOOH, CAT, NOx, MDA, AOPP	HC, BPI, BPII, MDD	2.20	18/320	0.004	0.104
		ND_3groups	1.64	12/226	0.078	0.081
		Age	1.17	6/113	0.328	0.058
		Sex	4.98	6/113	< 0.001	0.209
		BMI	1.46	6/113	0.197	0.072
		Education	2.90	6/133	0.011	0.133
Between-subject effects	SOD1	HC, BPI, BPII, MDD	3.95	3/118	0.010	0.091
	LOOH	HC, BPI, BPII, MDD	1.97	3/118	0.178	0.041
	CAT	HC, BPI, BPII, MDD	0.77	3/118	0.516	0.019
	NOx	HC, BPI, BPII, MDD	2.38	3/118	0.083	0.055
	MDA	HC, BPI, BPII, MDD	1.02	3/118	0.296	0.031
	AOPP	HC, BPI, BPII, MDD	3.05	3/118	0.031	0.072
Multivariate #2	zLOOH+SOD,	HC, BPI, BPII, MDD	2.49	15/315	0.002	0.095
	zLOOH+SOD-CAT,	ND_3groups	1.54	10/228	0.126	0.063
	zLOOH+SOD+NOx,	Age	0.79	5/114	0.558	0.034
	zLOOH+SOD+NOx+MDA,	Sex	6.02	5/114	< 0.001	0.209
	zLOOH+SOD+NOx+AOPP	BMI	1.71	5/114	0.137	0.070
		Education	3.37	5/114	0.007	0.129
Between-subject effects	zLOOH+SOD	HC, BPI, BPII, MDD	5.21	3/118	0.002	0.117
	zLOOH+SOD-CAT	HC, BPI, BPII, MDD	2.88	3/118	0.039	0.068
	zLOOH+SOD+NOx	HC, BPI, BPII, MDD	7.35	3/118	< 0.001	0.157
	zLOOH+SOD+NOx+MDA	HC, BPI, BPII, MDD	8.18	3/118	< 0.001	0.172
	zLOOH+SOD+NOx+AOPP	HC, BPI, BPII, MDD	7.35	3/118	< 0.001	0.157
Multivariate #3[a]	zLOOH+SOD,	HC, BPI, BPII, MDD	1.99	15/312	0.016	0.080
	zLOOH+SOD-CAT,	HAM-D	3.30	5/113	0.008	0.127
	zLOOH+SOD+NOx,					
	zLOOH+SOD+NOx+MDA,					
	zLOOH+SOD+NOx+AOPP					
Between-subject effects	zLOOH+SOD	HC, BPI, BPII, MDD	5.53	3/117	0.001	0.124
	zLOOH+SOD-CAT	HC, BPI, BPII, MDD	3.27		0.024	0.077
	zLOOH+SOD+NOx	HC, BPI, BPII, MDD	7.25		< 0.001	0.157
	zLOOH+SOD+NOx+MDA	HC, BPI, BPII, MDD	8.29		< 0.001	0.175
	zLOOH+SOD+NOx+AOPP	HC, BPI, BPII, MDD	7.08		< 0.001	0.148
	zLOOH+SOD+NOx+AOPP	HAM-D	7.08	1/117	< 0.001	0.057
Multivariate #4[a]	zLOOH+SOD,	BPI, BPII, MDD	3.48	10/134	< 0.001	0.206
	zLOOH+SOD-CAT,	Dichotomized HAM-D (< 7 versus > 7)	1.05	5/67	0.398	0.072
	zLOOH+SOD+NOx,					
	zLOOH+SOD+NOx+MDA,					
	zLOOH+SOD+NOx+AOPP					
Between-subject effects	zLOOH+SOD	BPI, BPII, MDD	9.07	2/71	< 0.001	0.204
	zLOOH+SOD-CAT	BPI, BPII, MDD	6.96	2/71	0.002	0.164
	zLOOH+SOD+NOx	BPI, BPII, MDD	10.46	2/71	< 0.001	0.228
	zLOOH+SOD+NOx+MDA	BPI, BPII, MDD	15.25	2/71	< 0.001	0.301
	zLOOH+SOD+NOx+AOPP	BPI, BPII, MDD	11.73	2/71	< 0.001	0.248
Multivariate #5[a]	zLOOH+SOD,	BPI, BPII, MDD in patients with HAM-D < 11 only	3.17	10/74	0.003	0.306
	zLOOH+SOD-CAT,					
	zLOOH+SOD+NOx,					
	zLOOH+SOD+NOx+MDA,					
	zLOOH+SOD+NOx+AOPP					
Between-subject effects	zLOOH+SOD	BPI, BPII, MDD	4.35	2/40	0.020	0.179
	zLOOH+SOD-CAT	BPI, BPII, MDD	6.07	2/40	0.005	0.233
	zLOOH+SOD+NOx	BPI, BPII, MDD	5.86	2/40	0.006	0.227
	zLOOH+SOD+NOx+MDA	BPI, BPII, MDD	8.02	2/40	0.001	0.286
	zLOOH+SOD+NOx+AOPP	BPI, BPII, MDD	8.84	2/40	0.001	0.306

zLOOH+SOD computed as z transformation of LOOH (zLOOH) + zSOD

zLOOH+SOD-CAT computed as zLOOH + zSOD − zCAT

zLOOH+SOD+NOx computed as zLOOH + zSOD + zNOx

zLOOH+SOD+NOx+MDA computed as zLOOH + zSOD + zNOx + zMDA

zLOOH+SOD+NOx+AOPP computed as zLOOH + zSOD + zNOx + zAOPP

SOD superoxide dismutase, *LOOH* lipid hydroperoxides, *CAT* catalase, *NOx* nitric oxide metabolites, *MDA* malondialdehyde, *AOPP* advanced oxidation protein products

[a] All multivariate and univariate GLM analyses are adjusted for age, sex, nicotine dependence (ND), years of education and BMI

Table 3 Model-generated estimated marginal mean (SE) values (expressed in z values) obtained by the general linear model analyses shown in Table 2

Variables	Healthy controls^A	BPI^B	BPII^C	MDD^D
SOD1	− 0.09 (0.15)^D	+ 0.04 (0.20)	− 0.52 (0.23)^D	+ 0.46 (0.18)^{A,C}
LOOH	+ 0.19 (0.15)	+ 0.16 (0.20)	− 0.06 (0.23)	+ 0.57 (0.18)
CAT	− 0.36 (0.16)	− 0.06 (0.22)	− 0.17 (0.25)	− 0.05 (0.20)
NOx	− 0.24 (0.17)	− 0.16 (0.22)	− 0.28 (0.26)	+ 0.39 (0.20)
MDA	+ 0.23 (0.15)	+ 0.10 (0.20)	− 0.10 (0.23)	+ 0.44 (0.18)
AOPP	+ 0.04 (0.13)^B	+ 0.51 (0.17)^{A,C}	+ 0.04 (0.19)^B	+ 0.18 (0.15)
zLOOH+SOD	+ 0.10 (0.21)^D	+ 0.20 (0.29)^C	− 0.58 (0.33)^{B,D}	+ 1.02 (0.26)^{A,B,C}
zLOOH+SOD-CAT	+ 0.46 (0.25)	+ 0.26 (0.35)	− 0.40 (0.39)^D	+ 1.06 (0.31)^C
zLOOH+SOD+NOx	− 0.14 (0.26)^D	+ 0.04 (0.36)^D	− 0.85 (0.41)^D	+ 1.41 (0.32)^{A,B,C}
zLOOH+SOD+NOx+MDA	+ 0.10 (0.30)^{C,D}	+ 0.14 (0.41)^D	− 0.96 (0.46)^{A,B,D}	+ 1.85 (0.37)^{A,B,C}
zLOOH+SOD+NOx+AOPP	− 0.18 (0.30)^D	+ 0.55 (0.40)^C	− 0.81 (0.46)^{B,D}	+ 1.59 (0.37)^{A,C}

zLOOH+SOD computed as z transformation of LOOH (zLOOH) + zSOD

zLOOH+SOD-CAT computed as zLOOH + zSOD − zCAT

zLOOH+SOD+NOx computed as zLOOH + zSOD + zNOx

zLOOH+SOD+NOx+MDA computed as zLOOH + zSOD + zNOx + zMDA

zLOOH+SOD+NOx+AOPP computed as zLOOH + zSOD + zNOx + zAOPP

SOD superoxide dismutase, *LOOH* lipid hydroperoxides, *CAT* catalase, *NOx* nitric oxide metabolites, *MDA* malondialdehyde, *AOPP* advanced oxidation protein products

composite scores, we entered the latter in regression #2, Table 2. There was no significant effect of ASSIST hypnotics ($F = 1.06$, $df = 5/113$, $p = 0.386$), while ASSIST alcohol resulted in a significant effect ($F = 2.83$, $df = 5/113$, $p = 0.019$) although none of the univariate effects was significant. There were no significant effects of the drug state of the patients on the five composite scores, namely antidepressants ($F = 0.90$, $df = 5/107$, $p = 0.483$), lithium ($F = 0.70$, $df = 5/106$, $p = 0.628$), mood stabilizers ($F = 0.79$, $df = 5/106$, $p = 0.556$), and atypical antipsychotics ($F = 1.00$, $df = 5/107$, $p = 0.421$).

