HEBER ODEBRECHT VARGAS

ASSOCIAÇÃO DE MARCADORES DE ESTRESSE OXIDATIVO E INFLAMATÓRIO EM FUMANTES E EM DEPRESSIVOS

Londrina
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Tese apresentada ao Programa de Pós-Graduação em Ciências da Saúde do Centro de Ciências da Saude da Universidade Estadual de Londrina.

Orientadora: Profª. Drª. Sandra Odebrecht Vargas Nunes.

Co-orientador: Prof. Dr. Décio Sabbatini Barbosa.

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Tese apresentada ao Programa de Pós-Graduação em Ciências da Saúde do Centro de Ciências da Saúde da Universidade Estadual de Londrina, como um dos requisitos para obtenção do título de Doutor.

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Londrina, 26 de setembro de 2013.

Dedico este trabalho

à minha família que soube apoiar-me
até mesmo nos momentos em que eu
estava mais fragilizado.
A meu pai, que, por ter sido sempre
ligado à academia, ficaria orgulhoso ao
ver o trabalho de seus filhos e que
tinha o seguinte lema de vida:

"É preciso ter feito tantas coisas como se
hoje fosse nosso último dia de vida, assim
como é preciso ter tantos projetos como
se pudéssemos viver eternamente".

Heber Soares Vargas

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RESUMO

Objetivo: O presente estudo teve como objetivo geral avaliar as características sócio-demográficas, clínicas, biomarcadores de estresse oxidativo e inflamatórios em fumantes, com e sem depressão, e compará-los a indivíduos nunca fumantes depressivos e não depressivos. **Método**: Foram avaliadas as características sócio-demográficas, clínicas, história tabagística e exames laboratoriais de marcadores de estresse oxidativo e inflamatórios em fumantes e nunca fumantes. Foram selecionados 150 fumantes ambulatoriais (72 depressivos e 78 não depressivos) recrutados do Centro de Referência de Abordagem e Tratamento do Tabagismo (CRATT), da Universidade Estadual de Londrina (UEL) e 191 nunca fumantes (68 depressivos e 123 não depressivos) recrutados na mesma instituição. A pesquisa foi aprovada pelo Comitê de Ética em Pesquisa da UEL. Os participantes foram submetidos a um questionário estruturado para avaliar as características sócio-demográficas, clínicas, risco do uso do álcool e história tabagística. O transtorno depressivo e o transtorno por uso do tabaco foi avaliado pela entrevista clínica estruturada, versão clínica (SCID-I), baseada no DSM-IV. Os marcadores inflamatórios foram mensurados pelos seguintes exames laboratoriais: proteína C reativa, velocidade de hemosedimentação (VHS), homocisteína e fibrinogênio. O estresse oxidativo foi avaliado pelos seguintes exames: determinação de dialdeído malônico (MDA), determinação de hidroperóxidos lipídicos, determinação de metabólitos do Óxido Nítrico (NOx), determinação do potencial antioxidante total plasmático (TRAP) e determinação dos produtos avançados de oxidação proteica (AOPP). **Resultados**: Os fumantes depressivos apresentaram alterações nas concentrações de NOx, hidroperóxidos lipídicos, AOPP, TRAP, fibrinogênio, maior incapacidade laboral, maior gravidade dos sintomas depressivos e taxas maiores de tentativas de suicídio ao serem comparados aos fumantes não depressivos e, nunca fumantes depressivos e não depressivos. Fumantes deprimidos tinham níveis significativos de estresse oxidativo e marcadores inflamatórios após o ajuste para sexo, idade, anos de escolaridade, deficiência de trabalhar, e as medidas de laboratório. Indivíduos com histórico de tentativas de suicídio tiveram significativamente níveis mais elevados de NOx e de hidroperóxidos lipídicos e mais baixas concentrações de TRAP, em comparação com indivíduos sem tentativas de suicídio. **Conclusão**: O presente estudo sugeriu que a comorbidade do transtorno depressivo e transtorno por uso de tabaco estava associada com maior gravidade dos sintomas depressivos, maiores taxas de tentativas de suicídio, maior incapacidade laboral e alterações de marcadores inflamatórios e de estresse oxidativo. Desse modo, no planejamento terapêutico dos fumantes depressivos deve-se levar em conta estes achados para prevenir morbidade e mortalidade de ambas enfermidades.

Palavras-chave: Transtorno por uso do tabaco. Transtorno depressivo. Inflamação. Estresse oxidativo

VARGAS, Heber Odebrecht. **Oxidative stress and inflammatory markers are associated with smokers and depression**. 2013. 86f. Thesis (Post-Graduation in Health Science) - Londrina State University, Londrina, 2013.

ABSTRACT

Objective: The present study aimed to assess the socio-demographic, clinical, inflammatory and oxidative stress biomarkers in depressed and non-depressed smokers comparing them to depressed and non-depressed never smokers subjects. **Methods**: We evaluated the socio-demographic, clinical, smoking history and laboratory biomarkers of inflammation and oxidative stress in smokers and never smokers. We selected 150 smokers (72 depressed and 78 non-depressed) recruited from outpatients at the Centre of Approach and Treatment for Smokers (CRATT), at the Londrina State University (UEL). We also selected 191 never smokers (68 depressed and 123 non-depressed) recruited from the same institution. All subjects had given informed consent to participate in this study, which was approved by the Ethics Research Committee at UEL. A structured questionnaire was used to gather information on socio-demographic, clinical risk screening of alcohol and s moking history. Depressive disorder and tobacco use disorder were assessed by structured clinical interview, clinical version (SCID-I), based on DSM-IV. Inflammatory markers were measured by the following laboratory tests: C-reactive protein, erythrocytes sedimentation rate, homocysteine and fibrinogen. Oxidative stress was evaluated by the following tests: determination of malonic dialdehyde (MDA), lipid hydroperoxide determination, determination of metabolites of nitric oxide (NOx), determination of plasma total antioxidant potential (TRAP) and determination of advanced oxidation protein products (AOPP). **Results**: Depressed smokers exhibited altered concentrations of NOx, lipid hydroperoxides, AOPP, TRAP, fibrinogen. They also were more unable to work, showed more severe depressive symptoms and hi gher suicide attempts rates as compared to non depressed smokers, depressed and non-depressed never smokers. Depressed smokers had s ignificant levels of oxidative stress and i nflammatory biomarkers after adjusting for gender, age, years of education, disability for work, and laboratory measures. Individuals with a hi story of suicide attempts had significantly higher levels of NOx and lipid hydroperoxides and lowered TRAP as compared to individuals without suicide attempts. **Conclusion:** This study suggested that the comorbidity of depressive disorder and tobbaco use disorder was associated with greater severity of depressive symptoms, suicide attempts, more unable to work and altered levels of inflammation and oxidative stress biomarkers. Thus, in the treatment planning of depressive smokers should consider these findings to prevent morbidity and mortality in both deseases.

Keywords: Tobacco use disorder. Depressive disorder. Inflammation. Oxidative Stress.

LISTA DE ABREVIATURAS E SIGLAS

AHC	Ambulatório do Hospital de Clínicas
AOPP	Produtos avançados de oxidação proteica
AST	Aspartato aminotransferase
ALT	Alanina aminotransferase
ASSIST	Teste de triagem do envolvimento com álcool, tabaco e outras substâncias
CRATT	Centro de Referência de Abordagem e Tratamento do Tabagismo
CRF	Fator liberador de corticotrofina
DSM-IV	Manual Diagnóstico e Estatístico dos Transtornos Mentais- 4 edição
DNA	Ácido desoxiribonucleico
DSM-IV	Diagnostic and Statistical Manual of Mental Disorders, fourth ed.
EROs	Espécies reativas de oxigênio
ERN	Espécies reativas de nitrogênio
GABA	Ácido gama-aminobutírico
Gama GT	Gama glutamil transferase
FTND	Teste de dependência de Nicotina de Fagerström
HHA	Eixo hipotálamo-hipófise-adrenal
HIV	Vírus da imunodeficiência humana
HUL	Hospital Universitário de Londrina
IDO	Indolamina 2-3- dioxygenase
INCA	Instituto de Câncer do Brasil
IL-1	Interleucina 1
IL-6	Interleucina 6
MAO	Mono Amino Oxidase,
MDA	Dialdeído malônico
MS	Ministério da Saúde
NOx	Metabólitos do Óxido Nítrico
PICs	Citocinas pró-inflamatórias
PCR	Proteína C-reativa
SCID-I	Entrevista clínica estruturada, versão clínica
SNC	Sistema nervoso central
SOD	Superóxido dismutase

TBARS	Espécies reativas ao ácido tiobarbitúrico
TGO	Transaminase glutâmico oxalacética
TGP	Transaminase glutâmico pirúvica
TNFα	Fator de necrose tumoral alfa
TRAP	Potencial antioxidante total plasmático
UEL	Universidade Estadual de Londrina
VHS	Velocidade de hemossedimentação
α4β2	Receptores nicotínicos alfa 4 beta 2
5-HIAA	Ácido 5-hidroxindolacético

SUMÁRIO

1 INTRODUÇÃO

O transtorno por uso de tabaco é uma importante causa de mortalidade em todo mundo e entre as doenças médicas mais freqüentes relacionadas ao seu uso estão o câncer de pulmão e outros tipos de cânceres, doença pulmonares, doenças cardiovasculares entre outras enfermidades[1]. O tabagismo e a exposição ao tabaco resultam em aproximadamente 443.000 mortes prematuras por ano nos Estados Unidos[2] e é a maior causa de morte prevenível em todo o mundo. No Brasil, sua prevalência é estimada em 16,2% da população[3].

Os transtornos depressivos, segundo o Diagnostic and Statistical Manual of Mental Disorders 5ª edição (DSM-V), incluem um grupo amplo de enfermidades nas quais, destacam-se os transtornos bipolares I e II, que nos Estados Unidos, apresentam uma prevalência em doze meses estimada de 0.6% e 0.8%, respectivamente, e transtorno depressivo maior com uma prevalência em um ano, estimada em 7%[4]. Associado às altas prevalências os transtornos depressivos são caracterizados por altas taxas de recorrências, evolução crônica e incapacidade[5], o que gera, além de sofrimento, altos custos econômicos e sociais para os governos, devido aos gastos com tratamentos para a população e às perdas de produtividade[6].

Neste grupo de enfermidades de alta prevalência foram descritas alterações similares de estresse oxidativo e inflamação, que contribuem para a neuroprogressão[7-9]. As alterações de estresse oxidativo e inflamatórias em transtornos depressivos abrem novos caminhos para o desenvolvimento de outras terapêuticas que poderão ser efetivas no transtorno depressivo, como, por exemplo, agentes que modulam o estresse oxidativo[10].

Nos Estados Unidos, cerca de 20% da população fuma, sendo que esta porcentagem aumenta para 30% entre as pessoas que consultam um médico regularmente e, aproximadamente, mais de 50% dos cigarros fumados são consumidos por pacientes com transtornos psiquiátricos[11].

A dependência de nicotina é um fator de vulnerabilidade para o desenvolvimento de sintomas depressivos e de ansiedade, que cursam com maior gravidade, prejudicando, desta forma, a recuperação dessas enfermidades[12]. O inicio precoce do consumo de cigarro está associado a um aumento do risco para o desenvolvimento de depressão[13]. Por outro lado, sintomas depressivos estão

comumente associados com a dependência da nicotina e podem ter um importante papel no desenvolvimento de comorbidades e manutenção da dependência do cigarro[14] e, consequentemente, pior evolução no tratamento dos transtornos depressivos[15].

Em um estudo com 167 fumantes que buscaram tratamento no Centro de Referência de Abordagem e Tratamento do Tabagismo (CRATT), localizado no Ambulatório do Hospital de Clínicas (AHC), da Universidade Estadual de Londrina (UEL), e com 272, nunca fumantes que procuraram, voluntariamente, o hemocentro do Hospital Universitário de Londrina (HUL) para doação de sangue, verificou-se que 50% dos fumantes eram depressivos e, estatisticamente, havia mais depressão entre fumantes do que entre pessoas que nunca fumaram[16].

Nos indivíduos com transtorno por uso de tabaco ocorre liberação de substâncias que afetam funções celulares como aquelas relacionadas aos ribossomos e às mitocôndrias, gerando as espécies reativas de oxigênio (EROs) e as espécies reativas de nitrogênio (ERN). A formação das EROs que são pró-oxidantes é um fenômeno necessário para a vida aeróbia e para manter a homeostase do nosso organismo sendo igualmente importante uma contínua regeneração da capacidade antioxidante[17]. O estresse oxidativo pode ser resultado da diminuição dos níveis dos antioxidantes e/ou aumento da produção de espécies reativas a partir do oxigênio ou de nitrogênio[18]. O estresse oxidativo aumenta a lipoperoxidação, podendo gerar danos às proteínas, lipídeos, carboidratos e ao ácido desoxiriboxinocleico (DNA), em uma sucessão de eventos que poderia iniciar a morte celular[19].

A dependência crônica de tabaco provoca aumento das EROs o que poderia gerar uma toxicidade extra pulmonar, assim como a oxidação de lipídeos na membrana que com o tempo, esgotaria os níveis de antioxidantes no plasma, gerando uma resposta inflamatória, tanto nos tecidos pulmonares como em outros órgãos[20].

O estresse oxidativo, particularmente o aumento da lipoperoxidação lipídica e dos metabólitos de oxido nítrico, bem como uma diminuição na capacidade antioxidante assim como o fibrinogênio podem estar associados a uma maior incapacidade para o trabalho, gravidade dos sintomas depressivos e um histórico de tentativas de suicídio[21].

Detectar os marcadores inflamatórios e de estresse oxidativo, em indivíduos fumantes, com e sem depressão, e nunca fumantes, com e sem depressão, poderá contribuir para que, no futuro, possamos disponibilizar intervenções mais específicas que resultam na redução de morbidade e mortalidade atribuídas ao transtorno do uso de tabaco e transtorno depressivo.

O presente estudo teve como objetivo entender melhor o papel quantificar os marcadores inflamatórios e do estresse oxidativo em fumantes depressivos e não depressivos e nunca fumantes depressivos e não depressivos, para saber se o estresse oxidativo e a inflamação estão associados ao tabaco, à depressão ou a ambas comorbidades. Também foram avaliados neste estudo, a gravidade dos sintomas depressivos, taxas de tentativa de suicídio, incapacidade laboral, risco para o consumo do álcool em indivíduos fumantes, com e sem depressão, e nunca fumantes, com e sem depressão. Este é o primeiro estudo em população brasileira de marcadores de estresse oxidativo e inflamatórios em transtorno por uso de tabaco e em transtorno depressivo.

2 REFERENCIAL TEÓRICO

A Organização Mundial de Saúde em sua décima versão da classificação internacional das doenças classifica como F.17, os transtornos mentais e de comportamento decorrentes do uso de tabaco[22]. Em sua última versão o DSM-V classifica como transtorno por uso de tabaco, um padrão problemático de uso de tabaco que piora significativamente o funcionamento do indivíduo no último ano[4]. Este é um transtorno que além de ser a maior causa evitável de doença e morte prematura em todo o mundo, gera como consequência grande impacto econômico devido ao potencial de anos de vida perdidos, perda de produtividade e custos elevados com mortes e cuidados de saúde[23].

O transtorno depressivo é classificado nas mesmas diretrizes diagnósticas da Organização Mundial de Saúde como transtonos do humor (F.30-39)[22], e é igualmente uma doença extremamente grave, levando a risco de cronicidade, incapacidade progressiva e morte prematura[24]. As pessoas portadoras de transtornos depressivos estariam mais envolvidas com o transtorno por uso de tabaco, do que aquelas que nunca tiveram depressão[25] e, paralelamente, o transtorno por uso de tabaco situam-se entre os vários comportamentos de risco associados com a depressão[26-27]. Quando a depressão e o transtorno por uso de tabaco coexistem, a qualidade e a quantidade de vida são duplamente agredidas.

A comorbidade entre transtorno depressivo e transtorno por uso de tabaco pode ser explicada por uma fisiopatogenia comum e/ou por fatores genéticos e ambientais. Há evidências de que inflamação e estresse oxidativo compartilhariam as mesmas vias fisiopatológicas nas duas desordens. O estresse oxidativo e a inflamação estão relacionados ao dano e/ou morte celular, o que poderia contribuir para a neuroprogressão dos transtornos depressivos e a dependência de nicotina. A patofisiologia de ambas as doenças incluem alterações de neurotransmissores, desregulação do eixo hipotálamo-hipófise-suprarrenal, aumento de citocinas inflamatórias e das proteínas de fase aguda, ativação da micróglia, disfunção mitocondrial, aumento do estresse oxidativo e nítrico e o decréscimo dos níveis de antioxidante, o que gera danos de lipídeos, proteína e no DNA com consequente modificação da função do gene[28]. Há também evidências de que o transtorno do uso de tabaco está associado a vias inflamatórias ativadas[28] e igualmente associado ao estresse oxidativo[29].

2.1 Comorbidade de Transtorno por Uso de Tabaco e Transtorno Depressivo

A comorbidade entre transtorno por uso de tabaco e transtorno depressivo é alta. Cerca de 60% de fumantes, atuais ou que fumaram no passado, apresentam história de depressão, comparados com 39% dos fumantes sem depressão[30]. Ainda não está claro na literatura se, é o transtorno por uso de tabaco que aumentaria o risco para a depressão ou se, o transtorno depressivo elevaria o risco para fumar cigarros[31]. No entanto, um estudo de coorte no qual, 1265 crianças foram avaliadas aos 18, 21 e 25 anos para o trantorno depressivo e para o transtorno por uso de tabaco, foi demonstrado que fumar cigarros aumentou o risco de sintomas depressivos[32].