Best Predictions of O&NS Biomarkers

In order to delineate the best predictors of the biomarkers, we have carried out multiple regression analyses with the

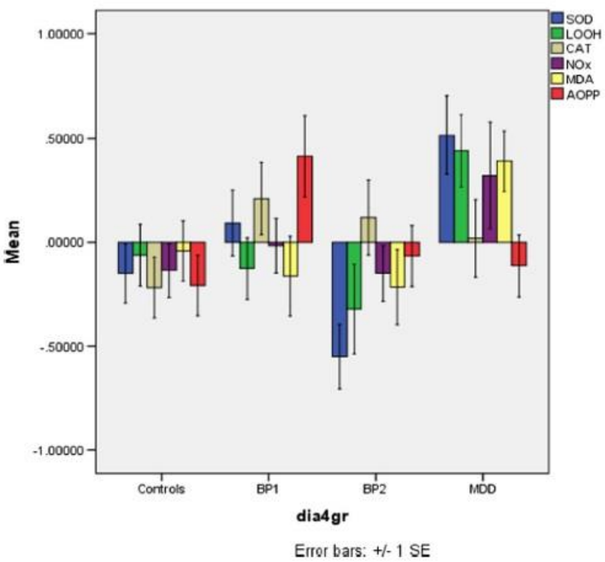

Fig. 2 The z-transformed values of the O&NS biomarkers in the four study groups

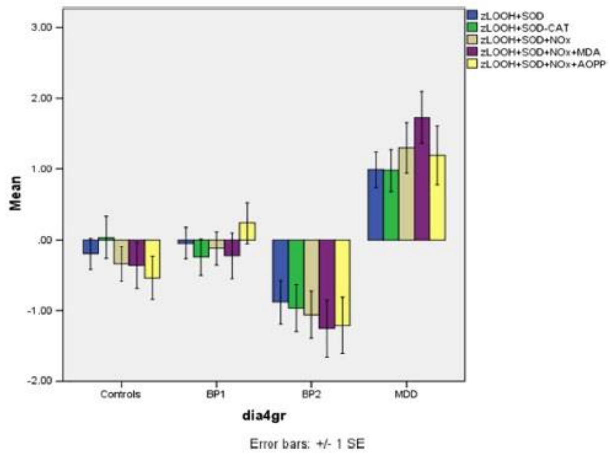

Fig. 3 The z transformed values of the five composite scores in the four study groups

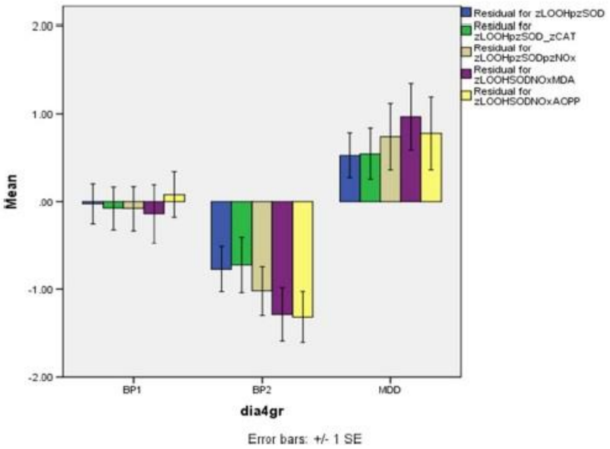

Fig. 4 The residualized composite scores in the three mood disorder subgroups after adjusting for age, sex, body mass index, education, nicotine dependence, and the dichotomized HAM-D score (cut-off value 7)

biomarkers as dependent variables and the diagnostic groups (entered as four dummy variables: namely controls versus mood disorders, BPI versus the rest, BPII versus the rest, and depression versus the rest), HAM-D score, nicotine dependence (entered as three dummy variables, namely dependence versus no-dependence, mild dependence that is Fagerstrom score between 2 and < 6, and severe dependence, that is Fagerstrom score ≥ 6), age, sex, education, and BMI as explanatory variables. Table 4 shows that 21.4% of the variance in SOD1 was predicted by BPII, education (inversely associated), and age (positively). Of the variance in LOOH levels, 26.1% was explained by the HAM-D score, mild and severe nicotine dependence (positively), BPI and BPII (both negatively), and male sex. NOx was associated with major depression and nicotine dependence (positively). Of the variance in MDA, 15.2% was explained by the regression on major depression and years of education, while 22.6% of the variance in AOPP levels was explained by HAM-D score, male sex, and BMI (all positively associated).

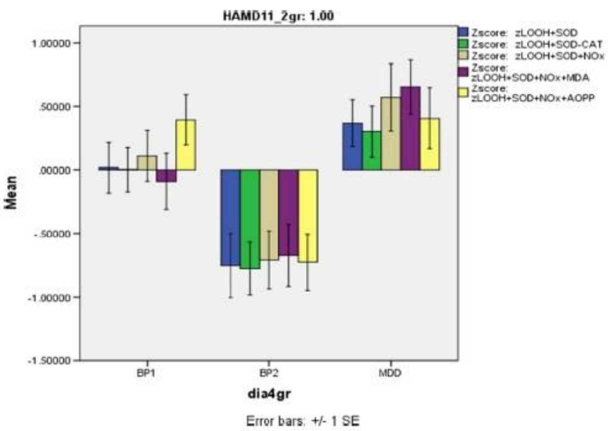

Fig. 5 z transformations of the five composite scores in patients with a HAM-D score < 11

Of the variance in zLOOH+SOD, 29.8% was explained by major depression (positively), BPII (inversely), male sex, and years of education (inversely). Of the variance in zLOOH+SOD-CAT, 30.4% was explained by the regression on BPII (inversely), mild and severe nicotine dependence (positively), male sex, and education (inversely). Of the variance in zLOOH+SOD+NOx, 23.4% was explained by the regression on depression (positively), BPII, and education (both inversely). zLOOH+SOD+NOx+MDA was best predicted by major depression (positively), male sex, education, and BPII (both inversely). Of the variance in zLOOH+SOD+NOx+AOPP, 28.6% was explained by the regression on depression, HAM-D, BMI (all three positively), male sex, and BPII (inversely). The number of depressive and manic episodes was not significant in these regressions. There were also no significant univariate correlations between any of the biomarkers or composite scores and number of depressive and manic episodes.

Table 5 shows the outcome of a multiple regression analysis with the HAM-D as dependent variable and all 11 biomarkers together with age, sex, education, and number of episodes as explanatory variables. We found that 32.2% of the variance in HAM-D was explained by AOPP, number of depressive episodes, female sex, and education.

Discussion

The first major finding of this study is that patients with major depression in (partial) remission show significant aberrations in O&NS biomarkers as compared with HCs. These findings extend the results of recent meta-analyses indicating that depression is accompanied by indices of O&NS stress, including increased MDA or TBARS [35, 36]. Nevertheless, these meta-analyses reported that treatment with antidepressants significantly suppresses MDA/TBARS levels, while in our study, lipid peroxidation was also elevated in patients with depression who were in (partial) remission (HAM-D scores < 11). In addition, our study found higher SOD1 levels but no significant changes in catalase activity in participants with depression relative to HCs. The meta-analysis conducted by Liu et al. [35] reported a trend toward increased SOD levels in depression and no difference in catalase activity between patients with depression and controls. SOD catalyzes the partition of superoxide radicals into oxygen and hydrogen peroxides, which are further degraded into H_2O by catalase. SOD is a major protective antioxidant enzyme against the damaging effects of increased superoxide levels and additionally protects against peroxynitrite formation [66]. Nevertheless, SOD activity is accompanied by increased formation of hydrogen peroxides, which can generate other ROS, including

Table 4 Results of multivariate regression analyses with oxidative and nitrosative stress biomarkers as dependent variables and diagnosis, nicotine dependence (ND), age, sex, body mass index, Hamilton Depression Rating Scale (HAM-A), and years of education as additional explanatory variables

Dependent variables	Explanatory variables	*t*	*p*	R (model)	*F*	*df*	*p*
SOD1	BPII	−2.97	0.004	0.214	10.69	3/118	< 0.001
	Age	+2.07	0.041				
	Education	−3.60	< 0.001				
LOOH	BPI	−2.41	0.018	0.261	6.79	6/115	< 0.001
	BPII	−2.49	0.014				
	Mild-ND	+2.86	0.005				
	Severe-ND	+2.29	0.030				
	HAM-D	+3.11	0.002				
	Sex	+2.92	0.004				
NOx	MDD	+2.73	0.007	0.070	4.75	2/120	0.013
	ND	+2.20	0.030				
MDA	MDD	+2.17	0.032	0.152	11.48	2/128	< 0.001
	Education	−3.88	< 0.001				
AOPP	HAM-D	+3.70	< 0.001	0.226	11.59	3/119	< 0.001
	Sex	+4.44	< 0.001				
	BMI	+2.17	0.032				
LOOH+SOD	MDD	+3.45	0.001	0.298	12.31	4/116	< 0.001
	BPII	−2.70	0.008				
	Sex	+2.23	0.028				
	Education	−3.83	< 0.001				
LOOH+SOD-CAT	BPII	−2.47	0.015	0.304	9.98	5/114	< 0.001
	Mild-NC	+2.51	0.014				
	Severe-NC	+2.80	0.006				
	Sex	+3.10	0.002				
	Education	−2.88	0.005				
LOOH+SOD+NOx	MDD	+3.91	< 0.001	0.234	11.92	3/117	< 0.001
	BPII	−2.26	0.026				
	Education	−2.32	0.022				
LOOH+SOD+NOx+MDA	MDD	+4.48	< 0.001	0.357	16.08	4/116	< 0.001
	BPII	−2.65	0.009				
	Sex	+2.38	0.027				
	Education	−4.17	< 0.001				
LOOH+SOD+NOx+AOPP	MDD	+3.65	< 0.001	0.286	9.23	5/115	< 0.001
	BPII	−2.86	0.005				
	HAM-D	+2.50	0.014				
	Sex	+3.69	< 0.001				
	BMI	+2.21	0.029				

zLOOH+SOD computed as z transformation of LOOH (zLOOH) + zSOD

zLOOH+SOD-CAT computed as zLOOH + zSOD − zCAT

zLOOH+SOD+NOx computed as zLOOH + zSOD + zNOx

zLOOH+SOD+NOx+MDA computed as zLOOH + zSOD + zNOx + zMDA

zLOOH+SOD+NOx+AOPP computed as zLOOH + zSOD + zNOx + zAOPP

Diagnosis is entered as four dummy variables, namely controls versus mood disorders, bipolar I (BPI) versus the rest, BPII versus the rest, and depression (MDD) versus the rest. Nicotine dependence is entered as three dummy variables, namely dependence versus no-dependence, mild dependence (that is Fagerstrom score between 2 and < 6) versus the rest, and severe dependence (that is Fagerstrom score > 6) versus the rest

SOD superoxide dismutase, *LOOH* lipid hydroperoxides, *CAT* catalase, *NOx* nitric oxide metabolites, *MDA* malondialdehyde, *AOPP* advanced oxidation protein products

the very reactive hydroxyl or metal-associated radicals [66, 67]. Moreover, oxidative stress and inflammatory triggers (e.g., T cell activation) may enhance SOD activity as well as its de novo synthesis especially in early stages of an injury [68, 69]. This explains why enhanced SOD activity may constitute a compensatory mechanism protecting against overwhelming amounts of superoxide.

In order to decipher the association between mood disorders and nitro-oxidative pathways, we computed specific composite scores that may reflect ROS and LOOH formation (zLOOH+SOD) and the protective effects of catalase on ROS/LOOH production (zLOOH+SOD-CAT). The increased levels of zLOOH+SOD and zLOOH+SOD-CAT found in depressed patients indicate an increased production of ROS/

Table 5 Results of multivariate regression analyses with the Hamilton Depression Rating Scale (HAM-D) score as dependent variable and number of depressive episodes, oxidative and nitrosative stress biomarkers, nicotine dependence, age, sex, body mass index, and years of education as explanatory variables

Dependent variables	Explanatory variables	*t*	*p*	*R* (model)	*F*	*df*	*p*
HAM-D	No. of depression episodes	+ 5.16	< 0.001	0.322	14.36	4/121	< 0.001
	AOPP	+ 3.04	0.003				
	Male sex	− 2.43	0.017				
	Education	− 2.13	0.035				

AOPP advanced oxidation protein products

LOOH, which appears to be insufficiently neutralized by catalase. These findings are in agreement with the meta-analysis by Liu et al. [35], which reported higher peroxide levels and no significant changes in catalase levels in individuals with depression compared to HCs.

This study detected a significant (albeit weak) association between depression and increased NOx levels. Although an increased NO production was not observed in the meta-analysis of Liu et al. [35], some other studies reported increased NOx in depression. Gomes et al. [40] found that depression co-occurring with chronic apical periodontitis is accompanied by increased NO metabolites which are significantly correlated with depression severity. Furthermore, in pregnant women, increased NOx levels are significantly associated with IgM responses to NO-adducts, indicating amplified nitrosylation [70], while NOx as well as IgM responses directed to NO-adducts are significantly associated with a history of mood disorders. Several levels of evidence point to a role of NO in the pathophysiology of depression [2, 3, 71]. Nitrosylation with increased S-nitrosothiol (SNO) levels regulate neuroimmune systems and when moderate may protect neuronal systems, whilst hypernitrosylation may contribute to neuroprogressive and neurodegenerative processes [44, 72].

We also constructed a new composite score reflecting increased ROS/RNS production (namely zLOOH+SOD+NOx). The latter score was increased in depression (even after adjusting for HAM-D scores) indicating increased nitro-oxidative stress and a higher potential to generate hydroxyl radicals and peroxynitrite, which have caused damage to lipid membranes [64]. These findings may explain previous evidence that depression is accompanied by signs of nitro-oxidative damage to fatty acid membranes [18 20, 22, 24, 25] as well as higher protein oxidation and nitration [2, 3].

We also found that major depression is characterized by increased composite scores reflecting activation of the pathway from formation of ROS and lipid peroxidation to reactive aldehydes with long-lasting detrimental consequences (zLOOH+SOD+NOx+MDA) and protein nitro-oxidation (zLOOH+SOD+NOx+AOPP). These findings confirm that depression is accompanied by increased nitro-oxidative stress that may drive damage to proteins and lipids. In addition, our results indicate that that over-activation of these pathways may be evident even during (partial) remission. Previous research detected that TBARS is also increased in the euthymic phase of depression and, therefore, TBARS was described as a trait biomarker of depression rather than a state marker [8]. Our findings are also in agreement with the knowledge that depression is characterized by lowered antioxidant levels which (a) prevent and protect against lipid peroxidation, including lowered LCAT activity, HDL-cholesterol, vitamin E, as well as coenzyme Q10 and paraoxonase 1 activities [9 12, 16, 17, 35], and (b) remove and repair lipid hydroperoxide lesions, including glutathione peroxidase, HDL cholesterol, and LCAT [3, 9, 10, 35, 63].

The second major finding of this study is that there are significant differences in O&NS pathways between patients with BPI and depression as well as HCs. Recent meta-analyses show that BD is accompanied by signs of lipid peroxidation, protein oxidation (as indicated by elevated protein carbonyl levels), NO production, and increased nitrotyrosine levels, indicating enhanced nitration [37, 38, 73]. A previous study reported higher SOD activity in euthymic BD patients [45], while we established increased SOD1 activity in MDD patients only. The results of the current study show that protein oxidation (AOPP levels) is significantly increased in patients with BPI and depression as compared with HCs, but that the nitro-oxidative pathway from ROS+RNS to MDA formation is less activated in BPI than in MDD. Previously, it was shown that increased TBARS in BD is a biomarker of acute depressive and manic episodes and also a possible stage biomarker of BD [8, 74]. Thus, the inclusion of patients in (partial) remission (this study) may explain the lower MDA levels in BPI herein observed. Increased protein oxidation was previously observed in BD as measured with protein carbonyls, expressed as C=O contents [38]. Nevertheless, protein carbonylation (C=O) and formation of AOPP (O=C) are different pathways. Protein carbonyls are generated from direct protein carbonylation via the effects of hydrogen peroxides, while an indirect mechanism involves non-oxidative reactions with oxidized lipids that contain carbonyl radicals and cleavage of protein backbones as well as α-amidation [75 77]. AOPPs, on the other hand, are generated via increased production of ROS and peroxynitrite coupled with increased myeloperoxidase activity during chlorine stress [42, 76]. The results of the current study suggest that increased protein oxidation with generation of AOPPs is associated with BPI and MDD even in (partial) remission, while illness severity seemed to be accompanied by higher AOPP levels.