A nicotina age diretamente nos receptores colinérgicos nos circuitos de recompensa do cérebro, e é a substância psicoativa que mais leva à dependência. Estima-se que, cerca de 20% da população geral fuma, sendo que 40 a 50% dos pacientes em uso de psicofármacos fumam, incluindo cerca de 60 a 85% dos pacientes com transtornos mentais[11]. Cerca de 80% dos indivíduos dependentes de nicotina tentam parar em algum momento de sua vida, mas somente 5% permanecem em abstinência por toda vida[4].

As comorbidades psiquiátricas mais frequentemente associadas ao transtorno do uso de tabaco são o consumo de álcool e outras drogas, ansiedade, transtornos depressivos, transtorno de hiperatividade e déficit de atenção. Fumantes dependentes de tabaco têm 2,7 a 8,1 vezes mais probabilidade de ter algum tipo de enfermidade psiquiátrica do que nunca fumantes ou ex-fumantes[4].

A comorbidade entre o transtorno por uso de tabaco e o transtorno depressivo tem sido demonstrada em alguns estudos epidemiológicos[30,33]**.** O risco de depressão é quatro vezes maior para fumantes pesados em comparação aos nunca fumantes[34]. A associação do tabagismo com os processos depressivos ocorrem mais do que em outros diagnósticos psiquiátricos. Esta associação é acompanhada de uma dificuldade maior de parar o consumo de cigarros e sintomas depressivos podem ocorrer na abstinência dessa dependência[35]. Existem hipóteses que poderiam explicar esta associação, entre elas, a da "auto-medicação" que se concentra nas propriedades químicas estimulantes da nicotina, sugerindo que o tabagismo poderia reduzir a disforia e os sintomas depressivos.[36]

Em um estudo em gêmeos, o consumo médio diário de cigarros foi fortemente relacionado à prevalência uma maior prevalência depressão maior, em grande parte devido à fatores familiares, provavelmente genéticos e que predispõem para a dependência de nicotina e depressão maior[37]. Estudos comportamentais e de genética molecular forneceram evidências para a base etiológica genética comum entre transtorno por uso de tabaco e transtorno depressivo, ainda que os estudos estejam em fase inicial, pesquisas futuras são necessárias para identificar outros genes promissores[38].

Entre as outras teorias que poderiam explicar a comorbidade entre transtorno por uso de tabaco e transtorno depressivo estariam aquelas que, seriam decorrentes das ações dos neurotransmissores. A estimulação de receptores colinérgicos nicotínicos libera uma variedade de neurotransmissores no cérebro como a dopamina, a serotonina e o glutamato. Os neurónios dopaminérgicos na área tegmental ventral do cérebro e no nucleus accumbens são importantes para aumentar o prazer e a recompensa, efeitos que, promovem a auto-administração de nicotina[39]. A nicotina ativa o receptor nicotínicos α7, o qual libera glutamato, e consequentemente dopamina no núcleo accumbens, responsável pela sensação do prazer[40]. Os transtornos depressivos apresentam em sua patogênese, alterações de neurotransmissores monoaminérgicos tais como: noradrenalina, serotonina e dopamina[11]. Também foram observados receptores de glutamato periféricos disfuncionais nos processos depressivos[41].

A exposição crônica à nicotina resulta em neuroadaptação, caracterizada por um aumento do número de receptores colinérgicos nicotínicos α4β2, mais associados dependências, através da liberação de dopamina no núcleo acumbens[11]. Dessa forma, a neuroadaptação associa-se às mudanças na expressão do gene e a síntese de proteínas com a geração de novas conexões sinápticas[42]. Este fenômeno de novas conexões, conhecido como aumento da plasticidade sináptica, participa do processo de aprendizagem, memória e atenção[43]. Há, também, uma redução de dopamina como consequência do processo de adaptação dos receptores nicotínicos pelo uso crônico de cigarros[11]. A dopamina tem uma ação bem conhecida na regulação do humor e sua diminuição pode ser comprovada com a mensuração do ácido homovanílico que é seu principal metabólito[44]. O antidepressivo bupropiona tem uma ação dopaminérgica, sendo utilizado para o tratamento da cessação do tabagismo[45].

Na abstinência de tabaco, ocorre a presença de sintomas depressivos e ansiosos decorrentes da redução de dopamina, da disfunção do eixo hipotálamo-hipófise-adrenal (HHA) e do aumento dos niveis do fator liberador de corticotrofina (CRF)[46]. O efeito negativo que caracteriza a resposta à abstinência de nicotina, provavelmente, resultaria em parte de uma cascata que envolveria o aumento dos níveis de CRF[47]. A liberação do CRF no núcleo central da amígdala pode provocar ansiedade e estresse[39]. A ativação deste eixo estaria associada aos transtornos depressivos[48] e também implicaria na patofisiologia da dependência de nicotina[49].

A atividade da monoaminooxidase (MAO) no sistema nervoso central (SNC) pode estar diminuída em fumantes de cigarros, e esta atividade modifica o sistema serotoninérgico e noradrenérgico relacionados com depressão e ansiedade. No SNC, a MAO não só pode desempenhar um papel fisiológico na inativação metabólica dos neurotransmissores de monoamina (catecolaminas e serotonina), mas também atuar na desintoxicação de aminas xenobióticas. A MAO tem, ainda, um papel patofisiológico, ao gerar radicais livres citotóxicos, uma vez que o produto final de sua metabolização é o peróxido de hidrogênio, cujo, papel oxidante atua nos processos de envelhecimento e nas doenças neurodegenerativas[50]. A formação dos metabólitos da serotonina através da MAO é acompanhada pela geração de EROs em fumantes e ex-fumantes[50], o que pode contribuir para as alterações de estresse oxidativo.

A relação entre a comorbidade entre o transtorno por uso de tabaco e os transtornos depressivos pode ser explicada pela atividade da MAO, pois sua inibição tem propriedades antidepressivas. Fumantes mostraram redução dos níveis da MAO A e B no cérebro, comparados aos não fumantes e aos ex–fumantes. A inibição da MAO A e B pode ter um efeito antidepressivo, o que contribuiria para as altas taxas de fumantes em indivíduos com transtorno depressivo[47]. Esta atividade da MAO reduzida no cérebro de fumantes poderia contribuir para o desenvolvimento da dependência de nicotina[51]. No entanto, a exposição crônica de nicotina leva a neuroadaptação e a redução da função dos neurotransmissores nos circuitos cerebrais[46].

O sistema serotoninérgico está envolvido no transtorno por uso de tabaco e no transtorno depressivo decorrente, em parte, pelo envolvimento do polimorfismo genético do transportador de serotonina[52-53]. A relação do

comportamento de fumar com a disfunção do sistema serotonérgico podem ser avaliadas em dois momentos: na administração aguda de nicotina, que resultaria na liberação de serotonina, e por outro lado, seu uso crônico estaria associado à diminuição da função serotoninérgica cerebral. Esta atividade mostrou-se mais baixa em fumantes depressivos que tentaram suicídio, demonstrada pela redução do metabólito da serotonina, o ácido 5-hidroxindolacético (5-HIAA) no liquor e pela resposta da prolactina à fenfluramina[54].

A relação entre os transtornos depressivos e o transtorno do uso de tabaco também tem impacto na prática clínica quando foi observado aumento do risco de desenvolver depressão entre fumantes[13], do mesmo modo que pacientes com enfermidades psiquiátricas, como a depressão, estariam associados a um início precoce e uso diário de tabaco[55].

O transtorno por uso de tabaco e o transtorno depressivo causam graves problemas de saúde pública, com múltiplas dimensões etiológicas e de prognósticos. Embora cada uma das condições seja importante por si só, ambas as enfermidades potencializam e agravam, uma a outra, sendo essencial entender a natureza de seu relacionamento.

2.2 Transtorno por Uso de Tabaco, Transtorno Depressivo e Marcadores Inflamatórios

Na literatura existem poucos estudos que examinaram a relação da inflamação na co-ocorrência de transtorno depressivo e transtorno por uso de tabaco. Não está claro se essa associação é causal ou é devido a fatores confundidores como atividade física, peso, consumo de álcool e gênero.

Homens e mulheres evoluem com diferentes padrões de uso da substância, o que sugere que, intervenções específicas, são necessárias para o tratamento. Fumantes deprimidos do sexo masculino usam mais cigarros por dia, referem maior consumo de álcool e apresentam níveis mais baixos de colesterol de alta densidade e marcadores inflamatórios elevados, como o fator de necrose tumoral (TNFα), comparados aos fumantes deprimidos e não deprimidos do sexo feminino[56].

A associação do transtorno por uso de tabaco e do transtorno depressivo pode gerar alterações de circuitos neurais que levam a alterações inflamatórias, aumento do estresse oxidativo e redução dos antioxidantes[28].

Há evidências de que existem vias inflamatórias comuns que poderiam associar o transtorno por uso do tabaco e o transtorno depressivo. Em fumantes deprimidos, existem níveis elevados de citocinas pró-inflamatórias (PICs) e mais hospitalizações do que em fumantes não depressivos[57].

Níveis elevados da proteina C reativa (PCR) foram encontrados em transtorno depressivo e permaneceram significativos, mesmo quando ajustados por fatores como: gênero, idade, tabagismo, atividade física, peso, o uso de medicamentos e condições médicas que poderiam influenciar os níveis de inflamação[58-60].

Muitos estudos confirmaram que os níveis elevados de PCR estariam relacionados a um risco aumentado de desenvolvimento de transtorno depressivo[61-63]. Foi observada uma associação entre a gravidade dos sintomas depressivos atuais e aumento dos níveis de marcadores inflamatórios, como a interleucina-6 (IL-6) e PCR, no entanto somente a IL-6 estava correlacionada a um modelo biométrico genético em gêmeos [64]. A cessação do tabagismo não reduziria os níveis de PCR[65].

Elevadas taxas de homocisteína plasmática têm sido relacionadas aos processos depressivos e à piora cognitiva em especial, em depressivos com idades avançadas[66]. Por outro lado, nem sempre foi possível avaliar a relação dos processos inflamatórios com ou sem quadros psiquiátricos, assim como não foram visualizados níveis diminuídos da atividade das células *Natural Killers*, ou o aumento de IL-6 nos depressivos que fumavam, comparados com nunca fumantes, nem com depressivos com ou sem história de álcool[67].

Foram observados níveis elevados de PCR, fibrinogênio e células brancas do sangue em indivíduos que estavam deprimidos e que foram maltratados durante a primeira década de vida (idade entre 3-11 anos). Essa associação não foi explicada por fatores de risco associados, tais como a recorrência de depressão, baixo nível socioeconômico na infância ou na idade adulta, problemas de saúde, ou fumar. No entanto, os indivíduos deprimidos e maltratados eram mais propensos a fumar[68]. Outro estudo também confirma a resposta inflamatória aumentada naqueles indivíduos que tiveram maus-tratos na infância[69]. As disfunções no eixo hipotálamo-hipófise-adrenal (HPA) que contribuem para o processo inflamatório são observadas

em estresse de vida precoce[70-71], na depressão[72] e em individuos com transtorno por uso de tabaco[73]. Indivíduos, com transtorno de humor, com comorbidade com transtorno de uso de substâncias psicoativas e estresse de vida precoce têm um efeito cumulativo de risco o que, poderia contribuir para alterar o sistema imunológico. Esta observação está de acordo com o modelo de sobrecarga alostática do transtorno depressivo[74].

A vulnerabilidade genética associada ao estresse de vida precoce podem ativar os circuitos do SNC, incluindo o eixo HPA e citocinas pró-inflamatórias, entre elas, o TNFα, interleucina1(IL-1) e IL-6. Estas citocinas levam ao aumento da atividade da enzima Indolamina 2-3- dioxygenase (IDO), reduzindo o triptofano, e consequentemente, alterando a função do sistema serotoninérgico[75]. Estas alterações estimulam a liberação de glutamato, o qual está associado à inibição do fator neurotrófico derivado do cérebro (BDNF), ao declínio nos fatores neuroprotetores e ao aumento do estresse oxidativo[76].

Os pacientes com transtornos depressivos refratários tinham níveis subsequentes elevados de marcadores inflamatórios (PCR, IL-6 e fibrinogênio), mas esta associação também ocorreu em outros comportamentos não saudáveis, como tabagismo, sedentarismo e obesidade[77].

A maioria dos estudos citados relataram alterações em marcadores inflamatórios, tanto no transtorno depressivo como em transtorno por uso de tabaco, sem esclarecer se estas alterações estariam relacionadas a ambas comorbidades ou individualizadas em cada transtorno[28].

2.3 Co-ocorrência de Transtorno por Uso de Tabaco e Transtorno Depressivo com o Estresse Oxidativo

O transtorno depressivo maior é acompanhado por uma diminuição na capacidade antioxidante e um aumento do estresse oxidativo, o que pode causar neuroprogressão e neurodegeneração por apoptose, redução da neurogênese e neuroplasticidade, todos desempenhando um papel importante na fisiopatologia dos transtornos depressivos[8-9,78-79]. Estas alterações podem induzir danos cerebrais, o que pode ser um caminho para as doenças neurodegenerativas[19].

Também foi observada alteração no estresse oxidativo em pacientes com transtorno por uso de tabaco[29]. A nicotina ou outros componentes do tabaco no

SNC poderiam aumentar a lipoperoxidação e o óxido nítrico, bem como diminuiriam antioxidantes como a catalase, com consequente dano cerebral contribuindo para o aumento de risco de doenças psiquiátricas[80]. A nicotina é uma fonte de radicais livres e pode estar associada à lipoperoxidação e à oxidação proteica. Como existiria uma correlação entre elevados níveis de estresse oxidativo e os transtornos depressivos graves que retornariam ao normal após o tratamento, poder-se-ia concluir que a depressão seria uma sequela do estresse oxidativo gerado pela dependência de nicotina[10]. Esta dependência estaria ainda, associada a um início precoce, piorando a sintomatologia depressiva nos transtornos bipolares, além de aumentar o risco de suicídio e de outras dependências[81].

O SNC é propenso ao estresse oxidativo, assim como é adequadamente equipado com sistemas de defesa antioxidantes para prevenir o dano oxidativo, mas não está preparado para o aumento do dano oxidativo causado pela disfunção mitocondrial que leva à morte celular e, consequentemente, à neurodegeneração[19]. O aumento de indicadores de estresse oxidativo no transtorno afetivo bipolar, que pode ser demonstrado pelo aumento da peroxidação lipídica e níveis de 3-nitrotirosina, já no início da doença e tendem a aumentar nas fases tardias[82]. O transtorno depressivo está associado a um aumento dos níveis do estresse oxidativo, bem como à diminuição das enzimas antioxidantes (superoxido dismutase, catalase e glutationa peroxidase), e isto poderia ser a possível contribuição para ajudar a explicar o processo de neurodegeneração da doença[8].

Os transtornos depressivos podem ser considerados como uma doença neurodegenerativa, porque estão associados à diminuição da plasticidade sináptica, à perda neuronal e redução da neurogênese. As alterações que levam à deficiência de plasticidade sináptica e à resiliência celular nos transtornos depressivos incluem as alterações de função mitocondrial[83]. Eventos que podem comprometer a função e integridade mitocondrial incluem dano ou mutação do DNA mitocondrial, aumento das EROs, e a elevação anormal de cálcio, que em conjunto levam ao excesso do glutamato e morte celular[83]. A disfunção mitocondrial também é encontrada em transtorno por uso de tabaco, pois o monóxido de carbono (CO), que é um transmissor gasoso endógeno, apresenta várias funções biológicas e está envolvido na manutenção da homeostase celular. Em condições fisiológicas, as mitocôndrias são as principais fontes de EROs, as quais são neutralizadas pelo sistema de defesa antioxidante[84]. No entanto, em situações, nas quais existem um

desequilíbrio entre pró-oxidantes e antioxidantes, as EROs podem acumular e levar à morte celular[19].

Contagem de células brancas do sangue significativamente elevadas foram encontradas entre os indivíduos com depressão moderada e grave, no entanto, não foi possível associar essas alterações com marcadores correlatos de avaliação de estresse oxidativo como vitamina C e ácido úrico, mesmo quando outras variáveis foram ajustadas[85].

Em um grupo de indivíduos depressivos, foram encontrados níveis aumentados de 8-hydroxy-2'-deoxyguanosine um biomarcador de dano em DNA[86], assim como alteração de 8 iso-$PGF_{2\alpha}$ que avalia dano lipídico por estresse oxidativo[87].

O estresse oxidativo em pacientes bipolares poderia acelerar o envelhecimento, com piora funcional e cognitiva, e poderia relacionar-se à morte prematura[88]. Os transtornos de humor possuem níveis séricos elevados de espécies reativas ao ácido tiobarbitúrico (TBARS), um marcador de lesão oxidativa dos lipídeos, bem como da atividade da superóxido dismutase (SOD). Esses dados sugerem que o entendimento dos mecanismos subjacentes à progressão do transtorno, pode ajudar no planejamento terapêutico e prognóstico, assim como a intervenção precoce para prevenir agravamento do transtorno do humor[18].

Diversos tratamentos para transtornos de humor podem reduzir o estresse oxidativo e inflamação[8]. Foi demonstrado que a atividade antioxidante tende a normalizar durante o tratamento com antidepressivos e compostos anti-inflamatórios incluindo substâncias naturais anti-estresse oxidativo com componentes, como por exemplo, vitamina E e zinco e que poderiam ter eficácia antidepressiva ou poderiam aumentar a eficácia dos antidepressivos, abrindo novas perspectivas no entendimento e manejo dessas enfermidades[8-9,89].

Na prática clínica, pacientes com transtornos depressivos e com dependência da nicotina são comuns, por isso, os médicos deverão tratar as duas comorbidades. Esta observação tem relevância clínica, pois ambos os transtornos pioram o prognóstico e podem levar a distúrbios neurodegenerativos.

No futuro, novos tratamentos poderão ser utilizados como as terapias anti- inflamatórias e antioxidantes, incluindo dietas, vitaminas , ácidos graxos, ômega-3 , ácido acetilsalicílico, ciclo- oxigenase, inibição de citocinas inflamatórias, minociclina e N-acetilcisteína. Esses tratamentos aumentam a eficácia clínica de

agentes estabelecidos e servem como novos tratamentos para comorbidade de de transtorno depressivo e transtorno por uso de tabaco[28] .

2.4 Co-ocorrência de Transtorno por Uso de Tabaco,Transtorno Depressivo e História de tentativa de suicídio com o Estresse Oxidativo

Nos processos depressivos, podem-se observar alterações, tanto de estresse oxidativo como de processos inflamatórios que poderiam contribuir para mudanças na função dopaminérgica e serotoninérgica, o que pode estar associado aos comportamentos suicidas[76, 90].

O transtorno por uso de tabaco estaria correlacionado ao risco de ansiedade, depressão e comportamento suicida[91-94]. Por outro lado, exitem estudos que relatam que a associação entre transtorno por uso do tabaco e comportamento suicida ocorreria independentemente do transtorno depressivo[95-96].

Em um estudo pós-morte em humanos foi evidenciado alteração da função serotoninérgica no córtex pré-frontal, em indivíduos com transtorno depressivo que cometeram suicídio[97]. Em outro estudo pós-morte, também em humanos, comparando fumantes e não fumantes, foi relatado que os fumantes tinham concentrações significativamente mais baixas de metabólito da serotonina, na formação hipocampal e na rafe mediana[98].

Outras alterações do comportamento como traços agressivos e impulsivos, alcoolismo, e outras dependências por outras substâncias de abuso têm sido associados a um elevado risco de suicídio e anormalidades serotoninérgicas[99].

Fumar cigarros também parece estar associado à disfunção do sistema serotoninérgico. A depleção de serotonina no cérebro estaria associada a maior risco de suicídio em fumantes com depressão. As alterações de serotonina poderiam modular, em parte, a ligação entre o transtorno por uso de tabaco e o comportamento suicida[100]. Evidência de baixas concentrações de 5-HIAA em indivíduos suicidas é consistente com a baixa atividade da MAO. A possibilidade de ligações neurobiológicas entre o tabagismo e suicídio sugere que novos estudos sejam realizados entre o tabagismo e os quadros psiquiátricos[100].

Existem evidências de que alterações no estresse oxidativo e nos processos inflamatórios poderiam levar a alterações nos neurotransmissores, incluindo a serotonina e a dopamina e estes poderiam estar associados ao

comportamento suicida[100-101]. Paralelalemente foi demonstrado que fumantes depressivos apresentavam maiores níveis de inflamação[57] e estavam associados a alterações de biomarcadores de estresse oxidativo[21].

O estresse oxidativo, mas não os marcadores inflamatórios, está relacionado à história de tentativas de suicídios. Indivíduos com histórico de tentativas de suicídio tiveram níveis significativamente mais elevados de NOx e de hidroperóxidos lipídicos e menores níveis de TRAP, em comparação com os indivíduos que não tentaram suicídio. A regressão logística mostrou que tanto transtorno afetivo unipolar e bipolar, sexo feminino, o comportamento de fumar e hidroperóxidos lipídicos foram significativamente associados com um histórico de tentativas de suicídio, mesmo quando ajustados a outros fatores clássicos de risco de suicídio[101].

3 OBJETIVOS

3.1 OBJETIVO GERAL

Avaliar as alterações inflamatórias e o estresse oxidativo em fumantes depressivos e não depressivos comparados com nunca fumantes com ou sem depressão.

3.2 OBJETIVOS ESPECÍFICOS

1) Analisar características sócio-demográficas, clínicas, gravidade dos transtornos depressivos, tentativas de suicídio, risco para o uso do álcool em fumantes depressivos e não depressivos e em nunca fumantes, com e sem depressão, da Universidade Estadual de Londrina (UEL).
2) Comparar a história tabagística, a gravidade da dependência de nicotina, número de cigarros fumados por dia, idade de início, anos/maços, hospitalizações clínicas, incapacidade de trabalhar e para atividades domésticas de tabagistas entre fumantes, com e sem depressão, e nunca fumantes com e sem depressão.
3) Comparar os níveis séricos de marcadores inflamatórios tais como, VHS, PCR, homocisteína e fibrinogênio entre fumantes depressivos e não depressivos e nunca fumantes, depressivos e não.
4) Avaliar o estresse oxidativo em fumantes, depressivos e não depressivos, e nunca fumantes, depressivos e não, utilizando técnicas específicas: determinação de dialdeído malônico (MDA), determinação de hidroperóxidos lipídicos (FOX), determinação de metabólitos do óxido nítrico (NOx), Potencial Antioxidante Total Plasmático (TRAP) e determinação dos produtos avançados de oxidação protéica (AOPP).

4 METODOLOGIA

4.1 CARACTERÍSTICAS DA POPULAÇÃO ESTUDADA

A amostra foi de conveniência de tempo e lugar, com a inclusão dos fumantes do Centro de Referência de Abordagem e Tratamento do Tabagismo (CRATT), localizado no Ambulatório do Hospital de Clínicas (AHC). O CRATT foi implantado com o apoio da Fundação Araucária em 15/08/2005, referência 5397 e atendendo ao Programa Nacional de Controle do Tabagismo do Ministério da Saúde (MS) / INCA, sendo credenciado para abordagem e tratamento do fumante, no Cadastro Nacional de Estabelecimentos de Saúde pelo MS, de acordo com a Portaria SAS/MS 442 de 13 de agosto de 2004, quando foi elaborado o Plano para Implantação da Abordagem e Tratamento do Tabagismo na Rede do Sistema Único de Saúde (SUS)[102-103]. Todos os participantes assinaram Termo de Consentimento Livre e Esclarecido, aprovado pelo Comitê de Ética da UEL, número 250/2010 (Anexo A).

O delineamento do estudo foi do tipo caso–controle com amostra de conveniência no qual, foram recrutados no período de março de 2011 a agosto de 2012. Foram avaliados 150 fumantes depressivos e não depressivos, recrutados no CRATT e comparados com 191 indivíduos do grupo controle não tabagista e não depressivo que trabalham na Universidade Estadual de Londrina. O cálculo do tamanho da amostra foi baseado em fumantes e nunca fumantes com ou sem depressão. O estudo para calcular o tamanho da amostra constatou que uma amostra de 34 nunca fumantes e 27 fumantes (N total=61) seria capaz de detectar 59% da prevalência de transtornos depressivos entre os fumantes, comparados com 17% de desordens depressivas entre não fumantes (OR= 7,03) com um intervalo de confiança com um nível de 0,95 e um poder de 95%. A razão entre casos e controles foi estimada em 1,27. Como foi possível recrutarmos 191 nunca fumantes e 150 dependentes de nicotina, o tamanho da amostra apresentava um poder adequado para detectar a prevalência de transtornos depressivos entre dependentes de nicotina e nunca fumantes, de acordo com a literatura[16].

Os critérios de inclusão foram: ambos os sexos, todas as raças, idade entre 18 e 60 anos, e consentimento de participação voluntária no estudo. Todos os participantes incluídos no estudo apresentavam os seguintes exames

laboratoriais normais: hemograma, aspartato aminotransferase (AST), alanina aminotransferase (ALT), ureia e creatinina. Os critérios de exclusão foram: presença de delírium, demência, amnésia e outros transtornos cognitivos, doenças infecciosas como Hepatite B e C, HIV, doenças crônicas como insuficiência renal, doença pulmonar obstrutiva e auto-imune, tratamento com interferon, uso patológico de substâncias psicoativas e consumo de substâncias antioxidantes. Estas situações poderiam afetar o processo inflamatório e/ou imunológico[78].

4.2 INSTRUMENTOS

4.2.1 Questionários

Os participantes responderam a um questionário constando os seguintes dados: sócio-demográficos, história de hospitalizações, história tabagística, história pregressa de doenças, capacidade de trabalho e para atividades domésticas, condicionamentos relacionados ao tabagismo, motivações para cessar o tabaco, história familial para o tabagismo, tratamentos efetuados anteriores, além de história de outras comorbidades médicas, psiquiátricas e tentativas de suicídio (Anexo B).

4.2.2 Avaliação do Diagnóstico do Transtorno Depressivo e de Dependência de Nicotina

Os critérios diagnósticos para pesquisa de episódio ou transtorno depressivo, bem como de dependência de nicotina foram avaliados por psiquiatra treinado de acordo com a Entrevista Clínica Estruturada para o D.S.M.-IV" – versão clínica traduzida e validada para o português[104].

4.2.3 Avaliação da Gravidade da Depressão

A avaliação da severidade da depressão entre os participantes do estudo foi realizada pela escala de depressão de Hamilton que foi traduzida e adaptada para a população brasileira[105].

4.2.4 Triagem de Uso de Substâncias Psicoativas

Utilizou-se o ASSIST (Alcohol, Smoking and Substance Involvement Screening Test) que é um questionário para rastreamento desenvolvido pela Organização Mundial da Saúde para pessoas que fazem uso de substâncias psicoativas, que abrange: tabaco, álcool, canabinoides, cocaína, estimulantes do tipo anfetamina, sedativos, alucinógenos, inalantes, opioides e outras drogas[106]. Esse teste foi traduzido e adaptado para o português por Henrique e colaboradores[107] e utilizado para o rastreio do uso do álcool, a seguinte pontuação: baixo risco (0-10), moderado risco (11-26) e alto risco (≥27). O ponto de corte utilizado neste trabalho foi o de alto risco, ou seja, uma pontuação ≥27.

4.2.5 Triagem da Gravidade da Dependência de Nicotina

O teste de Fagerström para Dependência da Nicotina (FTND) foi utilizado para avaliar a gravidade da dependência de tabaco[108]. Foi traduzido e adaptado para a língua portuguesa por Carmo e Pueyo[109]. O FTND possui uma escala de seis itens e a pontuação de 0 a 10. Os escores para dependência de nicotina permitem a classificação em cinco níveis: muito baixo (0 a 2 pontos); baixo (3 a 4 pontos); moderado (5 pontos); alto (6 a 7 pontos); muito alto (8 a 10 pontos) [110]. O ponto de corte de FTND para a dependência de nicotina foi ≥ 5[111].

4.3 AVALIAÇÕES LABORATORIAIS

As obtenções das amostras de sangue periférico para todos participantes foram realizadas às 8 horas da manhã após um jejum de 12 a 14 horas, em ambientes com temperatura local, no Laboratório de Análises Clínicas do Hospital Universitário de Londrina (HUL). As avaliações laboratoriais de rotina, tais como hemograma, lipidograma, glicose, creatinina, ácido úrico, AST, ALT, Gama GT, marcadores para hepatite C e B e sorologia para HIV foram realizados pelos setores de rotina do laboratório de análises clínicas do HUL.

4.3.1 Avaliação dos Marcadores Inflamatórios

A concentração da proteína de fase aguda (hs-CRP High-sensitivity) foi realizada pelas concentrações séricas por meio de um Nefalômetro BNII (Siemens®SystemBNTMII, Deerfield, IL, USA). A determinação quantitativa do fibrinogênio no plasma foi baseada no método de Clauss que mede a taxa de fibrinogênio derivado da conversão de fibrina na presença de excesso de trombina com a coagulação verificada em um analisador (Destiny Plus - Trinity Biotech GmbH, Lemgo, Germany). A determinação da homocisteína sérica foi realizada no ARCHITECT i System (Abbot, Wiesbaden, Germany). A velocidade de hemossedimentação (VHS) foi analisada em um analisador automático (MicroTest1X - Sire Analytical Systems, Udine, Italy).

4.3.2 Avaliação do Estresse Oxidativo

A avaliação do estresse oxidativo foi realizada utilizando-se dos seguintes métodos laboratoriais: 1-Determinação de dialdeído malônico (MDA), 2-Determinação de hidroperóxidos lipídicos (FOX), 3- Determinação de subprodutos do Óxido Nítrico (NOx), 4-Potencial Antioxidante Total Plasmático (TRAP), 5-Determinação dos produtos avançados de oxidação proteica (AOPP).

4.3.3 Determinação de Dialdeído Malônico (MDA) TBARS – Colorimétrico

O MDA é um produto secundário da peroxidação lipídica e pode ser considerado um biomarcador geral do dano oxidativo plasmático[112]. Este método consiste na medida de um cromógeno róseo formado pela reação do MDA com duas moléculas de ácido tiobarbitúrico, em meio ácido e em alta temperatura. A quantificação do MDA foi feita em um espectrofotômetro (Helios α Thermo Spectronic®, Waltham, MA, USA) nos comprimentos de onda de 535 e 572nm. Os valores encontrados foram ajustados pelo valor de proteína e apresentados na unidade nmol MDA/g de proteína.

Para avaliar os níveis de dialdeído malônico (MDA) foi utilizado a metodologia descrita por Jentzch et al.[113] que consiste em utilizar como reagentes o

ácido ortofosfórico, hidróxido de sódio (NaOH), ácido tiobarbitúrico (TBA) e cloreto de sódio (NaCl), tendo como padrão uma solução de 0,03 µmol MDA.

4.3.4 Determinação de Hidroperóxidos Lipídicos (FOX)

Para avaliar um biomarcador de dano lipídico ou peroxidação lipídica foi utilizado o método baseado na oxidação de íons ferrosos para íons férricos sob condições ácidas, que reagem com o corante indicador (xylenol orange) para produzir um complexo colorido. Para a quantificação de hidroperóxidos lipídicos foi utilizada uma adaptação da técnica descrita por Jiang et al.[114] que consiste em ter como reagente o ácido sulfúrico (H_2SO_4), o padrão tetraetóxipropano (TEP) e o reagente xylenol orange. Ao final do tempo de reação, esta foi lida em um espectrofotômetro (Helios α Thermo Spectronic®, Waltham, MA, USA), ajustando o comprimento de onda em 560nm.

4.3.5 Determinação de Subprodutos de Óxido Nítrico (NOx)

Para a estimativa da concentração de NOx (subprodutos de nitratos e nitritos) nas amostras foi realizada a técnica descrita por Navarro-Gonzálvez e colaboradores[115]. Íons nitratos presentes no meio de reação foram reduzidos a íons nitrito através de reação de oxi-rredução ocorrida entre o nitrato presente na amostra 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 deu-se em 550 nm em uma leitora de microplacas (Biochrom ASYS Expert Plus®, Holliston, MA, USA).

4.3.6 Potencial Antioxidante Total Plasmático (TRAP)

Esta metodologia detecta o total das defesas antioxidantes hidro e/ou lipossolúveis presentes no plasma. Este experimento foi conduzido em um contador β marca Beckman® (EUA) modelo LS 6000, em um modo de contagem não coincidente por 30 minutos e uma faixa de resposta entre 300 a 620nm. A capacidade antioxidante total plasmática (TRAP) foi avaliada por quimiluminescência (QL) em uma adaptação do método da técnica descrita por Repetto e

colaboradores[116] que consiste em usar como reagentes: ABAP (azobis), luminol, TROLOX em tampão glicina. O cálculo do TRAP foi realizado pela equação:

$$TRAP = 802 \times \frac{\text{Tempo da amostra}}{\text{Tempo de trolox}}$$

4.3.7 Determinação dos Produtos Avançados de Oxidação Proteica (AOPP)

As proteínas plasmáticas são alvos importantes para os agentes oxidantes. Para a quantificação de AOPP no plasma foi utilizado o método descrito por Witko-Sarsat et al.[117]. Sendo a leitura realizada em 340nm em um espectrofotômetro (Helios α Thermo Spectronic®, Waltham, MA, USA). A curva de calibração foi obtida a partir da solução estoque cloramina T 1 mM diluída com PBS. A concentração de AOPP foi expressa em μmoles/L de equivalente de cloramina T.

Todos os resultados das determinações acima descritas foram comparados entre o grupo dos fumantes depressivos, fumantes não depressivos, depressivos nunca fumantes e não depressivos nunca fumantes.

4.4 Análise Estatística

As análises estatísticas foram realizadas para examinar a relação entre não depressivos nunca fumantes comparando-os com os grupos dos depressivos fumantes e com os depressivos nunca fumantes. Foram feitas comparações entre estas medidas sócio-demográficas, clínicas e laboratoriais, utilizando testes paramétricos adequados nos quais os dados foram distribuídos normalmente e testes estatísticos não-paramétricos para os dados categóricos ou não-normal. A significância estatística adotada foi de 0,05.

Para a apresentação dos dados utilizou-se a média ($\bar{x}$), desvio padrão (±DP) para as variáveis com distribuição gaussiana, a mediana para as variáveis com distribuição não gaussiana, frequência bruta e percentual. As associações entre variáveis sócio-demográficas, clínicas e história tabagística foram analisadas utilizando o teste Qui-quadrado (χ^2) ou teste Exato de Fisher. Os testes estatísticos não paramétricos foram utilizados para dados ordinais e contínuos analisados pelo teste Kruskal-Wallis. A ANOVA foi aplicada quando as variáveis

apresentaram distribuição gaussiana e homogeneidade de variâncias, caso contrário, aplicou-se o teste Kruskal-Wallis.

Comparações univariadas foram inicialmente conduzidas e, em seguida, as variáveis que foram estatisticamente significativas foram incluídas nas análises multivariadas. A análise de regressão multinomial generalizada foi realizada com não-deprimidos nunca-fumantes, deprimidos nunca fumantes, fumantes não deprimidos e categorias fumantes deprimidos como variável dependente. A análise de regressão multinomial foi utilizada para estimar as chances de que nunca fumantes deprimidos, fumantes deprimidos ou fumantes sem depressão em comparação com os nunca fumantes não deprimidos estavam associados a cada uma das variáveis independentes. Todas as análises foram realizadas utilizando o programa SPSS (versão 20).