The third major finding of this study is that patients with BPII show significantly lower SOD1 and LOOH levels and lower zLOOH+SOD+NOx, zLOOH+SOD+NOx+MDA, and zLOOH+SOD+NOX+AOPP scores when compared to all other groups. In addition, BPII patients exhibited lower AOPP, zLOOH+SOD, zLOOH+SOD+NOx+MDA, and zLOOH+SOD+NOX+AOPP scores as compared to BPI patients and a significantly lower zLOOH+SOD+NOx+MDA score than HCs. These marked differences among patients with BPI and BPII in most O&NS pathways measured herein have not been previously reported. A previous study found that TBARS levels to be significantly increased in BPI and BPII patients compared to controls, while no significant differences in TBARS between both BD subtypes were observed [8]. These discrepant findings may be explained by differences in study samples regarding phase of illness (acute versus partial remission). Our results show that the O&NS pathway from ROS production to nitro-oxidative damage is attenuated in (partially) remitted BPII patients. One hypothesis is that patients with BPII could be protected against nitro-oxidative stress or display more adequate removal and repair mechanisms of lipid peroxide lesions and that this could explain its milder clinical phenotype as compared to BPI. Possible protective and repair mechanisms could be enhanced antioxidant defenses and better regulation through LCAT or IgM-mediated autoimmune responses [70]. However, no evidence is available to support this tentative hypothesis. It should be also noted that our findings are consistent with other levels of evidence that point to biological differences between BPI and BPII although this remains a relatively unexplored field [78, 79].

Another finding is that O&NS pathways in mood disorders could be modulated by effects of sex, education, and nicotine dependence. Thus, peroxides and AOPP levels and all composite scores were significantly higher in males than females. Previously, it was observed that plasma peroxides, but not IgG/IgM responses to oxidized LDL, are significantly greater in males than females [23] and that males show greater responses in ROS production than females [80]. The current study found that male sex is specifically associated with increased lipid hydroperoxide production, while there are no significant sex-linked differences in SOD1 or catalase activities. We observed that education is inversely associated with SOD1, the zLOOH+SOD composite score, and MDA formation, suggesting that education has a protective effect on the generation of ROS, thereby protecting against lipid peroxidation and reactive aldehyde production. Such effects may likely be explained by education resulting in a healthier lifestyle, which may increase protection or repair mechanisms through for example nutrition and exercise [81, 82]. Previous studies reported a strong impact of smoking and nicotine dependence on different O&NS pathways [83, 84] and that the increased risk for development of mood disorders in smokers is in part associated with smoking-induced oxidative stress pathways [85]. Nevertheless, in the current study, there were only mild associations between nicotine dependence (mild or severe) and increased LOOH levels, indicating that current smoking may somewhat increase lipid peroxidation without significant effects on reactive aldehyde formation and oxidative damage to proteins. Although age is significantly associated with increased levels of some, but not all, O&NS pathways and lowered antioxidant enzyme defenses [86, 87], the current study was unable to find relevant associations among age and the pathways measured herein. One explanation is that a stronger impact of diagnosis, sex, and education on O&NS pathways could have blurred the effects of age.

The results of the current study should be interpreted within its limitations. Firstly, this is a cross-sectional study, and therefore, no firm causal inferences can be established. Second, we assayed peripheral biomarkers and the extent to which these findings reflect brain alterations remain imprecise.

The current study showed that there is another fundamental problem with classifications of mood disorders, which often lump both BP types together, while in fact, our results show that both BPI and BPII may be quite different biological entities, which in addition differ from major depression. Current psychiatric nosological diagnoses are heavily debated [88] because mental disorders as defined by the DSM-IV-TR and DSM-5 lack statistical and biological validation [89 91]. Most diagnostic categories based on syndromal phenomenology will soon become a historical footnote [92, 93]. The delineation of trans-diagnostic phenotypes as defined in the NIMH Research Domain Criteria (RDoC) system may provide a somewhat better outcome [88, 94]. More specifically, physiosomatic symptoms have emerged as a new phenotype and the identification of this construct across mood disorders as well as somatizing and psychotic disorders has aided in the identification of more precise diagnostic biomarkers, including activated nitro-oxidative pathways [91, 95, 96]. Nevertheless, both consensus-based DSM classifications and the RDoC system miss our point that (a) classifications of psychiatric phenomenology should be derived from pattern recognition methods including supervised and unsupervised learning, which should be used to refute or consolidate existing classifications and detect new classifications, which additionally should be externally validated by biomarkers [91, 97], and (b) trans-diagnostic phenotypes should be derived by pattern recognition methods including deep learning to discover pathophysiologically delineated endophenotypes [96, 98]. Nevertheless, the sample size of the current study did not allow the conduction of unsupervised machine learning analyses in order to provide a more accurate indication of the possible clinical utility of the biomarkers herein measured as a means to aid in the differentiation of mood disorders or to detect relevant endophenotypes.

In conclusion, the current study indicates that alterations in specific nitro-oxidative pathways may differ among depression, BPI, and BPII. If replicated, these findings open relevant perspectives including the development of a panel of biomarkers that could aid in the differentiation of mood disorders. Furthermore, future studies should explore possible differences in mood disorders in other biomarkers related to O&NS including but not limited to myeloperoxidase, iNOS activity, protein carbonyls, and IgM-mediated autoimmune responses to oxidatively formed neopitopes.

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Author Contributions All authors contributed to the writing up of the paper. The work was designed by SOVN, MM, DSB, and HOV. Data were collected by SOVN and HOV. Laboratory analyses were conducted by KLB, NRM, and DSB. Statistics were performed by MM. AFC revised the manuscript and provided relevant intellectual content. All authors revised and approved the final draft.

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5.2 ARTIGO 2

Metabolic syndrome components might be associated with the oxidative stress alterations in patients with mood disorders.

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Abstract:
Bipolar Disorder (BD) – classified in types I and II – and Major Depressive Disorder (MDD) are part of a psychiatry disorders group defined as mood disorders. BD and MDD present a higher risk for metabolic syndrome (MetS), a cluster of risk factors to the development of cardiovascular. Since the oxidative stress (OS) has been reported in mood disorders and MetS, the aim of this study was to assess the interaction between OS biomarkers and MetS parameters in subjects with BD and MDD compared to controls. Controls and patients with mood disorders (BD I, BD II and MDD) were divided in groups with and without MetS. Protein oxidation was associated with triglycerides (TG) levels in patients with mood disorders and MetS and it seems to be the main OS biomarker involved with the MetS in BD, mainly in BD I. Correlations between antioxidant defenses and MetS parameters were found in BD II and MDD. In addition, glucose levels were associated with lipid peroxidation in patients with mood disorders and MetS. In conclusion, the OS in patients with BD and MDD seems to be closely associated with MetS parameters.

Keywords: Bipolar disorder, depressive disorder, comorbidity, metabolic disorder, oxidants.

1. Introduction

Bipolar Disorder (BD) and Major Depressive Disorder (MDD) are part of a psychiatry disorders group defined as mood disorders. A lifetime prevalence for BD is around 5% including subjects with the BD spectrum, and 16% for MDD (Rakofsky and Rapaport, 2018). BD and MDD are among the leading causes of years lived with disability worldwide (Global Burden of Disease Study 2013 Collaborators, 2015). Besides, subjects with psychiatry disorders present a higher risk of suicide and self-harm than the general population (Bolton et al., 2015).

MDD is characterized only by depressive episodes, while subjects with BD present mood swings. In BD episodes of mania, hypomania and depression are alternated or mixed. Based on the manifestation of the symptoms, BD is classified in types I and II. Even though the symptoms seem to be more severe in BD I, the impairment in quality of life is higher in BD II, because of the elevated episode frequency, presence of psychiatric comorbidities and recurrent suicidal behavior (Grande et al., 2016).

A meta-analysis showed in subjects with psychiatry disorders, including BD and MDD, a higher risk for metabolic syndrome (MetS) in comparison with the general population (Vancampfort et al., 2015). MetS is a cluster of risk factors to the development of cardiovascular disease including abdominal obesity, decreased high-density lipoprotein (HDL) levels and increased triglycerides (TG), glucose and blood pressure (BP) levels (Grundy et al., 2005). A previous study supports that the prevalence of MetS is higher in subjects with BD than in subjects with MDD (Silarova et al., 2015). A possible reason for the link between MetS and psychiatric disorders is the poor lifestyle observed in these subjects, such as smoking, alcohol intake, sedentary and an unhealthy nutrition (Penninx and Lange, 2018).