4.5 ASPECTOS ÉTICOS

Em consonância com a Resolução 196/96 do Conselho Nacional de Saúde/Comissão Nacional de Ética em Pesquisa e buscando atender a Resolução 196/96, do Conselho Nacional de Saúde de 1998, este estudo foi encaminhado ao Comitê de Ética em Pesquisa da UEL. A pesquisa foi aprovada pelo Comitê de Ética em Pesquisa da UEL número 250/2010 e todos os indivíduos da amostra receberam, previamente, todas as informações pertinentes à pesquisa, asseguramento sobre o sigilo dos dados e a possibilidade de retirar o consentimento na participação, a qualquer momento, sem sofrer nenhuma censura. Nessa ocasião também tiveram sanadas todas suas dúvidas e assinaram o Termo de Consentimento Livre e Esclarecido em duas vias (Anexo A).

A pesquisa fez parte do projeto 07517, cadastrado na Pró-Reitoria de Pesquisa e Pós-Graduação da UEL, intitulado Marcadores Biológicos em Fumantes de um Centro de Referência de Abordagem e Tratamento do Tabagismo.

5 RESULTADOS

A revisão da literatura, a elaboração de um banco de dados e análise estatística, permitiu-nos resolver os objetivos propostos inicialmente e, finalmente, com uma parceria com outros pesquisadores nacionais e internacionais foi possível a realização dos seguintes artigos publicados:

Artigo 1 – Oxidative stress and inflammatory markers are associated with depression and nicotine dependence

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Oxidative stress and inflammatory markers are associated with depression and nicotine dependence☆

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HIGHLIGHTS

- Oxidative stress markers are associated with both depression and smoking.
- Increased nitric oxide and fibrinogen levels are seen in smokers and depression.
- There is a correlation between depressed smokers and lipid hydroperoxides.
- Depressed smokers have an elevated disability for work.
- Depressed smokers have a higher severity of depression and more suicide attempts.

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ABSTRACT

To determine if oxidative stress and inflammation are linked with major depressive disorder, nicotine dependence and both disorders combined. This study comprised 150 smokers and 191 never smokers. The instruments were: a socio-demographic questionnaire, diagnoses of mood disorder and nicotine dependence according to DSM-IV, (SCID-IV), and the Alcohol, Smoking and Substance Involvement Screening Test. Laboratory assessments included: nitric oxide metabolites (NOx), lipid hydroperoxides, malondialdehyde (MDA), total reactive antioxidant potential (TRAP), advanced oxidation protein products (AOPP), fibrinogen concentrations, homocysteine, erythrocytes sedimentation rate (ESR) and high-sensitivity C-reactive protein (hs-CRP) were assayed from blood specimens. Statistically significant differences were found among depressed smokers who had more severe depressive symptoms, a higher risk of alcohol consumption, more suicide attempts, and more disability for work than non-depressed never smokers. Depressed smokers had significantly higher levels of NOx, fibrinogen, hs-CRP, AOPP, ESR and lower levels of TRAP compared to non-depressed never smokers. Depressed smokers had significant levels of oxidative stress and inflammatory biomarkers after adjusting for gender, age, years of education, disability for work, and laboratory measures. The levels of NOx, lipid hydroperoxides, AOPP, and fibrinogen were substantially higher, whereas levels of TRAP were lower in depressed smokers compared to non-depressed never smokers. (1) Depressed smokers exhibited altered concentrations of NOx, lipid hydroperoxides, AOPP, TRAP, and fibrinogen. (2) Depressed smokers were more unable to work, showed more severe depressive symptoms and attempted suicide more frequently.

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1. Introduction

Tobacco use is a risk factor for disability and morbidity, as well as the most preventable cause of death in the world [32]. Smokers more often presented with impaired work/domestic functionality, more frequent hospitalization, more depressive disorders, more sedative use and a family history of mental disorders, as well as scoring lower on the quality of life scale compared with never smokers [6]. Smoking with nicotine dependence increases the risk of developing major depressive disorders [25], contributes to more severe depressive symptoms [3,14] and is associated with suicidal behavior [4,20].

Major depressive disorder is accompanied by an increase in oxidative stress and a decrease in antioxidant status, which damages neurons and has an important role in the pathophysiology of depressive disorders [2,10,17,19,22]. There is also data linking oxidative stress and nicotine dependence [28]. Oxidative and nitrosative stress and inflammation induced damage to fatty acids, proteins, DNA, mitochondria and consequent autoimmune reactions, which link to neuroprogression and degenerative processes that occur in depressive disorders [2,19]. Nitric oxide plasma concentration is associated with cognitive impairment in patients with recurrent depressive disorders [29]. These alterations may lead brain damage, which may be a pathway to neurodegenerative diseases [13], as well as neuroprogression of mood disorders [2,19].

The purpose of this study was to elucidate the involvement of oxidative and nitrosative stress and inflammatory markers, including nitric oxide metabolites (NOx), lipid hydroperoxides, malondialdehyde (MDA), advanced oxidation protein products (AOPP), total reactive antioxidant potential (TRAP) and inflammatory biomarkers (high-sensitivity C-reactive protein (hs-CRP), fibrinogen, homocysteine, erythrocytes sedimentation rate (ESR)) in depressed and non-depressed smokers compared to depressed and non-depressed never smokers. We also hypothesized that depressive smokers would have co-occurring disorders such as more risk of alcohol use, more suicide attempts, more physical diseases, more severe symptoms, as well as more disability for work than non-depressed never smokers.

2. Materials and methods

2.1. Study population

Smokers ($n = 150$) were recruited from outpatients at the Centre of Approach and Treatment for Smokers, at Londrina State University (UEL), Paraná, Brazil and never smokers ($n = 191$) were recruited from staff of UEL. The sample size calculation of was based on smokers and never smokers with and without depression. The study sample size calculation showed that a sample of 34 never smokers and 27 smokers (total $n = 61$) would be able to detect a 59% prevalence of depressive disorders among smokers compared to 17% depressive disorders among non-smokers (odds ratio = 7.03) with a confidence level of 0.95 and 95% power. It was assumed that the ratio of control to case was 1.27. However we are able to recruit 191 never smokers and 150 smokers and that sample size would be adequately powered to detect population level prevalence of depressive disorders among smokers and never smokers as reported in the literature [6,7].

Smokers and never smokers were men and women aged 18–60 and all ethnicities were accepted for this study. The study was conducted from March 2011 to July 2012.

Exclusion criteria were presence of medical comorbidities including chronic obstructive pulmonary disease, rheumatoid arthritis, systemic lupus erythematosus, inflammatory bowel disease, HIV infection, neurodegenerative and neuroinflammatory disorders, such as Alzheimer's, Huntington's and Parkinson's disorder, multiple sclerosis and stroke, conditions such as hemodialysis, use of interferon. All these conditions are known to share peripheral inflammation and cell-mediated immune activation [17]. All study participants needed to have normal blood values on the following laboratory tests: hemogram, aspartate transaminase (AST), alanine transaminase (ALT), urea, and creatinine.

All subjects had given written informed consent to participate in the study after the approval of this research by the Ethics Research Committee at Londrina State University (UEL), number 250/2010.

2.2. Instruments

2.2.1. Questionnaire

A self-reported questionnaire was used to gather information on socio-demographic, clinical characteristics and smoking status.

2.2.2. Major depressive disorder and nicotine dependence

The diagnoses of major depressive disorder and nicotine dependence were made at interview by a trained clinician using DSM-IV, criteria translated and validated into Portuguese [8].

2.2.3. Hamilton Depression Rating Scale

A translated and validated version of the Hamilton Depression Rating Scale, adapted to the Brazilian cultural context and Portuguese language [21] was used to measure the severity of depression in study participants who had been diagnosed with major depressive disorder.

2.2.4. The Alcohol, Smoking and Substance Involvement Screening Test (ASSIST)

The World Health Organization (WHO) developed Alcohol, Smoking and Substance Involvement Screening Test (ASSIST) is a questionnaire to screen for levels of risk for alcohol, smoking and substance use in adults. ASSIST scores were calculated for all participants. A risk score for alcohol was calculated as low risk (score 0–3), moderate risk (score 4–26) or high risk (score $\geq$27) [31].

2.3. Laboratory assessments

Peripheral blood samples were collected from all participants after 12–14 h overnight fasting. Lipid hydroperoxides were measured by the ferrous oxidation of xylenol assay using an adaptation of the technique described by Jiang [16]. The levels of nitric oxide metabolites (NOx) were assessed by measuring the plasma concentration of nitrite and nitrate, using an adaptation of the technique described by Navarro-Gonzálvez et al. [23]. TRAP was measured by chemiluminescence in an adaptation of the method described by Repetto et al. [27]. The quantification of advanced oxidation protein products (AOPP) in plasma used the method described by Witko-Sarsat et al. [30]. Malondialdehyde (MDA) is a secondary product of lipid peroxidation and was determined using the method described by Jentzch et al. [15].

We measured the serum concentration of hs-CRP by immunonephelometry system on a BNII analyzer (Siemens® System BNTMII, Deerfield, IL, USA). ESR was performed by an automatic analyzer for erythrocyte sedimentation rate determination (MicroTest1X – Sire Analytical Systems, Udine, Italy). The quantitative determination of fibrinogen in plasma was based on the Clauss method. This method measures the rate of fibrinogen to fibrin conversion in the presence of excess thrombin. This method was performed by a coagulation analyser (Destiny Plus – Trinity Biotech GmbH, Lemgo, Germany). The determination of serum homocysteine was performed on an ARCHITECT i System (Abbot, Wiesbaden, Germany).

Table 1
Demographic and clinical characteristics by smoking and depressive disorder.

Characteristics	Never-smoker				Smoker				*p*-Value[a]
	Non-depressed ($n=123$)		Depressed ($n=68$)		Non-depressed ($n=78$)		Depressed ($n=72$)		
	n	%	*n*	%	*n*	%	*n*	%	
Gender									0.008
Male	47	(38.2)	14	(20.6)*	35	(44.9)	20	(27.8)	
Female	76	(61.8)	54	(79.4)*	43	(55.1)	52	(72.2)	
Age									0.079
18–29	6	(4.9)	1	(1.5)	4	(5.1)	6	(8.3)	
30–39	18	(14.6)	8	(11.8)	8	(10.3)	12	(16.7)	
40–49	63	(51.2)	38	(55.9)	29	(37.2)	25	(34.7)*	
50–60	36	(29.2)	21	(30.9)	37	(47.4)**	29	(40.3)	
Ethnicity									0.524
Caucasian	84	(68.3)	48	(70.6)	53	(67.9)	48	(66.7)	
African	14	(11.4)	5	(7.4)	8	(10.3)	9	(12.5)	
Asian	9	(7.3)	3	(4.4)	4	(5.1)	0	(.0)*	
Mixed	16	(13.0)	12	(17.6)	13	(16.7)	15	(20.8)	
Marital status									0.289
Stable relationship	85	(69.1)	48	(70.6)	52	(66.7)	40	(55.6)	
Years of education									0.000
≤12 years	28	(22.8)	9	(13.2)	58	(74.4)**	51	(70.8)**	
≥13 years	94	(76.4)	55	(80.9)	19	(24.4)**	18	(25.0)**	
Disability for work									0.000
Yes	2	(1.6)	1	(1.5)	14	(17.9)**	19	(26.4)**	
Suicide attempt									
Yes	0	(.0)	2	(2.9)	2	(2.6)	22	(30.6)**	0.000
Hamilton scale (mean, SD)									
Yes	1.6	(2.3)	6.9	(7.9)	4.5	(4.6)	14.4	(9.4)**	0.000
Alcohol ASSIST									
Yes	1	(.8)	1	(1.5)	8	(10.3)**	16	(22.2)**	0.000

[a] The *p*-value tests based on Pearson Chi-square test. The Hamilton scale based Kruskal–Wallis test, other characteristics it is based on the Pearson Chi-square test.
* $p=0.05$.
** $p=0.01$.

2.4. Statistical analyses

Analyses were performed examining the relationship between the non-depressed never-smoker with depressed smokers, non-depressed smokers and depressed never smokers. Comparisons were made between these for socio-demographic, clinical and laboratory measurements, using appropriate parametric tests where data were normally distributed and non-parametric statistical tests for categorical or non-normal data. All tests were 2-tailed and a *p*-value of 0.05 was used for statistical significance.

Univariate comparisons were initially conducted and then variables that were statistically significant were included in the multivariate analyses. A generalized multinomial regression analysis was carried out using non-depressed never-smoker, depressed never-smoker, non-depressed smoker and depressed smoker categories as the dependent variable. The multinomial regression analysis was used to estimate the odds that depressed never-smokers, non-depressed smokers or depressed smokers, compared with non-depressed never-smokers, was associated with each of the independent variables. All analyses were performed using SPSS (Version 20).

3. Results

In examining socio-demographic and clinical variables, smoking and depressive status did not differ with respect to marital status, age and ethnicity. The depressed and non-depressed smokers were significantly different in having fewer years of education and more disability for work compared to non-depressed never smokers ($p<0.01$). Depressed smokers had significantly more disability for work than non-depressed never smokers ($p<0.01$). The mean age for all groups was 46.25 years (Table 1).

Depressed smokers showed statistically significant differences in consuming more alcohol, requiring more frequent hospitalization, attempting suicide more frequently, and being more unable to work than non-depressed never smokers. The depressed smokers also exhibited significant differences in severity of depression (higher Hamilton scores) compared with depressed never smokers (Table 1).

Depressed smokers had significantly higher levels of oxidative stress (NOx, AOPP) and lower levels of antioxidants (TRAP), higher levels of inflammatory biomarkers (fibrinogen, hs-CRP, ESR) compared to non-depressed never smokers However, smokers from all groups did not differ with respect to levels of homocysteine, lipid hydroperoxides and MDA (Table 2).

These results did not change when we analyzed these variables using multinomial regression analysis after adjusting for gender, age, years of education, disability for work, and laboratory measures. We found the levels of NOx, AOPP (OR = 1.02, 95% CI = 1.01–1.03), and fibrinogen were substantially higher, and levels of TRAP were lower (OR = 0.99, 95% CI = 0.99–1) in depressed smokers compared to non-depressed never smokers. However, the association between hs-CRP and ESR in depressed smokers was no longer significant after the multinomial regression analysis. On the other hand, we found significant differences in levels of lipid hydroperoxides (OR = 3.14, 95% CI = 1.25–7.88) (Table 3).

Using multinomial regression analysis, depressed never smokers comprised more women (odds ratio was 4.06, 95% CI = 1.58–10.43) compared to non-depressed never smokers. Depressed smokers were more unable to work (OR = 7.85, 95% CI = 1.29–47.90) compared to non-depressed never smokers.

4. Discussion

The current study demonstrated that depressed smokers had significantly higher levels of oxidative stress such as raised NOx (products of nitrates and nitrites), lipid hydroperoxides (a biomarker of oxidative damage to lipids), AOPP (a biomarker

Table 2
Comparison of laboratory measurements of oxidative stress and inflammatory biomarkers by smoking status and/or depressive disorder.

Laboratory measures	Never-smoker				Smoker				*p*-Value[a]
	Non-depressed		Depressed		Non-depressed		Depressed		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
CRP	2.8	(4.1)	2.5	(3.4)	4.1	(4.7)**	4.1	(4.7)**	0.000
NOx	3.3	(1.8)	3.3	(2.1)	5.3	(2.7)**	4.5	(2.1)**	0.000
Lipid hydroperoxides	0.9	(.4)	0.9	(.4)	1.0	(.4)	1.1	(.6)*	0.125
AOPP	98.5	(38.6)	103.5	(41.8)	109.9	(46.9)**	117.3	(48.0)**	0.018
TRAP	838.3	(131.7)	830.7	(135.3)	836.0	(142.5)	780.1	(126.8)**	0.020
MDA	16.0	(5.4)	14.8	(5.4)	16.0	(5.4)	16.4	(7.1)	0.501
Fibrinogen	327.9	(66.2)	323.4	(62.1)	368.1	(73.8)**	365.6	(67.9)**	0.000
ERS	12.3	(10.8)	11.5	(9.1)	14.6	(10.5)	17.8	(14.3)*	0.015

[a] The *p*-value for all the laboratory measurements except TRAP is based on the independent samples Kruskal–Wallis test and for TRAP it is based on the oneway ANOVA.
* Significance at the 0.05 level.
** Significance at the 0.01 level and the comparison is between the non-depressed never-smokers and the other three groups based on the Mann–Whitney *U* test for all variables except TRAP and for TRAP it is based on the independent sample *t*-test.

of oxidative damage to proteins) and lower levels of TRAP (a biomarker of anti-oxidants) than non-depressed never smokers. In depressed smokers we also found differences in inflammatory biomarkers such as fibrinogen compared to non-depressed never smokers. Furthermore, non-depressed and depressed smokers had significantly higher levels of NOx and fibrinogen compared to non-depressed never smokers. Our results were consistent with previous studies which have examined markers of oxidative stress disturbances in patients with depression, but which did not consider comorbid nicotine dependence [10,29]. Our study included depressed smokers with nicotine dependence.

This study provides evidence for an association between depressed smokers and higher levels of AOPP, a measure of oxidation damage to proteins [1]. AOPP correspond to highly oxidized proteins and specifically to albumin and might be formed during oxidative stress by reaction of plasma proteins with chlorinated oxidants. Thus, AOPP have been considered as a novel marker of oxidant-mediated protein damage [1].

Patients with both major depression and nicotine dependence also had higher levels of lipid hydroperoxides, a marker of oxidative damage. Consistent with previous research that found higher levels of serum F2a-isoprostanes (8-iso-PGF2a), we found high levels of lipid hydroperoxides, a biomarker of oxidative damage to lipids, in depressed compared to non-depressed individuals [33]. Furthermore, depressive smokers had significantly lower concentrations of TRAP, a measure of global antioxidant defenses. Cigarette smoking and depression are significant factors in alterations of the oxidant and antioxidant balance in blood, resulting in potent oxidative stress and in these conditions a lipoperoxidation process can occur [5,19].