Oxidative stress (OS) biomarkers have been reported in many diseases, including mood disorders (Brown et al., 2014; Mazereeuw et al., 2015) and MetS (Vona et al., 2019). Reactive oxygen species (ROS), for example, superoxide anion ($O_2^{\bullet-}$), hydroxyl radical ($OH^{\bullet}$) and hydrogen peroxide (H_2O_2), are produced during the cell metabolism. To neutralize the effect of ROS, the organism has an antioxidant system in which includes enzymes such as superoxide dismutase (SOD) and catalase (CAT). When there is an imbalance between ROS and antioxidant levels, favoring the action of ROS, the status defined as OS is established. ROS can lead to damage lipids, proteins, and DNA generating different products used to measure the OS (Birben et al., 2012).

Since MetS is a comorbidity commonly observed in subjects with mood disorders and the OS might be associated with both conditions, the aim of this study was to assess the interaction between OS biomarkers and MetS parameters in subjects with BD and MDD compared to controls.

2. Methods

2.1. Study design

This cross-sectional study had the participation of 185 subjects between controls and patients with mood disorders, including BD I, BD II and MDD. Controls and patients were allocated in four groups according to the diagnosis of MetS, namely as controls with MetS (G1, *n* = 20), controls without MetS (G2, *n* = 56), mood disorders with MetS (G3, *n* = 38) and mood disorders without MetS (G4, *n* = 71). All the patients were also divided according to the diagnosis of BD I (*n* = 39), BD II (*n* = 29) and MDD (*n* = 41) and subdivided again by the presence of MetS.

2.2. Subjects and clinical diagnosis

The patients were recruited at the Psychiatry Outpatient Clinic from the University Hospital of the State University of Londrina (UEL), Brazil, and the controls were recruited between students and staff from UEL. The written informed consent was obtained from all the participants and the Ethics Committee on Research from UEL (CAAE 34935814.2.0000.5231) approved this study.

For the diagnosis of BD I, BD II and MDD was used the validated Brazilian Portuguese version of the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-I) following the Diagnostic and Statistical Manual of Mental Disorders, 4th Edition, Text Revision (DSM-IV-TR) diagnostic criteria (American Psychiatric Association, 2000; Del-Ben et al., 2001). Brazilian Portuguese version of the 17-item Hamilton Depression Rating Scale (HAM-D) (Moreno and Moreno, 1998) and the Young Mania Rating Scale (YMRS) (Vilela et al., 2005) were used to measure the severity of depression and the severity of manic symptoms, respectively.

All the participants were from both sex (male and female) and between 20 to 68 years old. The exclusion criteria for patients and controls were hepatitis B and C virus and HIV infection, neurodegenerative (Alzheimer's disease, Parkinson's disease) and neuroimmune (multiple sclerosis) diseases, chronic obstructive pulmonary disease, chronic kidney disease, cancers, autoimmune diseases (type 1 diabetes, rheumatoid arthritis, systemic lupus erythematosus), another axis I diagnosis according to DSM-IV-TR criteria (autism, schizophrenia, schizo-affective disorder, psycho-organic syndromes) and pregnancy. Subjects in treatment with non-steroidal anti-inflammatory drugs, interferon, glucocorticoids, herbal supplements, antioxidants and omega-3 for the past 4 weeks before the study were also excluded.

2.3. MetS diagnosis

For the MetS diagnosis was used the National Cholesterol Education Program (NCEP) Adult Treatment Panel III (ATP III) criteria (Grundy et al., 2005). NCEP ATP III requires at least 3 of 5 risk factors: waist circumference (WC) ≥ 102 cm (men) and ≥ 88 cm (women); TG ≥ 150 mg/dL; HDL < 40 mg/dL (men) and < 50 mg/dL (women); systolic blood pressure (SBP) ≥ 130 mmHg or diastolic blood pressure (DBP) ≥ 85 mmHg or antihypertensive treatment; and fasting glucose ≥ 100 mg/dL or hypoglycemic treatment.

2.4. Body mass index, WC and BP assessment

The body mass index (BMI) was calculated following the formula: weight divided by square of height (kg/m^2); and WC (cm) was measured at the midway between the margin of the lower rib and the iliac crest (Madden and Smith, 2016). BP (mm/Hg) was assessed with the subjects at rest (Vischer and Burkard, 2017).

2.5. Biochemical parameters assays

After 12h of overnight fasting, blood samples from controls and patients were collected. Glucose, total cholesterol (TC), HDL, TG, uric acid (UA) and total protein levels were analyzed by a biochemical auto-analyzer (Dimension AR, Siemens, Berlin, Germany). Low-density lipoprotein (LDL) was calculated by Friedewald's equation: TC

- (HDL + TG/5) (Friedewald et al., 1972). Insulin levels were analyzed by automated methodology (Architect, Abbot Laboratories, Abbot Park, IL, USA). Glucose, TC, HDL, LDL and TG levels were expressed in mg/dL while insulin levels were expressed in μU/mL.

The homeostasis model assessment for insulin resistance (HOMA-IR) was calculated using the formula: fasting insulin (μU/mL) x fasting glucose (mmol/L)/22.5 (Haffner et al., 1997). The Castelli I and II indexes, used to assess the atherogenic risk, were calculated respectively by TC/HDL and LDL/HDL ratio (Millán et al., 2009).

2.6. OS biomarkers assays

Advanced oxidation protein products (AOPP) was performed to measure the levels of protein oxidation by spectrophotometry according to the method described by Hanasand et al. (2012). AOPP concentrations were expressed in μM. Lipid peroxidation was analyzed by lipid hydroperoxides (LOOH) and malondialdehyde (MDA). LOOH levels were measured by chemiluminescence, following the adapted method described by Gonzalez Flecha et al. (1991). The results were expressed in relative light units (RLU). MDA levels were measured by high performance liquid chromatography (HPLC) according to the method described by Bastos et al. (2012). MDA concentrations were corrected by total protein levels and the results expressed in μM/mg proteins.

Total radical-trapping antioxidant parameter (TRAP) was performed to analyze the antioxidant capacity by chemiluminscence, following the adapted method described by Repetto et al. (1996). The UA represents about 50% of the total antioxidant capacity (Ndrepepa, 2018), for this reason, TRAP levels were normalized by UA levels (TRAP/UA) and the results expressed in μM trolox/mg UA. The sulfhydryl (SH) group, an antioxidant, was measured using a colorimetric method previously described by HU (1994) and adapted for microplate by Taylan and Resmi (2010). SH concentrations were expressed in μM. The activity of the antioxidant enzyme SOD1 was analyzed by the pyrogallol method described by Marklund and Marklund (1974). SOD1 concentrations were expressed in U/mg hemoglobin (Hb).

2.7. Statistical analysis

Kolmogorov-Smirnov test was used to analyze the normal distribution of the variables. Pearson chi-squared test (analyses of contingency tables) was used for nominal variables and one-way analysis of variance (ANOVA) with Tukey's post hoc or Kruskal-Wallis test with Dunn's post hoc for scale variables. Multiple regression (for scale variables) and logistic regression (for nominal variables) were performed with the MetS parameters as dependent variables. Spearman and Pearson correlations were used between MetS parameters and OS biomarkers. A ROC curve was performed to analyze sensibility and specificity of the biomarker AOPP. Statistical significance was set at $p < 0.05$. SPSS Statistics, MedCalc and GraphPad InStat softwares performed the analyses.

3. Results

Table 1 shows the sociodemographic data, BMI, WC, BP, biochemical parameters, Castelli I and II indexes and HOMA-IR in controls and patients with mood disorders divided in groups with and without MetS. There was no statistical significance between the groups for sex ($p = 0.084$) and age ($p = 0.084$). BMI ($p < 0.001$), WC ($p < 0.001$), SBP ($p < 0.001$), DBP ($p < 0.001$), TC levels ($p = 0.007$), LDL levels ($p = 0.024$), HDL levels ($p < 0.001$), TG levels ($p < 0.001$), glucose levels ($p < 0.001$), insulin levels ($p < 0.001$), Castelli I index ($p < 0.001$), Castelli II index ($p < 0.001$) and HOMA-IR ($p < 0.001$) were statistically significant between the groups.

The post hoc test showed significant difference between G1 x G2, G1 x G4, G2 x G3 and G3 x G4 for BMI (respectively, $p < 0.001$), WC (respectively, $p < 0.001$), SBP (respectively, $p < 0.001$), DBP (respectively, $p < 0.01$), HDL levels (respectively, $p < 0.001$), TG levels (respectively, $p < 0.01$), glucose levels (respectively, $p < 0.001$), insulin levels (respectively, $p < 0.01$), Castelli I index (respectively, $p < 0.001$), Castelli II index (respectively, $p < 0.001$) and HOMA-IR (respectively, $p < 0.001$). BMI, WC, SBP, DBP, TG levels, glucose levels, insulin levels, Castelli I index, Castelli II index and HOMA-IR were increased while HDL levels were decreased in G1 and G3 compared to G2 and G4. In addition, the post hoc test showed significant difference between G1 x G2 for TC levels ($p = 0.025$) and LDL levels ($p = 0.026$) that were increased in G1 compared to G2. There was no significant difference between G1 x G3 and G2 x G4 for any of these variables.