In our study, fibrinogen concentrations were higher in smokers with and without depression compared to non-depressed never smokers. These findings are in accordance with other research that has found strong associations between fibrinogen, persistent depressive symptoms and smoking [12]. Fibrinogens and hs-CRP are inflammatory biomarkers. Thus, cigarette smoking and depressive disorders may increase the levels of inflammatory biomarkers and the production of oxidants, and may decrease the levels of antioxidants. An increase in inflammation and oxidative stress is documented in mood disorders [2,19]. There is also data linking oxidative stress and inflammation in cigarette smoking and lung disease [28].

Higher levels of CRP were consistently associated with depressive disorder, and remained significant after controlling for gender, age, smoking status, physical activity weight, as well as medication use and medical conditions potentially influencing inflammation levels [9,18,25]. Furthermore, in univariate analyses, there were higher levels of CRP in smokers with and without depression than non-depressed never smokers. However, in multinomial analysis our data did not suggest that the highest levels of CRP were a consequence of smoking or depressive disorder.

Our study found an association between higher levels of NOx and smokers with and without depression compared with non-depressed never smokers. Higher concentration of plasma NOx in patients with recurrent depressive disorder was associated with the severity of depressive symptom and cognitive impairment,

Table 3
Multinomial regression analysis of variables associated with smoking and/or depression.

Independent variables	Depressed never-smoker versus non-depressed never-smokers			Non-depressed smoker versus non-depressed never-smokers			Depressed smoker versus non-depressed never-smokers		
	OR[b]	95% CI	*p*-Value	OR[b]	95% CI	*p*-Value	OR[b]	95% CI	*p*-Value
Female[a]	4.06	(1.58–10.43)	0.00**	0.84	(0.34–2.08)	0.70	1.48	(0.55–3.98)	0.44
Age	1.02	(0.98–1.07)	0.29	1.02	(0.98–1.07)	0.30	0.99	(0.95–1.04)	0.74
Years of education	1.03	(0.96–1.12)	0.40	0.78	(0.72–0.86)	0.00**	0.82	(0.75–0.90)	0.00**
Disability for work,[a] yes	0.60	(0.04–8.32)	0.70	5.17	(0.86–30.99)	0.07	7.85	(1.29–47.9)	0.03*
CRP	0.98	(0.88–1.09)	0.72	0.94	(0.85–1.04)	0.25	0.93	(0.83–1.03)	0.16
NOx	1.03	(0.85–1.25)	0.75	1.45	(1.21–1.75)	0.00**	1.28	(1.05–1.57)	0.02*
Lipid hydroperoxides	1.56	(0.65–3.71)	0.32	1.41	(0.56–3.52)	0.47	3.14	(1.25–7.88)	0.01*
AOPP	1.01	(1.00–1.02)	0.15	1.01	(1.00–1.02)	0.10	1.02	(1.01–1.03)	0.00**
TRAP	1.00	(1.00–1.00)	0.56	1.00	(0.99–1.00)	0.07	0.99	(0.99–1.00)	0.00**
Fibrinogen	1.00	(0.99–1.01)	0.63	1.01	(1.01–1.02)	0.00**	1.01	(1.01–1.02)	0.00**
ERS	0.96	(0.92–1.01)	0.11	0.96	(0.92–1.00)	0.07	0.97	(0.93–1.01)	0.18

[a] Reference categories: gender, male; disability for work, no.
[b] Odds ratios (OR) mutually adjusted for each of the presented variables.
* $p = 0.05$.
** $p = 0.01$.

suggesting that an overproduction of nitric oxide via inducible nitric oxide synthase, inducible nitric oxide synthase (iNOS), which results in oxidative stress and cell damage [29]. Increased production of NO and peroxynitrite may cause nitration and nitrosylation of proteins that appears related to the pathogenesis of depression [22]. Induction of nitric oxide leads to an activation of nuclear factor kappa B (NF-κB), which is relevant to the development of depressive disorders [26].

Depressive smokers were more unable to work, showed more severe depressive symptoms and attempted suicide more frequently compared with non-depressed never smokers. Our data are consistent with previous reports that have shown that current smoking is associated with subsequent suicidal behavior [4] and more disability for work and more hospitalization [7,24].

The results of this study should be interpreted with regard to its strengths and limitations. The present study design was a cross-sectional study, which can only examine an exposure at a particular time, but cannot determine causal relationships [11]. In our study, the never smokers were highly educated; this could generally lead to healthy behavior patterns.

Despite of some limitations, our findings provide evidence of a link between depressed smokers and altered oxidative stress and inflammatory marker. In addition, our data suggest that depressed smokers were more unable to work, showed more severe depressive symptoms and attempted suicide more frequently.

5. Conclusion

Our results corroborate the inflammatory, oxidative and nitrosative stress theory of depression, and suggest that inflammatory, oxidative and nitrosative stress could be worsened by concomitant nicotine dependences. Depressed smokers exhibited altered concentrations of NOx, lipid hydroperoxides, AOPP, TRAP, and fibrinogen. Depressive smokers were more unable to work, showed more severe depressive symptoms and attempted suicide more frequently.

These findings need further studies to better understand the role of shared inflammatory, oxidative and nitrosative stress pathways in depressive disorders and nicotine dependence, which impacts on the course and severity of both diseases. The translational implications of these findings include the opportunity to identify subgroups of major depressive disorder with comorbidity with nicotine dependence, to potentially protect them from the effects of oxidants by applying new therapies.

References

[1] A. Barsotti, P. Fabbi, M. Fedele, S. Garibaldi, M. Balbi, G.P. Bezante, D. Risso, F. Indiveri, G. Ghigliotti, C. Brunelli, Role of advanced oxidation protein products and Thiol ratio in patients with acute coronary syndromes, Clin. Biochem. 44 (8–9) (2011) 605–611.
[2] M. Berk, F. Kapczinski, A.C. Andreazza, O.M. Dean, F. Giorlando, M. Maes, M. Yucel, C.S. Gama, S. Dodd, B. Dean, P.V.S. Magalhães, P. Amminger, P. McGorry, G.S. Malhi, Pathways underlying neuroprogression in bipolar disorder: focus on inflammation, oxidative stress and neurotrophic factors, Neurosci. Biobehav. Rev. 35 (2011) 804–817.
[3] N. Breslau, S.P. Novak, R.C. Kessler, Psychiatric disorders and stages of smoking, Biol. Psychiatry 55 (2004) 69–76.
[4] N. Breslau, L. Schultz, E. Johnson, E. Peterson, G. Davis, Smoking and the risk of suicidal behavior: a prospective study of a community sample, Arch. Gen. Psychiatry 62 (3) (2005) 328–334.
[5] A. Buico, C. Cassino, M. Ravera, P.-G. Betta, D. Osella, Oxidative stress in total antioxidant capacity in human plasma, Redox Rep. 14 (3) (2009) 125–131.
[6] M.R.P. Castro, T. Matsuo, S.O.V. Nunes, Clinical characteristics and quality of life of smokers at a referral center for smoking cessation, J. Bras. Pneumol. 36 (1) (2010) 67–74.
[7] M.R.P. Castro, T. Matsuo, S.O.V. Nunes, Characteristics of smokers in smoking cessation interventions: an analysis of sex differences, Addict. Disord. Their Treat. 9 (4) (2010) 135–142.
[8] C.M. Del Ben, J.A.A. Vilela, J.A.S. Crippa, J.E.C. Hallak, C.M. Labate, A.W. Zuardi, Confiabilidade da "Entrevista Clínica Estruturada para o D.S.M.-IV" – versão clínica traduzida para o português, Rev. Bras. Psiquiatr. 23 (3) (2001) 156–159.
[9] M. Elovainio, A.-M. Aalto, M. Kivimäki, S. Pirkola, J. Sundvall, J. Lönnqvist, A. Reunanen, Depression and C-reactive protein: population-based Health 2000 Study, Psychosom. Med. 71 (2009) 423–430.
[10] M.J. Forlenza, G.E. Miller, Increased serum levels of 8-hydroxy-2′-deoxyguanosine in clinical depression, Psychosom. Med. 68 (1) (2006) 1–7.
[11] D. Grimes, K.F. Schultz, An overview of clinical research the lay of the land, Lancet 359 (2002) 57–61.
[12] M. Hamer, G.J. Molloy, C. de Oliveira, P. Demakakos, Persistent depressive symptomatology and inflammation: to what extent do health behaviours and weight control mediate this relationship? Brain Behav. Immun. 23 (2009) 413–418.
[13] B. Halliwell, Oxidative stress and neurodegeneration: where are we now? J. Neurochem. 97 (2006) 1634–1658.
[14] M. Jamal, A.J. Willem Van der Does, P. Cuijpers, B.W.J.H. Penninx, Association of smoking and nicotine dependence with severity and course of symptoms in patients with depressive or anxiety disorder, Drug Alcohol Depend. 126 (1–2) (2012) 138–146.
[15] A.M. Jentzch, H. Bachman, P. Furst, H.K. Biesalski, Improved analysis of malondialdehyde in human body fluids, Free Radic. Biol. Med. 20 (1996) 251–256.
[16] Z. Jiang, A.C.S. Woollard, S.P. Wolff, Lipid hydroperoxide measurement by oxidation of Fe^{2+} in the presence of xylenol orange. Comparison with the TBA assay and an Iodometric method, Lipids 26 (10) (1991) 356–358.
[17] B. Leonard, M. Maes, Mechanistic explanations how cell-mediated immune activation, inflammation and oxidative and nitrosative stress pathways and their sequels and concomitants play a role in the pathophysiology of unipolar depression, Neurosci. Biobehav. Rev. 36 (12) (2012) 764–785.
[18] T. Liukkonen, S. Silvennoinen-Kassinen, J. Jokelainen, P. Räsänen, M. Leinonen, V.B. Meyer-Rochow, M. Timonen, The association between C-reactive protein levels and depression: Results from the northern Finland 1966 birth cohort study, Biol. Psychiatry 60 (2006) 825–830.
[19] M. Maes, Y.S. Chang, P. Galecki, M. Berk, A review on the oxidative and nitrosative stress (O&NS) pathways in major depression and their possible contribution to the (neuro)degenerative processes in that illness, Prog. Neuropsychopharmacol. Biol. Psychiatry 35 (2011) 676–692.
[20] K.M. Malone, C. Waternaux, G.L. Haas, T.B. Cooper, S. Li, J.J. Mann, Cigarette smoking, suicidal behavior, and serotonin function in major psychiatric disorders, Am. J. Psychiatry 160 (2003) 773–779.
[21] R.A. Moreno, D.H. Moreno, Hamilton and Montgomery & Ásberg depression rating scales, Rev. Psiquiatr. Clin. 25 (1998) 262–272.
[22] S. Moylan, M. Maes, N.R. Wray, M. Berk, The neuroprogressive nature of major depressive disorder: pathways to disease evolution and resistance, and therapeutic implications, Mol. Psychiatry 33 (2012), http://dx.doi.org/10.1038/mp.2012.
[23] J.A. Navarro-Gonzálvez, C. García-Benayas, J. Arenas, Semiautomated measurement of nitrate in biological fluids, Clin. Chem. 44 (1998) 679–681.
[24] S.O.V. Nunes, H.O. Vargas, J. Brum, E. Prado, M.M. Vargas, M.R.P. de Castro, S. Dodd, M. Berk, A comparison of inflammatory markers in depressed and nondepressed smokers, Nicotine Tob. Res. 14 (2012) 540–546.
[25] J.A. Pasco, L.J. Williams, F.N. Jacka, F. Ng, M.J. Henry, G.C. Nicholson, M.A. Kotowicz, M. Berk, Tobacco smoking as a risk factor for major depressive disorder: population-based study, Br. J. Psychiatry 193 (2008) 322–326.
[26] C.L. Raison, L. Capuron, A.H. Miller, Cytokines sing the blues: inflammation and the pathogenesis of depression, Trends Immunol. 27 (2006) 24–31.
[27] M. Repetto, C. Reides, M.L.G. Carretero, M. Costa, G. Griemberg, S. Llesuy, Oxidative stress in blood of HIV infected patients, Clin. Chim. Acta 255 (1996) 107–117.
[28] P. Rytilä, T. Rehn, H. Ilumets, A. Rouhos, A. Sovijärvi, M. Myllärniemi, V.L. Kinnula, Increased oxidative stress in asymptomatic current chronic smokers and GOLD stage 0 COPD, Respir. Res. 7 (2006) 69.
[29] M. Talarowska, P. Gałecki, M. Maes, A. Orzechowska, M. Chamielec, G. Bartosz, E. Kowalczyk, Nitric oxide plasma concentration associated with cognitive impairment inpatients with recurrent depressive disorder, Neurosci. Lett. 510 (2012) 127–131.
[30] V. Witko-Sarsat, M. Friedlander, C. Capeillere-Blandin, T. Nguyen-Khoa, A.T. Nguyen, J. Zingraff, P. Jungers, B. Deschamps-Latscha, Advanced oxidation protein products as a novel marker of oxidative stress in uremia, Kidney Int. 4 (1996) 1304–1313.
[31] World Health Organization (WHO), The Alcohol, Smoking and Substance Involvement Screening Test (ASSIST): development, reliability and feasibility, Addiction 97 (9) (2002) 1183–1194.
[32] World Health Organization (WHO). WHO report on the global tobacco epidemic, 2008: the MPOWER package. Geneva: World Health Organization; 2008.
[33] S. Yager, M.J. Forlenza, G.E. Miller, Depression and oxidative damage to lipids, Psychoneuroendocrinology 35 (2010) 1356–1362.

Artigo 2 – The shared role of oxidative stress and inflammation in major depressive disorder and nicotine dependence

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Review

The shared role of oxidative stress and inflammation in major depressive disorder and nicotine dependence

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ABSTRACT

Nicotine dependence is common in people with mood disorders; however the operative pathways are not well understood. This paper reviews the contribution of inflammation and oxidative stress pathways to the co-association of depressive disorder and nicotine dependence, including increased levels of pro-inflammatory cytokines, increased acute phase proteins, decreased levels of antioxidants and increased oxidative stress. These could be some of the potential pathophysiological mechanisms involved in neuro-progression. The shared inflammatory and oxidative stress pathways by which smoking may increase the risk for development of depressive disorders are in part mediated by increased levels of pro-inflammatory cytokines, diverse neurotransmitter systems, activation the hypothalamic–pituitary–adrenal (HPA) axis, microglial activation, increased production of oxidative stress and decreased levels of antioxidants. Depressive disorder and nicotine dependence are additionally linked imbalance between neuroprotective and neurodegenerative metabolites in the kynurenine pathway that contribute to neuroprogression. These pathways provide a mechanistic framework for understanding the interaction between nicotine dependence and depressive disorder.

Contents

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1. Introduction

Depressive disorder is highly comorbid with nicotine dependence. In the National Comorbidity Survey, nearly 59% of individuals with a life-time history of depression were current or past smokers, compared to less than 39% of those without a life-time history of depression (Lasser et al., 2000; Ziedonis et al., 2008). The comorbidity of nicotine dependence and depressive disorders can be explained either through common pathways or shared predisposing factors, such as genetic or environmental factors that may increase both smoking and depression. Smoking also appears to increase the risk for the development of mood disorders (Pasco et al., 2008; Pedersen and von Soest, 2009; Ziedonis et al., 2008). Depressive and anxiety disorders are an overwhelmingly strong predictor of daily smoking (Patton et al., 1998). Pre-existing psychiatric disorders predict the subsequent onset of daily smoking and progression to nicotine dependence (Breslau et al., 2004).

People with current nicotine dependence exhibit greater prevalence and severity of several depressive symptoms compared to people with no history of nicotine dependence (Leventhal et al., 2009). Smokers with nicotine dependence have more severe depressive and anxiety symptoms and recover more slowly (Jamal et al., 2012) such that smoking worsens treatment prognosis (Dodd et al., 2010).

There is significant literature describing the pathways that may explain the co-occurrence of depressive disorder and nicotine dependence. The effects of diverse neurotransmitters, particularly glutamate, serotonin, and dopamine, have been demonstrated in both addiction and mood regulation. Dopaminergic agents such as the antidepressant bupropion have been used successfully for the relief of smoking withdrawal syndromes (Danovitch, 2011). Dopamine plays a key role in the regulation of mood and in depression (Berk et al., 2007a; Malhi and Berk, 2007). Cigarette smoking is associated with decreased serotonin function (Malone et al., 2003), and there is extensive evidence of serotonergic abnormalities in depression (Stockmeier, 2003). Plasma levels of glutamate in patients with mood disorders were significantly higher than those in the control group (Hashimoto, 2011), and dysfunctional peripheral glutamate receptors are reported in depression (Berk et al., 2001).

There is also evidence that inflammatory pathways may link smoking and depression. Depressed smokers have higher levels of pro-inflammatory cytokines (PICs) than non-depressed smokers, including tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and acute phase proteins such as C-reactive protein (CRP) (Nunes et al., 2012).

Both depressive disorders and cigarette smoking are associated with increased levels of oxidative stress (Berk et al., 2011a,b; Maes et al., 2011; Rytilä et al., 2006; van der Vaart et al., 2004). Oxidative stress results from an oxidant–antioxidant imbalance, as either an excess of oxidants and/or a depletion of antioxidants, leading to potential protein, lipid, carbohydrate, and deoxyribonucleic acid (DNA) damage (Sies, 1991, 1997).

This article focuses on inflammation and oxidative stress in nicotine dependence and depressive disorders. Inflammation and oxidative stress may interact to increase the risk of neuroprogression of these diseases. In this context, we used studies to provide a better characterization of the shared role of inflammation and oxidative stress in individuals with co-occurring disorders that affect neurotransmitters and that activate the hypothalamic–pituitary–adrenal (HPA) axis and microglial cells. This review article also discusses the fact that both nicotine dependence and depressive disorders both diseases with brain dysfunction by increase the levels of inflammatory biomarkers, increase the production of oxidants, decrease the levels of antioxidants, alter mitochondrial function and modify gene function. Finally, this review provides some evidence for clinical practice.

2. Methods

A narrative review was performed to investigate studies showing explicit associations between depressive disorder, nicotine dependence, inflammation and oxidative stress. The sources used were identified in the electronic database Medline (PubMed) and were limited to English language articles published between January 2000 and July 2012. Using the MeSH (Medical Subject Headings), the following search terms were used: “depressive disorder” and “smoking” and “inflammation”, and “depressive disorder” and “smoking” and “oxidative stress”. Furthermore, review articles were searched, and other publications cross-referenced for additional published articles.

In this review, articles were excluded if inflammatory or immune abnormalities were accompanied by physical illnesses including diabetes, coronary artery disease, Crohn’s disease, rheumatoid arthritis, cancers, human immunodeficiency virus, and multiple sclerosis. These disorders are associated with an increased prevalence of major depressive disorder (MDD) (Benton et al., 2007). Medical comorbidities may include the following: cardiovascular disease, chronic obstructive pulmonary disease, rheumatoid arthritis, systemic lupus erythematosus, inflammatory bowel disease, HIV infection, diabetes and metabolic syndrome; neurodegenerative and neuroinflammatory disorders, such as Alzheimer’s, Huntington’s and Parkinson’s disorder, multiple sclerosis and stroke, conditions such as the postpartum period, hemodialysis, interferon-induced depression and psychological stressors. All of these conditions are known to involve peripheral inflammation and cell-mediated immune activation (Leonard and Maes, 2012). Inflammation in depressive disorders is closely linked with behavioral parameters such as exercise, sleep, alcohol abuse, and nicotine dependence, as well as with medical comorbidities including coronary artery disease, obesity and insulin resistance, osteoporosis, and pain (Goldstein et al., 2009).

Pro-inflammatory cytokines and acute phase proteins, which normally coordinate the local and systemic inflammatory response to microbial pathogens, also appear to act directly on the brain where they can cause behavioral symptoms, including sickness behavior (Dantzer et al., 2008; Leonard and Maes, 2012; Maes et al., 2012a,c).

The analysis also excluded depression in patients with hepatitis C receiving interferon alpha (IFN-α) treatment because it is associated with depression in 30–50% of patients (Asnis and De La Garza, 2006; Raison et al., 2006). Articles focusing on patients with depression due to previous brain injury were also excluded. Prolonged microglial hyperactivity may lead to neuronal apoptosis and brain injury, which are commonly observed in neurodegenerative disorders such as Parkinson’s disease, Alzheimer’s disease and schizophrenia (Monji et al., 2009).

The exclusion criteria were based on the following Medical Subject Heading (MeSH) categories “not”: (1) carried out in “animals”; (2) depression due to medical diseases (“Cardiovascular Disease”,

"Heart Failure", "Neoplasm", "Multiple Sclerosis", "Dementia", "Irritable Bowel Syndrome", "Acquired Immunodeficiency Syndrome", "Hepatitis", and "Herpes"); (3) depression due to drug treatments ("Interferons"); and (4) depression due to brain injury.

3. Shared oxidative stress pathways and inflammatory markers in both depressive disorder and nicotine dependence

The selected articles matched inclusion criteria regarding involvement of inflammatory biomarkers and oxidative stress in both depressive disorder and nicotine dependence. This review excluded medical comorbidities and medicines that are associated with excess inflammation. The results are summarized in Table 1.

A small number of studies have examined shared inflammation and oxidative stress pathways in both depressive disorder and nicotine dependence. It is unclear if this association is causal or is due to confounding and bias or modulated by factors such as physical activity, weight, alcohol consumption and gender.

Elevated levels of circulating CRP have been found in depression and remained significant when controlling for sex, age, smoking status, physical activity, weight, as well as medication use and medical conditions influencing inflammation levels (Danner et al., 2003; Elovainio et al., 2009; Ford and Erlinger, 2004; Liukkonen et al., 2006).

Many studies have confirmed that elevated levels of CRP predict an increased risk of development of depression (Almeida et al., 2007; Hamer et al., 2009a,b; Pasco et al., 2010) and precede the cognitive symptoms of depression (Gimeno et al., 2009). Smoking cessation does not reduce CRP (Asthana et al., 2010). Increased levels of CRP and the presence of clinically significant depressive symptoms can additionally be influenced by the presence of other factors, most notably poor physical health (Almeida et al., 2007) or weight gain (Hamer et al., 2009b) among men (Danner et al., 2003; Elovainio et al., 2009; Ford and Erlinger, 2004; Liukkonen et al., 2006). However, the presence of high CRP has also been shown to be a risk marker for major depressive disorder in women (Pasco et al., 2010). In particular, female hormones may protect tissues; pre-menopausal women experience fewer and less severe adverse cardiovascular events when compared with men of similar age or with post-menopausal women (Vassalle et al., 2011).

Adults with depressive symptoms had higher rates of smoking, had greater sleep disturbance, and higher levels of interleukin 1 receptor antagonist (IL-1RA) and IL-6 compared with non-depressed individuals (Lehto et al., 2010a). The pronounced secretion of the anti-inflammatory marker IL-1RA was thought to reflect the presence of compensatory mechanisms during a depression-related inflammatory state (Lehto et al., 2010a).

Significantly elevated white blood cell counts were found among subjects with moderate and severe depression. Oxidative stress and a medical history of inflammatory diseases did not appear to mediate this association (Kobrosly and van Wijngaarden, 2010). However, other studies failed to find either lower levels of Natural Killer (NK) activity or increased IL-6 in depressed smokers compared to depressed nonsmokers or in depressed patients with and without a history of alcohol abuse or dependence (Pike and Irwin, 2006).

An association was observed between the severity of current depressive symptoms and increased levels of the inflammatory markers IL-6 and C-reactive protein (CRP). Genetic modeling found a significant genetic correlation between IL-6 and depressive symptoms. There were no significant differences due to zygosity and current smoker status (Su et al., 2009).

The inflammatory response appears to be greater in those who suffered childhood abuse (Miller and Cole, 2012), suggesting a cumulative effect of contributory risks on the immune system. This observation is compatible with the allostatic load model of illness (Kapczinski et al., 2008). Depression is part of a family of interrelated disorders in the affective disorders spectrum, including anxiety disorders and fibromyalgia, where alterations in mitochondria, inflammation and neurodegeneration are observed (Gardner and Boles, 2011).

Elevated levels of CRP, fibrinogen, and white blood cells are found in individuals who were both depressed and maltreated during the first decade of life (age 3–11 years). This association was not explained by correlated risk factors such as depression recurrence, low socioeconomic status in childhood or adulthood, poor health, or smoking. Depressed and maltreated individuals were more likely to smoke. In turn, smoking was associated with elevated mean levels of the inflammatory factors (Danese et al., 2008).

Adolescents with depressive or anxiety disorders have significantly higher levels of interleukin-2 (IL-2), interleukin 1 beta (IL1-β) and interleukin-10 (IL-10) compared to adolescents without depressive disorders. However, higher levels of IL-6 and interferon-gamma (IFN-γ) were significantly related to more severe self-assessed symptoms of anxiety and depression after adjustment for use of tobacco (snuff and smoking of cigarettes) or intake of tea, coffee, caffeinated soft drinks or beta stimulant asthma medication (Henje Blom et al., 2012). The depressed participants who smoked had higher depression scores and lower levels of MCP-1, MIP-1β, and IL-8 than healthy controls. Low chemokine levels may lead to increased neurotoxicity, neuronal loss, or both (Lehto et al., 2010b).

One study demonstrated increased levels of immunologic and oxidative stress markers among individuals with depressive symptoms who smoked. Levels of the oxidative stress marker γ-glutamyltransferase were positively correlated with the severity of depression, after adjustment for oxidative stress measures, sex (male or female), age, smoking status and physical activity (Kobrosly and van Wijngaarden, 2010).

Serum levels of F(2α)-isoprostanes [8-iso-PGF(2α)], a biomarker of oxidative damage to lipids, were elevated in a group of depressed individuals, and this finding may represent a common pathophysiological mechanism by which depressed individuals become more vulnerable to atherosclerosis and its clinical sequelae (Forlenza and Miller, 2006; Yager et al., 2010). Depressed patients were significantly less educated and significantly more likely to be regular daily smokers (Yager et al., 2010).

Depression has also been shown to predict subsequent inflammation, but not vice versa. Patients with more persistent depression had higher subsequent levels of inflammatory markers (CRP, IL-6, and fibrinogen), but this association was also explained by unhealthy behaviors such as smoking, inactivity and obesity (Schroeder, 2011). Although research suggests that depression increases the risk for inflammatory markers in non-smokers, the majority of the studies cited in this review have reported an interaction between both major depressive disorder and nicotine dependence, which are highly comorbid, and the influence of oxidative stress and inflammatory markers in both conditions.

4. Inflammation and the involvement of neurotransmitters in both depressive disorder and nicotine dependence

The current understanding of the pathogenesis of depressive disorder has expanded significantly from the historical focus on the role of a monoamine deficit (e.g., noradrenaline and/or serotonin) and how that may be causally involved in the symptoms of illness (Baldessarini, 1975). Drugs that increase the synaptic availability of monoamines (serotonin, norepinephrine and dopamine) have been used to treat depression for more than 50 years.

Table 1
Inflammatory markers and oxidative stress pathways in both depressive disorder and smoking.

Author/year	PICs/APP IO&NS	Gender/age	Covariates
(1) Henje Blom et al. (2012)	IL-2, IL-4, IL-6, IL-10 TNFα, IFN-γ: IL-1β:	Adolescents with MDD or anxiety disorders	Adjustment for the use of tobacco
(2) Nunes et al. (2012)	IL-1β, IL-6, TNFα, CRP	Men and women (age 18–65 years) Depressive smokers (n = 77); non-depressive smokers (n = 78)	Adjustment for the use of tobacco and BMI
(3) Sublette et al. (2011)	KYN, TRP, neopterin	Men and women (age 18–73 years) Healthy volunteers (n = 31) Depressive with suicide attempt (n = 14) Depressive without suicide attempt (n = 16)	Adjustment for the use of tobacco and BMI
(4) Pasco et al. (2010)	CRP	Women (age 20–84 years) Depressive (n = 151), non-depressive (n = 671)	Adjustment for the use of tobacco, physical activity, and BMI
(5) Kobrosly and van Wijngaarden (2010)	CRP, GGTγ, vitamin C, bilirubin, uric acid platelet counts, WBC counts	Men and women (age 20 to 80+ years) No/mild depression (n = 3080), moderate depression (n = 705), severe depression (n = 82)	Adjustment for the use of tobacco, Socioeconomic status and physical activity
(6) Yager et al. (2010)	8-Iso-PGF(2α)	Men and women (age ±28 years) Depressed (n = 73); non-depressed (n = 72)	Adjustment for the use of tobacco, age, gender, years of education, physical activity, and BMI
(7) Lehto et al. (2010a)	IL-6, IL-10, IL-1RA	Men and women (age ±50 years) Elevated depressive symptoms (n = 44); non-depressed (n = 372)	Adjustment for the use of tobacco, physical activity, and BMI
(8) Lehto et al. (2010b)	MCP-1, MIP-1β, IL-8	Men and women (age 53± years) MMD (n = 61), non-depressed (n = 61)	adjusted for age, gender, BMI, smoking, and alcohol consumption
(9) Hamer et al. (2009b)	CRP, fibrinogen	3609 men aged 60.5 ± 9.2 years, 2 years of follow up Depression at baseline and persistent	Adjustment for BMI, smoking, alcohol, physical activity
(10) Su et al. (2009)	IL-6, CRP	188 male twins, age of 55 ± 2.75 years Past and current depressive symptoms	Adjustment for tobacco (pack-years), marital status, and education
(11) Elovainio et al. (2009)	CRP	Men and women (age > 30 years) men (n = 2748), women (3257) depressed and non-depressed	Adjustment for education, BMI, physical activity, alcohol consumption, smoking status, long-term illness,
(12) Danese et al. (2008)	CRP, fibrinogen, WBC	Men and women (age > 32 years) Non-depressed, non-maltreated (n = 673) Depressed-only (n = 109) Maltreated-Only (n = 56) Depressed and maltreated (n = 27)	Adjustment for the use of tobacco, socioeconomic status, gender, medication
(13) Almeida et al. (2007)	CRP	Older male Non depressive (n = 5098) Depressive (n = 340). 28.7	Adjustment for the use of tobacco and BMI
(14) Pike and Irwin (2006)	NK, IL-6, s IL-2R	Men (age non-depressed ±45, depressed ±42 years) Depressed (n = 25), Non-depressed (n = 25)	Adjustment for the use of tobacco and BMI
(15) Liukkonen et al. (2006)	CRP	Men and women (was born from 1st January and 31st December in 1966) Men (n = 2688), Women (n = 2837)	Adjustment for the use of tobacco, physical exercise, and BMI
(16) Forlenza and Miller (2006)	8-OHdG	Men and women (age ±28 years) Depressed (n = 84); non-depressive (n = 85)	Adjustment for the use of tobacco, BMI, age, gender, ethnicity, years of education, alcoholic drinks and physical activity
(17) Ford and Erlinger (2004)	CRP	Men and women (age 18–39 years) Men (n = 3154), Women (n = 3760) MDD and non-depressed	Adjustment for the use of tobacco and BMI
(18) Douglas et al. (2004)	CRP	Men and women (age 39–45 years) n = 696, US army 18% women 18% depressed, 82% non-depressed	Adjustment for the use of tobacco and BMI
(19) Danner et al. (2003)	CRP	Men and women (age 17–39 years), men (n = 2981), women (n = 3119) past and current of MDD	Adjustment for the use of tobacco and BMI

The macrophage hypothesis suggests that depressive disorder is associated with innate immune system activation due to abnormal secretion of cytokines, such as IL-1 and interferon-alpha (IFN-α) (Smith, 1991). This notion has expanded to include the interrelationship between inflammation and oxidative stress and has been termed the inflammatory and oxidative and nitrosative stress (O&NS) theory of depression.

Inflammation might regulate brain functions, including neurotransmitter systems, neuroendocrine functions, synaptic plasticity, and the neural circuitry of mood (Salim et al., 2012).

The alterations of neurotransmitters by inflammation and oxidative stress suggest that antidepressant treatments may have an anti-inflammatory and antioxidant effect. Previous studies have reported that antioxidant activity normalizes during subchronic treatment with antidepressants and anti-inflammatory compounds. Furthermore, natural anti-oxidative stress substances may augment the efficacy of antidepressants or may have antidepressant efficacy (Maes et al., 2009, 2011, 2012b). Diverse treatments for mood disorders reduce oxidative stress and inflammation (Berk et al., 2011a). Antidepressants and lithium enhance ATPase activity, improving mitochondrial dysfunction and inflammation (Gardner and Boles, 2011).

The mechanisms underlying the association between nicotine dependence and MDD appear to involve neurotransmitter pathways that are linked to both conditions. Monoamine oxidases catalyze the metabolism of dopamine, norepinephrine, and serotonin. Cigarette smoke inhibits the activity of monoamine oxidase type A (MAO A) and B (MAO B) (Benowitz, 2010).

Smoking also appears to be associated with dysfunction of the serotonergic system. Depletion of serotonin and lowered brain

serotonin is associated with a higher risk for suicide and attempted suicide in smokers with depression. Acute administration of nicotine may result in the release of serotonin as well as dopamine, and chronic nicotine administration has been shown to decrease the concentrations and biosynthesis of serotonin. Impaired serotonergic function was found in smokers after fenfluramine challenge (Malone et al., 2003). The lowered MAO activity observed in the brain of smokers may contribute to addiction in that disorder (Talhout et al., 2007). Lowered MAO activity, which may play a role in central nervous system (CNS) serotonin metabolism, could modulate, in part, the link between cigarette smoking and suicidal behavior (Breslau et al., 2005).

Dopamine is a shared and robust biomarker for depressive disorder as well as smoking. Individuals with depressive disorder have a decreased turnover of homovanillic acid, the primary metabolite of dopamine (Berger et al., 1980; Lambert et al., 2000). Bupropion is an atypical antidepressant that helps to normalize noradrenaline and dopamine and is effective in smoking cessation and depressive disorder (Cox et al., 2012).

Nicotine has diverse effects throughout the CNS, acting on multiple forms of nicotinic acetylcholine receptors. Nicotine can hijack synaptic plasticity mechanisms in key brain circuits, most importantly in the mesolimbic dopamine system, which is central to reward processing in the brain (Dani and Bertrand, 2007).

Neuroadaptation and tolerance involve changes in nicotinic receptors and neural plasticity, which could cause nicotine dependence (Benowitz, 2010). Addictive drugs elicit or modify synaptic plasticity in many of the key brain regions involved in addiction, and these synaptic modifications have important consequences (Dani and Bertrand, 2007). Self-reported measures of tolerance, loss of control, and other behaviors such as relapse during a quit attempt and withdrawal symptoms must be present for an individual to receive a diagnosis of tobacco/nicotine dependence (Fagerstrom and Eissenberg, 2012).

$\alpha4\beta2$ receptors appear to be crucial to the effects of nicotine on mood and the development of dependency. Nicotine activates $\alpha4\beta2$ receptors in the ventral tegmental area, resulting in dopamine release in the shell of the nucleus accumbens (Benowitz, 2010). Dopamine D2 and D3 receptors in the striatum are down regulated by smoking. Activation of dopaminergic neurons in the ventral tegmental area is enhanced by excitatory glutamatergic projections and inhibited by γ-aminobutyric acid (GABA) projections that are also stimulated by nicotine (Benowitz, 2008). Thus, stimulation of nicotine cholinergic receptors releases dopamine, glutamate, and GABA, which affects the development of neuroadaptation and increases levels of corticotrophin-releasing factor (CRF). These alterations may play a key role in withdrawal. The negative effect that typifies the response to nicotine withdrawal most likely results in part from a cascade involving increased levels of CRF. The release of CRF in the central nucleus of the amygdala causes anxiety and stress (Benowitz, 2010).