Table 2 shows the OS biomarkers in controls and patients with mood disorders divided in groups with and without MetS. AOPP levels and TRAP/UA levels were the only OS biomarkers with statistical significance between the groups. The post hoc test showed significant difference between G1 x G2, G1 x G4, G2 x G3 and G3 x G4 for AOPP levels (respectively, $p < 0.05$) that were increased in G1 and G3 compared to G2 and G4. In addition, the post hoc test showed significant difference between G1 x G2 and G1 x G4 for TRAP/UA levels (respectively, $p < 0.01$) that were decreased in G1 compared to G2 and G4. There was no significant difference between G1 x G3 and G2 x G4 for these OS biomarkers.

Table 3 shows the multiple regression with glucose, HDL and TG as dependent variables and AOPP, TRAP/UA, SOD1 and MDA as independent variables in patients with mood disorders and MetS (G3). It was found an association between AOPP and TG levels (R^2 *adjusted* 0.438; $p < 0.001$). MDA levels were associated with glucose levels (R^2 *adjusted* 0.305; $p < 0.001$). Logistic regression was performed with BP and WC as dependent variables and glucose, HDL, TG, AOPP and TRAP/UA as independent variables in the group of patients with mood disorders and MetS (G3). The analyses did not show any significant result between the variables (data not shown).

Table 4 shows the correlations between WC, glucose, HDL and TG with AOPP, TRAP/UA, SOD1 and MDA in patients with MetS divided in BD I, BD II and MDD. In BD I patients, it was found a strong correlation of 0.871 between AOPP and TG levels ($p < 0.0001$). In BD II patients, TRAP/UA levels were inversely correlated with glucose levels ($p = 0.040$) and SOD1 activity were directly correlated with HDL levels ($p = 0.031$). In MDD patients, TRAP/UA levels were inversely correlated with WC ($p = 0.045$), but SOD1 activity were directly correlated with TG levels ($p = 0.048$).

Figure 1 shows the ROC curve performed with AOPP as a biomarker for MetS in all the patients divided in BD I, BD II and MDD. The ROC curve analysis showed a significant result for AOPP in BD I and BD II. The area under the curve (AUC) was more significant in BD I (*AUC*: 0.883; $p < 0.0001$) than in BD II (*AUC*: 0.833; $p < 0.001$), suggesting that AOPP was a biomarker with high sensitivity and specificity for BD I.

Table 1: Sociodemographic data, BMI, WC, BP, biochemical parameters, Castelli I and II indexes and HOMA-IR in controls and patients with mood disorders divided in groups with and without MetS.

	Controls		**Mood disorders**		***p* value**
MetS	**Yes (G1, *n* = 20)[A]**	**No (G2, *n* = 56)[B]**	**Yes (G3, *n* = 38)[C]**	**No (G4, *n* = 71)[D]**	
Sex (male/female)[§]	10/10	18/38	8/30	17/54	0.084
Age (years)[†]	43.0 (22.0 – 64.0)	44.5 (20.0 – 68.0)	48.5 (24.0 – 65.0)	38.0 (20.0 – 62.0)	0.084
BMI (kg/m^2)[†]	31.36[B,D] (24.87 – 35.79)	24.04[A,C] (18.61 – 45.17)	29.92[B,D] (21.64 – 39.08)	24.61[A,C] (17.44 – 46.07)	**< 0.001**
WC (cm)[‡]	105.50[B,D] (± 8.92)	87.50[A,C] (± 13.01)	102.00[B,D] (± 8.44)	89.00[A,C] (± 13.02)	**< 0.001**
SBP (mmHg)[†]	130.00[B,D] (110.00 – 180.00)	110.00[A,C] (80.00 – 150.00)	130.00[B,D] (90.00 – 150.00)	110.00[A,C] (90.00 – 160.00)	**< 0.001**
DBP (mmHg)[†]	85.00[B,D] (70.00 – 120.00)	70.00[A,C] (60.00 – 90.00)	80.00[B,D] (60.00 – 100.00)	70.00[A,C] (10.00 – 110.00)	**< 0.001**
TC (mg/dL)[‡]	210.25[B] (± 42.73)	182.23[A] (± 31.79)	202.05 (± 43.51)	186.27 (± 37.34)	**0.007**
LDL (mg/dL)[†]	135.50[B] (66.00 – 193.00)	106.00[A] (66.00 – 196.00)	117.00 (58.00 – 212.00)	107.00 (62.00 – 216.00)	**0.024**
HDL (mg/dL)[†]	35.00[B,D] (23.00 – 62.00)	50.00[A,C] (28.00 – 116.00)	35.50[B,D] (21.00 – 72.00)	50.00[A,C] (23.00 – 86.00)	**< 0.001**
TG (mg/dL)[†]	138.00[B,D] (61.00 – 629.00)	82.50[A,C] (27.00 – 259.00)	187.50[B,D] (81.00 – 417.00)	93.00[A,C] (14.00 – 188)	**< 0.001**

Glucose (mg/dL)†	101.50[B,D] (83.00 – 142.00)	89.00[A,C] (70.00 – 155.00)	103.00[B,D] (74.00 – 250.00)	87.00[A,C] (74.00 – 116.00)	**< 0.001**
Insulin (μU/mL)†	12.40[B,D] (6.70 – 24.90)	7.55[A,C] (2.80 – 17.20)	10.00[B,D] (3.20 – 57.20)	6.45[A,C] (2.30 – 20.30)	**< 0.001**
Castelli I index†	5.59[B,D] (3.04 – 8.96)	3.49[A,C] (2.00 – 8.54)	5.40[B,D] (2.73 – 9.44)	3.56[A,C] (1.90 – 6.92)	**< 0.001**
Castelli II index†	3.87[B,D] (1.29 – 6.91)	2.02[A,C] (0.81 – 7.00)	3.42[B,D] (1.36 – 6.89)	2.16[A,C] (0.78 – 5.14)	**< 0.001**
HOMA-IR†	3.25[B,D] (1.52 – 5.90)	1.54[A,C] (0.58 – 3.99)	2.85[B,D] (0.71 – 14.55)	1.46[A,C] (0.46 – 5.55)	**< 0.001**

MetS: Metabolic syndrome; BMI: Body mass index; WC: waist circumference; BP: Blood pressure; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; TC: Total cholesterol; LDL: Low-density lipoprotein; HDL: High-density lipoprotein; TG: Triglycerides; HOMA-IR: Homeostasis model assessment for insulin resistance. §Pearson chi-squared test. †Kruskal-Wallis test, data expressed in median (minimum and maximum). ‡One-way analysis of variance (ANOVA), data expressed in means (± SD). [A,B,C,D]Significant difference between the groups after post hoc test.

Table 2: OS biomarkers in controls and patients with mood disorders divided in groups with and without MetS.

	Controls		**Mood disorders**		***p* value**
MetS	**Yes (G1, *n* = 20)[A]**	**No (G2, *n* = 56)[B]**	**Yes (G3, *n* = 38)[C]**	**No (G4, *n* = 71)[D]**	
AOPP (µM)†	83.94[B,D] (36.75 – 350.36)	61.83[A,C] (31.78 – 162.36)	90.91[B,D] (43.41 – 251.65)	61.90[A,C] (38.71 – 231.77)	**< 0.001**
MDA (µM/mg protein)†	208.63 (152.81 – 281.69)	208.16 (165.27 – 271.57)	209.08 (161.15 – 359.00)	219.56 (150.40 – 310.69)	0.397
LOOH (RLU)†	$1.61x10^6$ ($8.39x10^5$ – $9.34x10^6$)	$1.37x10^6$ ($3.81x10^5$ – $6.05x10^6$)	$1.27x10^6$ ($4.90x10^5$ – $7.11x10^6$)	$1.55x10^6$ ($3.80x10^5$ – $5.42x10^6$)	0.224
TRAP/UA (µM trolox/mg UA)†	167.98[B,D] (124.68 – 232.59)	213.54[A] (119.42 – 353.03)	184.39 (104.62 – 429.38)	203.79[A] (126.74 – 566.97)	**0.001**
SH (µM)†	374.00 (298.99 – 499.39)	388.44 (267.69 – 506.33)	383.27 (299.87 – 495.65)	400.31 (268.07 – 565.28)	0.750
SOD1 (U/mg Hb)‡	99.51 (± 36.58)	100.37 (± 29.14)	89.22 (± 33.93)	100.72 (± 48.75)	0.615

OS: Oxidative stress; MetS: Metabolic syndrome; AOPP: Advanced oxidation protein products; MDA: Malondialdehyde; LOOH: Lipid hydroperoxide; RLU: Relative light units; TRAP: Total radical-trapping antioxidant parameter; UA: Uric acid; SH: Sulfhydryl group; SOD1: Superoxide dismutase activity; Hb: Hemoglobin. †Kruskal-Wallis test, data expressed in median (minimum and maximum). ‡One-way analysis of variance (ANOVA), data expressed in means (± SD). [A,B,C,D]Significant difference between the groups after post hoc test.

Table 3: Multiple regression with glucose, HDL and TG as dependent variables and AOPP, TRAP/UA, SOD1 and MDA as independent variables in patients with mood disorders and MetS (G3).

	Glucose		**HDL**		**TG**	
	R^2 ***adjusted*****:** 0.305		R^2 ***adjusted*****:** 0.056		R^2 ***adjusted*****:** 0.438	
	Coefficient	***p* value**	**Coefficient**	***p* value**	**Coefficient**	***p* value**
AOPP	-0.029	0.838	-0.064	0.187	1.125	**< 0.001**
TRAP/UA	-0.141	0.135	-0.005	0.877	-0.140	0.432
SOD1	-0.185	0.301	0.041	0.508	0.206	0.536
MDA	0.538	**< 0.001**	-0.087	0.090	0.157	0.555

OS: Oxidative stress; MetS: Metabolic syndrome; HDL: High-density lipoprotein; TG: Triglycerides; AOPP: Advanced oxidation protein products; TRAP: Total radical-trapping antioxidant parameter; UA: Uric acid; SOD1: Superoxide dismutase activity; MDA: Malondialdehyde.

Table 4: Correlations between WC, glucose, HDL and TG with AOPP, TRAP/UA, SOD1 and MDA in patients with MetS divided in BD I, BD II and MDD.

		WC		Glucose		HDL		TG	
		r	***p* value**	***r***	***p* value**	***r***	***p* value**	***r***	***p* value**
BD I	AOPP	-0.223§	0.463	-0.271§	0.348	-0.486§	0.066	0.871§	**< 0.0001**
	TRAP/UA	0.150†	0.622	-0.063§	0.828	-0.046†	0.868	-0.417†	0.121
	SOD1	0.328†	0.426	-0.619§	0.115	0.117†	0.764	0.002†	0.995
	MDA	0.132†	0.753	0.381§	0.359	-0.282†	0.462	-0.222†	0.564
BD II	AOPP	0.307†	0.358	0.077§	0.817	-0.111†	0.743	0.315†	0.345
	TRAP/UA	-0.046†	0.892	-0.637§	**0.040**	-0.327†	0.325	-0.090†	0.790
	SOD1	0.152†	0.718	0.428§	0.299	0.751†	**0.031**	-0.699†	0.053
	MDA	0.586†	0.126	0.619§	0.115	-0.250†	0.550	0.195†	0.642
MDD	AOPP	-0.098†	0.772	0.105†	0.743	-0.144†	0.653	0.185†	0.564
	TRAP/UA	-0.642†	**0.045**	-0.075†	0.824	-0.025†	0.940	-0.171†	0.614
	SOD1	0.353†	0.285	-0.064†	0.843	0.465†	0.127	0.580†	**0.048**
	MDA	-0.102†	0.764	0.544†	0.067	-0.223†	0.485	0.311†	0.325

OS: Oxidative stress; MetS: Metabolic syndrome; BD I: Bipolar I disorder; BD II: Bipolar II disorder; MDD: Major depressive disorder; WC: Waist circumference; HDL: High-density lipoprotein; TG: Triglycerides; AOPP: Advanced oxidation protein products; TRAP: Total radical-trapping antioxidant parameter; UA: Uric acid; SOD1: Superoxide dismutase activity; MDA: Malondialdehyde. §Spearman correlation. †Pearson correlation.

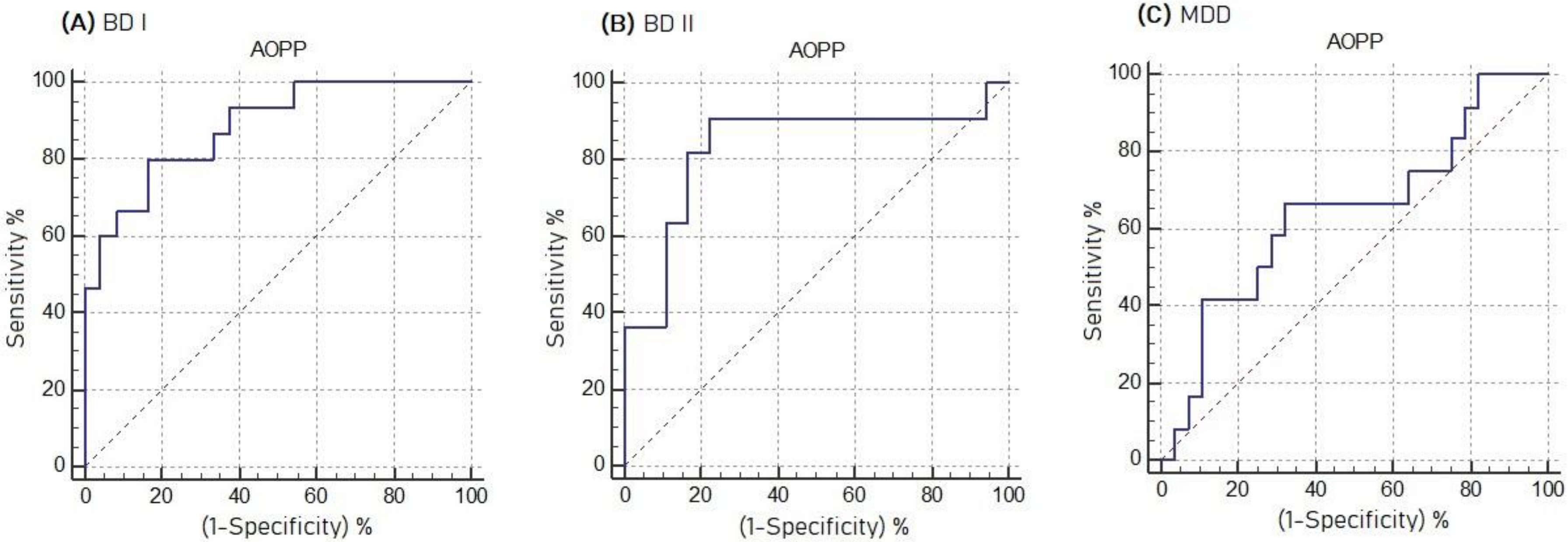

Figure 1: ROC curve performed with AOPP as a biomarker for MetS in all the patients divided in BD I, BD II and MDD. **(A)** *AUC*: 0.883; 95% *CI*: 0.740 to 0.964; Significant level (Area = 0.5): ***p* < 0.0001**. **(B)** *AUC*: 0.833; 95% *CI*: 0.649 to 0.945; Significant level (Area = 0.5): ***p* < 0.001**. **(C)** *AUC*: 0.643; 95% *CI*: 0.476 to 0.788; Significant level (Area = 0.5): $p = 0.160$. BD I: Bipolar I disorder; BD II: Bipolar II disorder; AOPP: Advanced oxidation protein products; *AUC*: Area under the curve; *CI*: Confidence intervals.

4. Discussion

One of the most important findings from our study is that AOPP levels were significantly elevated in subjects with MetS, both controls and patients with mood disorders. AOPP levels were significant associated with TG levels in patients with mood disorders and MetS and the correlation analysis also showed TG levels directly correlated with AOPP levels in BD I. AOPP seems to be the main OS biomarker involved with the MetS in BD, mainly BD I, confirmed by the ROC curve analysis.

Hypertriglyceridemia was already independent associated with AOPP levels in a previous study, suggesting that AOPP is associated with atherosclerosis (Klafke et al., 2015). TG are involved with the development of atheroma plaque and this process is followed by increased ROS production (Peng et al., 2017) which may lead to AOPP formation. Besides, AOPP might be responsible for many mechanisms in the atherosclerosis process. An example is the AOPP ability to impair HDL metabolism by blocking the scavenger receptor class B type I (SR-BI) and inhibiting the reverse cholesterol transport (Ou et al., 2017). High TG levels in patients with mood disorders and MetS observed in our study is a strong indicator of atherogenic risk together with increased AOPP levels, especially for BD I patients in which a strong direct correlation between AOPP and TG levels were present.