There is growing evidence that the glutamatergic system plays an important role in the neurobiology and treatment of depressive disorders (Berk et al., 2011a,b). In nicotine dependence, the rewarding effect of nicotine can be attenuated by administering compounds that reduce glutamate transmission (D'Souza and Markou, 2011).

5. Stress in individuals with depressive disorders and nicotine dependence

Nicotine dependence and depressive disorders could be linked by the role of stress in the activation of the HPA axis. Psychological stress can activate the HPA axis, inducing secretion of corticotrophin-releasing factor (CRF) and subsequent increases in adrenocorticotrophic hormone (ACTH) and cortisol (Bateman et al., 1989).

Stressful life events (personal loss, infection, trauma, childhood maltreatment) and genetic vulnerability to stress may alter the HPA axis, which can activate the release of pro-inflammatory cytokines. The increased secretion of CRF, a key factor involved in the stress response, has been implicated in the pathophysiology of both nicotine dependence and depressive disorder. Patients with depressive disorders exhibit higher rates of CRF neuronal activation and increased levels of cortisol compared to age-matched controls (Nemeroff and Vale, 2005). In addition, many depressive symptoms can be induced by intracerebroventricular injections of CRF (Holsboer et al., 1992). Stressors facilitate the initiation of smoking, decrease the motivation to quit, and increase the risk of relapse. The role of brain stress systems in nicotine addiction indicates that CRF plays a pivotal role in nicotine addiction (Bruijnzeel, 2012).

Dysfunction of the HPA axis, with amygdala hyperfunction and decreased activity of the hippocampus (defective glucocorticoid-negative feedback), has been reliably observed in patients with depressive disorder (Pariante and Miller, 2001), as well as patients who suffered childhood sexual abuse (Heim et al., 2000; Nunes et al., 2010). Similar findings are observed in nicotine dependence (Rohleder and Kirschbaum, 2006).

A central tenet of the glucocorticoid cascade hypothesis is that excess glucocorticoid results in damage to key brain structures involved in HPA axis restraint, including, most notably, the hippocampus (Raison and Miller, 2003).

The pathways by which inflammatory cytokines produce depressive disorder include activation of the HPA axis, which causes microglial activation. This pathway is reactive to stressful life events, infection, trauma, toxins and immunogenetics. This activation releases pro-inflammatory cytokines and free radicals. These mediators are known to cause neuronal degeneration and decreased neurogenesis, which may be an important factor in the pathophysiology of depressive disorder (Moylan et al., 2012).

6. Epigenetic effects in both depressive disorder and nicotine dependence

Epigenetic modification of gene function may be related to depressive disorder and nicotine dependence. Chronic exposure to nicotine results in changes in gene expression and protein synthesis, including the generation of new synaptic connections, analogous to other forms of learning (Kauer and Malenka, 2007). The identification of single candidate genes associated with MDD and nicotine dependence has been difficult because of the likelihood that complex psychiatric illnesses are under polygenic influence and are associated with interactions between genetic variants and environmental exposures (Uher, 2009).

The importance of serotonin in the depression-nicotine dependence nexus indicates that pro-inflammatory cytokines may also affect serotonin neurotransmission. Altered levels of cytokines are known to stimulate production of kynurenine (KYN) from its precursor, tryptophan (TRP), and may thus potentially deplete TRP, leading to reduced levels of the TRP metabolite serotonin (Sublette et al., 2011).

The short ("s") allele in the promoter region of the serotonin transporter gene (5-HTTLPR) is associated with lower transcriptional efficiency of the promoter compared with the long ("l") allele. The hypothesis of a gene-by-environment interaction showed that childhood maltreatment predicted adult depression only among individuals carrying an "s" allele but not among l/l homozygotes (Caspi et al., 2003). This original observation has been replicated by many subsequent studies. Individuals with the "s" allele in the promoter region of the serotonin transporter gene (SLC6A4) are

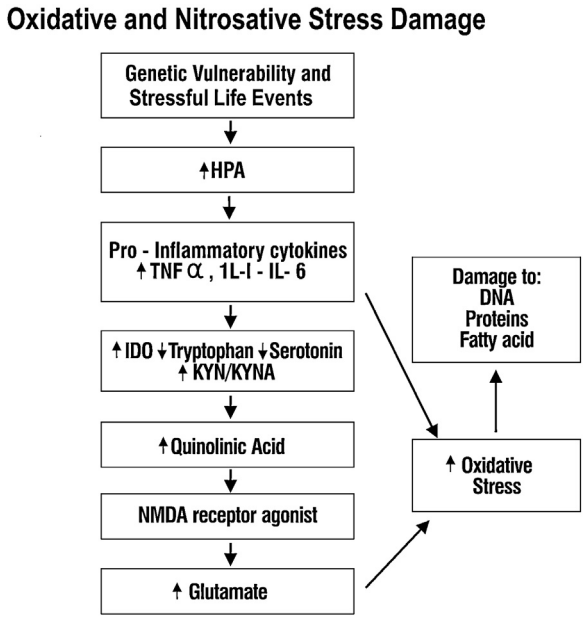

Fig. 1. Oxidative and nitrosative stress damage.

unusually vulnerable to the depressogenic effects of early life stress such as child abuse or neglect. Early life trauma and depression lead to chronic activation of the immune system and prolonged production of proinflammatory cytokines, as well as CRP (Saveanu and Nemeroff, 2012).

The serotonin pathway has been associated with smoking behavior, as well as several behavioral traits, such as neuroticism, novelty seeking and anxiety-related personality traits (Quaak et al., 2009). Genes involved in the serotonin pathway include insertion/deletion polymorphisms in the promoter region of the serotonin transporter gene (5-HTTLPR), which has been linked to vulnerability to smoking and the ability to quit (Sieminska et al., 2008).

Genetic polymorphisms and the 5-HTTLPR variant are associated with smoking-related phenotypes and diminished serotonin neurotransmission. A polymorphism in the promoter region of the 5-HTTLPR gene modulates the mRNA and protein levels, such that allelic variants may influence nicotine dependence. The presence of the short or long alleles appears to influence transcription regulation (Watanabe et al., 2011). Smoking is associated with epigenetic alteration of MAO-B by reducing methylation of its gene promoter. This alteration leads to increased production of MAO-B that persists long after smoking cessation (Launay et al., 2009).

7. Inflammation and oxidative stress are linked to central nervous system (CNS) dysfunction in both depressive disorder and nicotine dependence

7.1. Neuroprogression pathways in depression

Oxidative and nitrosative stress and inflammation are linked to neuronal cell injury or death, which contribute to the neuroprogression of depressive disorders, mediating changes in conjunction with genetic vulnerability and environmental factors (Fig. 1). In neurons, N-methyl-D-aspartate (NMDA) receptor (NMDAR) activation and subsequent Ca^{2+} influx can induce the generation of nitric oxide (NO)-via neuronal NO synthase, which leads to nitrosative stress, synaptic damage, and neuronal loss (Nakamura and Lipton, 2011). The excessive accumulation of these free radicals causes damage and can lead to alterations in the structure and function of membrane fatty acids and proteins. Furthermore, free radicals can alter the activity of proteins residing in the cell membrane and can alter or damage DNA and mitochondrial function, leading to cell death via necrosis or apoptosis (Maes et al., 2011).

An increase in inflammation-induced apoptosis, together with a reduction in the synthesis of neurotrophic factors caused by a rise in brain glucocorticoids and a reduction in the neuroprotective components of the kynurenine pathway, contributes to the pathological changes that are postulated to cause neuronal damage. This effect may predispose chronically depressed patients to neuroprogressive processes including dementia (Leonard and Myint, 2006).

The neurodegeneration hypothesis proposed that depressive disorder is a consequence of an imbalance between neuroprotective and neurodegenerative metabolites in the kynurenine pathway (Myint et al., 2007). Kynurenic acid (KYNA) is regulated by IDO (indoleamine 2-3-dioxygenase), which catalyzes the first step in the pathway, specifically the degradation from tryptophan to kynurenine (KYN). KYNA acts as a blocker of the glycine co-agonistic site of the NMDA receptor and as a noncompetitive inhibitor of the α7 nicotinic acetylcholine receptor, which has a role in cognitive disturbances. Depressive symptoms have been shown to be related to an increased ratio of KYN/KYNA (Müller and Schwarz, 2008).

Through stimulation of multiple inflammatory signaling pathways, including activation of nuclear factor kappa B (NF-κB) and p38 mitogen activated protein kinases (MAPKs), cytokines can activate IDO, which breaks down tryptophan (TRP), the primary precursor of serotonin, into quinolinic acid (QUIN), a potent NMDA agonist and stimulator of glutamate release (Miller et al., 2009).

Activation of NF-κB leads to an inflammatory response including the release of the pro-inflammatory cytokines. Once in the brain, cytokine signals participate in pathways known to be involved in the development of depression, including altered neurotransmitters such as serotonin and dopamine, activation of the HPA axis, and disruption of synaptic plasticity through alterations in relevant growth factors. NF-κB induction in the brain might contribute to alterations in neuronal growth and survival, especially through the induction of nitric oxide and, ultimately, oxidative stress, which has been shown to alter promoter function for several genes central to synaptic plasticity (Raison et al., 2006).

Microglia activated by excess inflammation, astroglial loss, and inappropriate glutamate receptor activation ultimately disrupt the delicate balance of neuroprotective versus neurotoxic effects in the brain, potentially leading to increased neurodegeneration and decreased neurogenesis (McNally et al., 2008). Biochemical factors including inflammatory, oxidative and nitrosative stress, mitochondrial dysfunction, epigenetic alterations, HPA axis dysregulation and disturbed neurotrophic function interact to cause cellular damage, stimulate apoptosis and decrease neuronal growth and survival (Moylan et al., 2012).

Mechanisms that may contribute to brain damage by oxidative and nitrosative stress are summarized in Fig. 1. Genetic vulnerability and stressful life events activate CNS circuitry, including the HPA axis and pro-inflammatory cytokines (TNFα, IL-1, IL-6), which in turn lead to the release of IDO, which breaks down (TRP), the primary precursor of serotonin. Tryptophan–kynurenine metabolism influences the ratio of KYN/KYNA (Müller and Schwarz, 2008), and this dysregulation of QUIN, a potent NMDA agonist, stimulates glutamate release. Glutamate release is associated with inhibition of BDNF, a decline in neuroprotective factors and increased oxidative stress (Miller et al., 2009).

7.2. Neuroprogression pathways in nicotine dependence

It is thought that smoking can predispose our brains to dementia or cognitive impairment by inflammatory, oxidative and nitrosative

stress pathways. These same pathways lead to neuroprogression through exposure to free radicals and by inducing direct cellular damage and inhibition of mitochondrial bioenergetics. Oxidative and nitrosative stress can result from either increased production of reactive species from nitrogen (RNS, such as nitric oxide) or oxygen (ROS), which damage neurons and promote the release of glutamate. Activation of synaptic NMDA can result in physiological ROS and RNS production (Nakamura and Lipton, 2011). Oxidative and nitrosative stress is manifested as increases in lipid peroxidation end products, leading to protein, lipid, carbohydrate and DNA damage. Thus, these events constitute a vicious cycle, and any one of them could initiate neuronal cell death (Halliwell, 2006).

Chronic cigarette smoking also affects the synapse through reducing the expression of pre-synaptic proteins that may induce synaptic changes and other neuropathological alterations. These changes might serve as evidence of early phases of neurodegeneration and may explain why smoking can predispose brains to Alzheimer's disease and dementia (Ho et al., 2012).

Cigarette smoking is known to be an important inducer of oxidative stress in multiple organs, including the brain (Rueff-Barroso et al., 2010). Several factors could explain the link between tobacco smoking and the higher risk of cognitive decline. For example, tobacco smoke and chronic lead exposure is a well-known risk factor for cognitive deterioration. Another ingredient of tobacco smoke, cadmium, also has neurotoxic properties. Furthermore, smokers have decreased levels of circulating antioxidants and increased levels of oxidative stress (Dome et al., 2010).

In cigarette smokers, there is an excess of pro-inflammatory cytokines such as CRP, IL-1-β, TNF-α and IL-6 (Yanbaeva et al., 2007). Cigarette smoke increases TNFα, which can induce histone acetylation, pro-inflammatory gene transcription and oxidative stress. Smoking curiously impacts NF-κB by phosphorylating IκB kinase α (IKKα) and causing enhancement of target gene expression. IKKα also reacts to cigarette smoke and plays a role in regulating histone modification (Chung et al., 2011). The direct exposure to oxidative stress from cigarette smoke also contributes to additional endogenous oxidant formation through effects on the inflammatory immune response pathway (Swan and Lessov-Schlaggar, 2007).

Multiple studies have shown that current smoking is a risk factor for Alzheimer's and Parkinson's diseases (Dome et al., 2010). However, contradictory research has suggested that smoking may be protective against the development of neurodegenerative diseases such as Alzheimer's disease and Parkinson's disease. In such cases, a local inflammatory response is sustained by microglial cells, which are associated with CNS nicotinic acetylcholine receptors and have recently been reported to inhibit TNF-α production in human macrophages as well as in mouse microglial cultures (de Simone et al., 2005).

Nicotine, on the other hand, may offer protective effects against dopaminergic cell damage induced by various neurotoxins and may also protect against aminochrome-induced toxicity in substantia-nigra derived RCSN-3 cells. These protective effects of nicotine may help to explain why smoking might reduce the incidence of Parkinson's disease (Muñoz et al., 2012).

There is an inverse relationship between cancer and neurodegeneration. The risk of Alzheimer's disease is lower among survivors of smoking-related cancers than among survivors of non-smoking-related cancers and cannot be explained by a survival bias (Driver et al., 2012).

In a meta-analysis of 43 studies, there was more evidence to suggest that smoking was associated with an increased risk for Alzheimer's disease. Moreover, 11 of the 43 studies were conducted by researchers with tobacco industry affiliations, although these affiliations were often not disclosed. Studies with tobacco industry affiliations have generally suggested that tobacco protects against Alzheimer's disease, whereas non-tobacco industry studies find that smoking is a risk factor for Alzheimer's disease (Cataldo et al., 2010).

7.3. Mitochondrial dysfunction may contribute to neuroprogression

CNS functions strongly depend on efficient mitochondrial function because brain tissue has a high-energy demand. Mutations in the mitochondrial genome, defects in mitochondrial dynamics, generation and presence of ROS, protein aggregate-associated dysfunctions and environmental factors may alter energy metabolism and in many cases are associated with neurodegenerative diseases (Federico et al., 2012).

Oxidative stress and mitochondrial involvement may be major triggering factors in neurodegenerative disorders. Neurodegenerative disorders lead to cellular energetic depression (CED), which is characterized by a decreased cytosolic phosphorylation potential that suppresses the cell's ability to do work and control intracellular Ca^{2+} homeostasis and its redox state. If progressive, CED leads to cell death, whose type is linked to the functional status of the mitochondria (Seppet et al., 2009).

Mitochondria are responsible for the energy supply of cells. Mitochondria house the oxidative phosphorylation machinery, which enables aerobic ATP generation, and multiple metabolic pathways, such as β-oxidation of fatty acids and the tricarboxylic acid and urea cycles. Indeed, over 90% of cellular energy generation takes place in mitochondria. In addition, mitochondria have important biosynthetic activities, control intracellular Ca^{2+} metabolism and signaling, regulate thermogenesis, generate most cellular reactive species from oxygen (ROS) and serve as gatekeepers of the cell for programmed cell death (apoptosis). Several events can compromise mitochondrial function and integrity. These include damage or mutation of mitochondrial DNA, increases in ROS and abnormal elevation of Ca^{2+} through NMDA (Manji et al., 2012). Mitochondrial dysfunction is associated with an increasingly large proportion of inherited human disorders and is implicated in common diseases, such as neurodegenerative disorders (Nunnari and Suomalainen, 2012).

There is a growing body of evidence to suggest that impaired mitochondrial function may affect key cellular processes, thereby altering synaptic functioning and contributing to the atrophic changes that underlie the deteriorating long-term course of major psychiatric illnesses such as mood disorders and schizophrenia. Because the brain is the body's most metabolically active tissue, it is not surprising that the majority of mitochondrial disorders have a neurological phenotype (Manji et al., 2012).

Mitochondria are the key organelle involved in the control of two cellular processes: cell metabolism and cell death. The action of carbon monoxide (CO) on mitochondria is involved in cell death (Queiroga et al., 2012). Thus, in the case of CO-mediated oxidative stress exposure, neurons are especially sensitive to oxidative lesions, which could be the basis for later memory impairment (Garrabou et al., 2011). Evidence has shown that mitochondrial dysfunction in cells is induced by tobacco smoke components. In particular, CO could be responsible for impaired mitochondrial function in smokers (Miró et al., 1999).

Mitochondrial dysfunction has been linked to neuron loss in ischemia, traumatic brain injury and neurodegenerative diseases (Monji et al., 2009). Smoking is associated with a decrease in neurogenesis, as well as white matter abnormalities in the brain. Chronic nicotine dependence is related to global brain atrophy and to structural and biochemical abnormalities in anterior frontal regions, subcortical nuclei and commissural white matter. Chronic nicotine dependence may also be associated with an increased risk for various forms of neurodegenerative diseases (Durazzo et al., 2010).

7.4. Autoimmune disorders may contribute to neuroprogression

It has been postulated that autoimmune dysfunction may be part of the cognitive impairment in MDD. It is noteworthy that humoral immunity dysfunction is frequently described in patients with depressive disorder, as indicated by increased autoantibody levels. MDD could be another autoimmune disease from the view of autoantibodies (Chen et al., 2009). High levels of autoantibodies were detected in serum of depressed patients (Laske et al., 2008; Maes et al., 1993, 2011, 2012a; Nemeroff et al., 1985).