AOPP results mainly from the oxidation of albumin by hypochlorous acid (HOCl) produced through myeloperoxidases (MPO) from neutrophils (Ou et al., 2017). The ROC curve analysis showed AOPP as a sensitive and specific biomarker for MetS in both BD subtypes, but this result was more significant in BD I than in BD II. Although protein oxidation biomarkers, for example protein carbonyl, have been reported in BD, lipid peroxidation products are suggested as potential biomarkers for BD (Brown et al., 2014; Scola and Andreazza, 2014). Therefore, our findings indicate that AOPP seems to be a better representative for MetS than the BD itself.

Although the TRAP/UA levels were significant reduced only in controls with MetS, correlations between TRAP/UA and SOD1 activity with MetS parameters were found in BD II and MDD patients, being another important finding from our study. TRAP/UA levels were inversely correlated with glucose levels in BD II patients. Our study is in accordance with a previous one that found an inverse correlation between glucose levels and antioxidant defenses in subjects with and without MetS diagnosis (Awadallah et al., 2019). Still in BD II patients, SOD1 activity were directly correlated with HDL levels. The HDL presents antioxidant activity given by different components, including the enzyme paraoxonase 1 (PON1). Its antioxidant activity can prevent LDL oxidation by free radicals and represents an antiatherogenic effect of HDL (Brites et al., 2017). This correlation may suggest a necessity of the body to increase antioxidant defenses against ROS levels in BD II patients.

The mitochondrial electron transport chain (ETC) is the major physiological source for ROS production in the cells. The $O_2^{\bullet-}$ is released from the mitochondria and is converted into H_2O_2 by the enzyme SOD; then, H_2O_2 can be converted into water and oxygen by the enzyme CAT, but if H_2O_2 react with transition metals, $OH^{\bullet}$ is produced. In BD, mitochondrial dysfunction observed by reduction in the expression of some subunits from mitochondrial complex I suggests an inclination of these patients to ETC deregulation that can lead to increase ROS production and, consequently, to OS (Fraunberger et al., 2016; Scola and Andreazza, 2014).

In MDD patients SOD1 activity were directly correlated with TG levels. Increased SOD1 activity could be a compensatory effect of the body trying to neutralize ROS produced due the high TG levels. Another study found increased SOD activity in MDD patients in an acute phase compared to healthy subjects and suggested a compensatory mechanism as well (Tsai and Huang, 2016) which may indicate a response characteristic of the disease. In the meantime, TRAP/UA levels were inversely correlated with WC in MDD patients. The study conducted by Awadallah et al. (2019) aforementioned also found an inverse correlation between WC and antioxidant defenses. Besides, the same study showed that the WC seems to be an important determinant to OS (Awadallah et al., 2019).

Our study also found that glucose levels were significantly associated with MDA levels in patients with mood disorders and MetS. Hyperglycemia and insulin resistance were previously associated with higher MDA levels in subjects with MetS or presenting high risk for MetS, suggesting both parameters as determinants for lipid peroxidation (Moreto et al., 2014). Excess of glucose leads to overproduction of NADH that can overwhelm the mitochondria and increase ROS production; then, ROS are able to impair the glucose metabolism and, as consequence, activate other pathways, such as the advanced glycation end products (AGEs), that are involved with ROS production too (Yan, 2014). High glucose levels, as observed in our patients with MetS, might be responsible to induce lipid peroxidation by increasing the levels of ROS and leading to decreased antioxidant defenses.

As expected, our study found all the MetS parameters changed in both controls and patients with mood disorders diagnosed with MetS. The subjects with MetS also presented elevated values for BMI, HOMA-IR and Castelli I and II indexes. Even though BMI and HOMA-IR are not required for the MetS diagnosis based on the NCEP ATP III criteria (Grundy et al., 2005), these parameters are pivotal factors involved with the MetS. Concerning to Castelli I and II indexes, considered better predictors for atherogenic risk than the cholesterol parameters apart (Millán et al., 2009), our findings suggest that the subjects with MetS are in higher risk.

It is very important to list the limitations of this study. First, because this is a cross-sectional study, our data not allow us to establish causality. Second, the sample size (n) that was small. Third, in patients with mood disorders, some cofounding variables were not considered for the analyses, for example, use of medication and disease stage/severity. Fourth, the study did not consider the subjects lifestyle that may be a contributing factor for MetS.

5. Conclusion

In conclusion, the OS in patients with mood disorders seems to be closely associated with MetS parameters. The presence of MetS in patients with BD and MDD might be responsible for the alterations concerning to OS biomarkers in these patients. In addition, BD I, BD II and MDD showed that the OS biomarkers could respond in a different way for each mood disorder. Therefore, more studies are necessary to show the reliability of these OS biomarkers.

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6 CONCLUSÃO

O presente estudo mostrou que os biomarcadores de EO/EN podem estar alterados de forma diferente para os diagnósticos de TDM, TAB I e TAB II. Da mesma forma, alterações nos biomarcadores de EO em relação aos parâmetros da SM também foram diferentes dependendo do transtorno de humor. Esses resultados sugerem que o TDM, TAB I e TAB II podem apresentar características específicas no que diz respeito ao EO/EN e, por isso, mais estudos são necessários para avaliar a aplicabilidade dos biomarcadores de EO/EN.

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APÊNDICES

APÊNDICE A

Termo de Consentimento Livre e Esclarecido (TCLE)

O Senhor (a) está sendo convidado a participar de um projeto de pesquisa chamado “Avaliação dos marcadores biológicos em pacientes em tratamento por transtorno afetivo bipolar e por transtorno por uso de tabaco”.

O estudo vai investigar as alterações de biomarcadores de estresse oxidativo, síndrome metabólica e atividade inflamatória em pacientes portadores de transtorno afetivo bipolar e transtorno por uso de tabaco, na fase basal do tratamento e após o tratamento de 6 meses e 1 ano, com a terapia convencional e associado ao tratamento adjuvante do antioxidante N-acetilcisteína (NAC). A realização da pesquisa será na Universidade Estadual de Londrina (UEL).

Justificativa: A redução dos marcadores biológicos em transtorno por uso de tabaco e transtorno afetivo bipolar preserva a saúde e a qualidade de vida. Estratégias visando a redução no agravamento das duas enfermidades, com intervenções eficazes e de baixo custo, são uma prioridade-chave da saúde pública.

Objetivo: Avaliar a eficácia do tratamento convencional e com adjuvante com a NAC na redução das alterações do biomarcadores relacionados à síndrome metabólica, à inflamação e ao estresse oxidativo em transtorno do humor e transtorno por uso de tabaco.

Procedimentos: O estudo implica em: 1) responder um questionário com dados sócio demográficos e clínicos; 2) responder escalas para diagnósticos; 3) coletar sangue para analisar a síndrome metabólica, o estresse oxidativo e a inflamação em tratamento convencional com e sem adjuntivo da NAC na fase basal, 6 meses e 12 meses de tratamento.

Custos: A pesquisa é gratuita e, portanto, não envolve qualquer custo para os participantes, não haverá qualquer gratificação financeira pela participação.

Riscos: Nenhum dos procedimentos utilizados constitui risco direto para a integridade física ou moral dos participantes. Além disso, os participantes poderão abandonar o estudo em qualquer momento que se achar conveniente, sem qualquer prejuízo em nenhum sentido, além da garantia de permanência no tratamento de cessão de tabagismo AHC/UEL se assim for o seu desejo.

Sigilo: Embora os resultados da pesquisa possam ser divulgados em publicações e eventos científicos, a identidade dos participantes será sempre preservada de maneira sigilosa, ou seja, em segredo.

Caso o Sr. (a) aceite o convite e concorde voluntariamente em participar do estudo assinando este termo de consentimento, consideramos que o Sr. (a) acredita que foi suficientemente informado (a) pelo (a) pesquisador (a) ______________________________ sobre a pesquisa, os procedimentos envolvidos nela, assim como os possíveis riscos e benefícios dessa participação.

Ressaltamos novamente que o Sr. (a) pode retirar seu consentimento a qualquer momento, sem que isto leve a qualquer prejuízo em nenhum sentido.

Londrina, _____ de _________________ de ______.

Assinatura do participante: ____
Assinatura do responsável: ____
Nome do pesquisador: ____
Assinatura do pesquisador: ____

Colocamo-nos à disposição para qualquer esclarecimento que se fizer necessário nos telefones (43) 33715791, (43) 33712234 ou pessoalmente no Ambulatório do Hospital de Clínica, da Universidade Estadual de Londrina (AHC/UEL), Rodovia Celso Garcia Cid, Br 445, Campus Universitário, Km 380. Se você tiver alguma consideração ou dúvida sobre a ética do estudo, entre em contato com o comitê de ética em pesquisa (CEP) da UEL/Hospital Universitário através do telefone (43) 33712490.

Atenciosamente,
Prof. Dr. Heber Odebrecht Vargas
Coordenador do Projeto