7.5. Neuroimaging studies have demonstrated brain abnormalities

Nicotine-dependent depressive disorder could be conceptualized as a neurodegenerative disease because it is associated with impairment of synaptic plasticity, neuron loss and reduced neurogenesis (Manji et al., 2012).

Neuroimaging studies have demonstrated brain abnormalities that link nicotine dependence and depressive disorders, particularly involving portions of the prefrontal cortex. Magnetic resonance imaging has shown volume loss in regions of the orbitofrontal cortex and the medial prefrontal cortex in MDD, concordant with post-mortem-derived evidence of tissue loss. MDD patients with a family history of affective illness showed left hemisphere gray matter loss in a region immediately ventral to the genu of the corpus callosum – the subgenual anterior cingulate cortex (Savitz and Drevets, 2009).

Neuroimaging studies have also shown cigarette smoking to be associated with numerous structural brain changes, including a reduction in the integrity of the cerebral white matter microstructure (Gons et al., 2011) and reduced gray matter volumes in the prefrontal cortices (Brody et al., 2004; Zhang et al., 2011). Other brain abnormalities in cigarette smokers include ventricular enlargement and atrophy (Brody et al., 2004), as well as volumetric changes and atrophy (Brody et al., 2004; Gons et al., 2011; Zhang et al., 2011). One possible explanation may be the stimulating effect of nicotine on nicotine receptors expressed in oligodendrocyte precursor cells, which could result in microstructural alterations of white matter integrity in cigarette smokers, leading to cognitive decline (Gons et al., 2011).

8. Conclusions and implications for clinical practice

The depression-nicotine dependence nexus may increase the levels of inflammatory markers and oxidative and nitrosative stress. The exact pathways that underpin the common pathophysiology of both diseases are still not well defined. Some hypotheses have been postulated for common pathways for nicotine dependence and depressive disorders that have implications for neuroprogression. These pathways include alterations of neurotransmitters, HPA axis dysregulation, increased pro-inflammatory cytokines and levels of acute phase proteins, increased oxidative and nitrosative stress, decreased levels of antioxidants leading to damage to lipids, proteins, and DNA, microglia activation, mitochondrial dysfunction, and modification of gene function.

Smokers with serious mental disorders are at risk of dying prematurely, on average, 25 years earlier than the general population. Clinicians need to intervene to encourage their patients to cease smoking (Schroeder, 2011). Smoking is a risk factor in psychiatric disorders, in addition to causing a legion of other documented health problems. Aggressive targeting of smoking cessation must be a part of routine care (Berk, 2007b).

Patients with co-occurring depressive disorders and nicotine dependence in clinical practice are common; therefore, clinicians may have to treat these patients as having co-occurring disorders. This observation has clinical relevance because both disorders worsen the prognosis and could be regarded as neurodegenerative disorders.

In the future, anti-inflammatory treatments that promote neurogenesis and neuronal survival could be used by people with co-occurring disorders. Treatments with anti-inflammatory and antioxidant-target therapies, including diets, vitamins, omega-3 fatty acids, acetylsalicylic acid, cyclo-oxygenase, inhibiting inflammatory cytokines, minocycline and N-acetyl cysteine, may augment the clinical efficacy of established agents and serve as novel treatments in depressive disorder and nicotine dependence.

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References

Almeida, O.P., Norman, P., Hankey, G.J., Jamrozik, K., Flicker, L., 2007. The association between C-reactive protein concentration and depression in later life is due to poor physical health: results from the Health in Men Study (HIMS). Psychol. Med. 37, 1775–1786.
Asnis, G.M., De La Garza, R., 2006. Interferon-induced depression in chronic hepatitis C: a review of its prevalence, risk factors, biology, and treatment approaches. J. Clin. Gastroenterol. 40, 322–335.
Asthana, A., Johnson, H.M., Piper, M.E., Fiore, M.C., Baker, T.B., Stein, J.H., 2010. Effects of smoking intensity and cessation on inflammatory markers in a large cohort of active smokers. Am. Heart J. 160, 458–463.
Baldessarini, R.J., 1975. The basis for amine hypotheses in affective disorders. A critical evaluation. Arch. Gen. Psychiatry 32, 1087–1093.
Bateman, A., Singh, A., Kral, T., Solomon, S., 1989. The immune–hypothalamic–pituitary–adrenal axis. Endocr. Rev. 10, 92–112.
Benowitz, N.L., 2008. Clinical pharmacology of nicotine: implications for understanding, preventing, and treating tobacco addiction. Clin. Pharmacol. Ther. 83, 531–541.
Benowitz, N.L., 2010. Nicotine addiction. N. Engl. J. Med. 362, 2295–2303.
Benton, T., Staab, J., Evans, D.L., 2007. Medical co-morbidity in depressive disorders. Ann. Clin. Psychiatry 19, 289–303.
Berger, P.A., Faull, K.F., Kilkowski, J., Anderson, P.J., Kraemer, H., Davis, K.L., Barchas, J.D., 1980. CSF monoamine metabolites in depression and schizophrenia. Am. J. Psychiatry 137, 174–180.
Berk, M., Plein, H., Ferreira, D., 2001. Platelet glutamate receptor supersensitivity in major depressive disorder. Clin. Neuropharmacol. 24, 129–132.
Berk, M., Dodd, S., Kauer-Sant'anna, M., Malhi, G.S., Bourin, M., Kapczinski, F., Norman, T., 2007a. Dopamine dysregulation syndrome: implications for a dopamine hypothesis of bipolar disorder. Acta Psychiatr. Scand. Suppl., 41–49.
Berk, M., 2007b. Should we be targeting smoking as a routine intervention? Acta Neuropsychiatr. 19 (2), 131–132.
Berk, M., Kapczinski, F., Andreazza, A.C., Dean, O.M., Giorlando, F., Maes, M., Yucel, M., Gama, C.S., Dodd, S., Dean, B., Magalhães, P.V.S., Amminger, P., McGorry, P., Malhi, G.S., 2011a. Pathways underlying neuroprogression in bipolar disorder: focus on inflammation, oxidative stress and neurotrophic factors. Neurosci. Biobehav. Rev. 35, 804–817.
Berk, M., Johansson, S., Wray, N.R., Williams, L., Olsson, C., Haavik, J., Bjerkeset, O., 2011b. Glutamate cysteine ligase (GCL) and self reported depression: an association study from the HUNT. J. Affect. Disord. 131, 207–213.
Breslau, N., Novak, S.P., Kessler, R.C., 2004. Psychiatric disorders and stages of smoking. Biol. Psychiatry 55, 69–76.
Breslau, N., Schultz, L., Johnson, E., Peterson, E., Davis, G., 2005. Smoking and the risk of suicidal behavior: a prospective study of a community sample. Arch. Gen. Psychiatry 62 (3), 328–334.
Brody, A.L., Mandelkern, M.A., Jarvik, M.E., Lee, G.S., Smith, E.C., Huang, J.C., Bota, R.G., Bartzokis, G., London, E.D., 2004. Differences between smokers and nonsmokers in regional gray matter volumes and densities. Biol. Psychiatry 55, 77–84.
Bruijnzeel, A.W., 2012. Tobacco addiction and the dysregulation of brain stress systems. Neurosci. Biobehav. Rev. 36, 1418–1441.
Caspi, A., Sugden, K., Moffitt, T.E., Taylor, A., Craig, I.W., Harrington, H., McClay, J., Mill, J., Martin, J., Braithwaite, A., Poulton, R., 2003. Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. Science 301, 386–389.

Cataldo, J.K., Prochaska, J.J., Glantz, S.A., 2010. Cigarette smoking is a risk factor for Alzheimer's disease: an analysis controlling for tobacco industry affiliation. J. Alzheimers Dis. 19 (2), 465–480, http://dx.doi.org/10.3233/JAD-2010-1240.
Chen, P., Jiang, T., Ouyang, J., Chen, Y., 2009. Depression, another autoimmune disease from the view of autoantibodies. Med. Hypotheses 73, 508–509.
Chung, S., Sundar, I.K., Hwang, J.W., Yull, F.E., Blackwell, T.S., Kinnula, V.L., Bulger, M., Yao, H., Rahman, I., 2011. NF-κB inducing kinase, NIK mediates cigarette smoke/TNFα-induced histone acetylation and inflammation through differential activation of IKKs. PLoS ONE 6 (8), e23488.
Cox, L.S., Nollen, N.L., Mayo, M.S., Choi, W.S., Faseru, B., Benowitz, N.L., Tyndale, R.F., Okuyemi, K.S., Ahluwalia, J.S., 2012. Bupropion for smoking cessation in African American light smokers: a randomized controlled trial. J. Natl. Cancer Inst. 104, 290–298.
Danese, A., Moffitt, T.E., Pariante, C.M., Ambler, A., Poulton, R., Caspi, A., 2008. Elevated inflammation levels in depressed adults with a history of childhood maltreatment. Arch. Gen. Psychiatry 65 (4), 409–415.
Dani, J.A., Bertrand, D., 2007. Nicotinic acetylcholine receptors and nicotinic cholinergic mechanisms of the central nervous system. Annu. Rev. Pharmacol. Toxicol. 47, 699–729.
Danner, M., Kasl, S.V., Abramson, J.L., Vaccarino, V., 2003. Association between depression and elevated C-reactive protein. Psychosom. Med. 65, 347–356.
Danovitch, I., 2011. The clinical assessment and treatment of nicotine dependence. Focus 9 (1), 15–24.
Dantzer, R., O'Connor, J.C., Freund, G.G., Johnson, R.W., Kelley, K.W., 2008. From inflammation to sickness and depression: when the immune system subjugates the brain. Nat. Rev. Neurosci. 9, 46–56.
de Simone, R., Ajmone-Cat, M.A., Carnevale, D., Carnevale, L., 2005. Activation of α7 nicotinic acetylcholine receptor by nicotine selectively up-regulates cyclooxygenase-2 and prostaglandin E2 in rat microglial cultures. J. Neuroinflammation 2 (1), 4, http://dx.doi.org/10.1186/1742-2094-2-4.
Dodd, S., Brnabic, A.J.M., Berk, L., Fitzgerald, P.B., de Castella, A.R., Filia, S., Filia, K., Kelin, K., Smith, M., Montgomery, W., Kulkarni, J., Berk, M., 2010. A prospective study of the impact of smoking on outcomes in bipolar and schizoaffective disorder. Compr. Psychiatry 51, 504–509.
Dome, P., Lazary, J., Kalapos, M.P., Rihmer, Z., 2010. Smoking, nicotine and neuropsychiatric disorders. Neurosci. Biobehav. Rev. 34 (3), 295–342.
Douglas, K.M., Taylor, A.J., O'Malley, P.G., 2004. Relationship between depression and C-reactive protein in a screening population. Psychosom. Med. 66, 679–683.
Driver, J.A., Beiser, A., Au, R., Kreger, B., Splansky, L.G., Kurth, T., Kiel, D.P., Lu, K.P., Seshadri, S.S., Wolf, P., 2012. Inverse association between cancer and Alzheimer's disease: results from the Framingham Heart Study. BMJ 344, e1442, http://dx.doi.org/10.1136/bmj.e1442.
D'Souza, M., Markou, A., 2011. Neuronal mechanisms underlying development of nicotine dependence: implications for novel smoking-cessation treatments. Addict. Sci. Clin. Pract. 6 (1), 4–16.
Durazzo, T., Meyerhoff, D., Nixon, D., 2010. Chronic cigarette smoking: implications for neurocognition and brain neurobiology. Int. J. Environ. Res. Public Health 7, 3760–3791.
Elovainio, M., Aalto, A.-M., Kivimäki, M., Pirkola, S., Sundvall, J., Lönnqvist, J., Reunanen, A., 2009. Depression and C-reactive protein: population-based Health 2000 Study. Psychosom. Med. 71, 423–430.
Fagerstrom, K., Eissenberg, T., 2012. Dependence on tobacco and nicotine products: a case for product-specific assessment. Nicotine Tob. Res., http://dx.doi.org/10.1093/ntr/nts007.
Ford, D.E., Erlinger, T.P., 2004. Depression and C-reactive protein in US adults: data from the Third National Health and Nutrition Examination Survey. Arch. Intern. Med. 164, 1010–1014.
Forlenza, M.J., Miller, G.E., 2006. Increased serum levels of 8-hydroxy-2′-deoxyguanosine in clinical depression. Psychosom. Med. 68, 1–7.
Federico, A., Cardaioli, E., Da Pozzo, P., Formichi, P., Gallus, G.N., Radi, E., 2012. Mitochondria, oxidative stress and neurodegeneration. J. Neurol. Sci. 322, 254–282.
Gardner, A., Boles, R.G., 2011. Beyond the serotonin hypothesis: mitochondria, inflammation and neurodegeneration in major depression and affective spectrum disorders. Prog. Neuropsychopharmacol. Biol. Psychiatry 35 (3), 730–743.
Garrabou, G., Inoriza, J.M., Morén, C., Oliu, G., Miró, Ò., Marti, M.J., Cardellach, F., 2011. Mitochondrial injury in human acute carbon monoxide poisoning: the effect of oxygen treatment. J. Environ. Sci. Health C 29, 32–51.
Gimeno, D., Kivimäki, M., Brunner, E.J., Elovainio, M., De Vogli, R., Steptoe, A., Kumari, M., Lowe, G.D.O., Rumley, A., Marmot, M.G., Ferrie, J.E., 2009. Associations of C-reactive protein and interleukin-6 with cognitive symptoms of depression: 12-year follow-up of the Whitehall II study. Psychol. Med. 39 (3), 413–423.
Goldstein, B.I., Kemp, D.E., Soczynska, J.K., McIntyre, R.S., 2009. Inflammation and the phenomenology, pathophysiology, comorbidity, and treatment of bipolar disorder: a systematic review of the literature. J. Clin. Psychiatry 70 (8), 1078–1090.
Gons, R.A., van Norden, A.G., de Laat, K.F., van Oudheusden, L.J., van Uden, I.W., Zwiers, M.P., Norris, D.G., de Leeuw, F.E., 2011. Cigarette smoking is associated with reduced microstructural integrity of cerebral white matter. Brain 134 (Pt 7), 2116–2124.
Halliwell, B., 2006. Oxidative stress and neurodegeneration: where are we now? J. Neurochem. 97, 1634–1658.
Hamer, M., Molloy, G.J., de Oliveira, C., Demakakos, P., 2009a. Leisure time physical activity, risk of depressive symptoms, and inflammatory mediators: the English Longitudinal Study of Ageing. Psychoneuroendocrinology 34, 1050–1055.
Hamer, M., Molloy, G.J., de Oliveira, C., Demakakos, P., 2009b. Persistent depressive symptomatology and inflammation: to what extent do health behaviours and weight control mediate this relationship? Brain Behav. Immun. 23, 413–418.
Hashimoto, K., 2011. The role of glutamate on the action of antidepressants. Prog. Neuropsychopharmacol. Biol. Psychiatry 35, 1558–1568.
Heim, C., Newport, D.J., Heit, S., Graham, Y.P., Wilcox, M., Bonsall, R., Miller, A.H., Nemeroff, C.B., 2000. Pituitary–adrenal and autonomic responses to stress in women after sexual and physical abuse in childhood. JAMA 284, 592–597.
Henje Blom, E., Lekander, M., Ingvar, M., Åsberg, M., Mobarrez, F., Serlachius, E., 2012. Pro-inflammatory cytokines are elevated in adolescent females with emotional disorders not treated with SSRIs. J. Affect. Disord. 136, 716–723.
Ho, Y.-S., Yang, X., Yeung, S.-C., Chiu, K., Lau, C.-F., Tsang, A.W.-T., Mak, C.-W., Chang, R.C.-C., 2012. Cigarette smoking accelerated brain aging and induced pre-Alzheimer-like neuropathology in rats. PLoS ONE 7 (5), e36752.
Holsboer, F., Spengler, D., Heuser, I., 1992. The role of corticotropin-releasing hormone in the pathogenesis of Cushing's disease, anorexia nervosa, alcoholism, affective disorders and dementia. Prog. Brain Res. 93, 385–417.
Jamal, M., Willem Van der Does, A.J., Cuijpers, P., Penninx, B.W.J.H., 2012. Association of smoking and nicotine dependence with severity and course of symptoms in patients with depressive or anxiety disorder. Drug Alcohol Depend. 126 (1/2), 138–146.
Kapczinski, F., Vieta, E., Andreazza, A.C., Frey, B.N., Gomes, F.A., Tramontina, J., Kauer-Sant'anna, M., Grassi-Oliveira, R., Post, R.M., 2008. Allostatic load in bipolar disorder: implications for pathophysiology and treatment. Neurosci. Biobehav. Rev. 32 (4), 675–692.
Kauer, J.A., Malenka, R.C., 2007. Synaptic plasticity and addiction. Nat. Rev. Neurosci. 8, 844–858.
Kobrosly, R., van Wijngaarden, E., 2010. Associations between immunologic, inflammatory, and oxidative stress markers with severity of depressive symptoms: an analysis of the 2005–2006 National Health and Nutrition Examination Survey. Neurotoxicology 31, 126–133.
Lambert, G., Johansson, M., Agren, H., Friberg, P., 2000. Reduced brain norepinephrine and dopamine release in treatment-refractory depressive illness: evidence in support of the catecholamine hypothesis of mood disorders. Arch. Gen. Psychiatry 57, 787–793.
Laske, C., Zank, M., Klein, R., Stransky, E., Batra, A., Buchkremer, G., Schott, K., 2008. Autoantibody reactivity in serum of patients with major depression, schizophrenia and healthy controls. Psychiatry Res. 158, 83–86.
Lasser, K., Boyd, J.W., Woolhandler, S., Himmelstein, D.U., McCormick, D., Bor, D.H., 2000. Smoking and mental illness: a population-based prevalence study. JAMA 284 (20), 2606–2610.
Launay, J.M., Del Pino, M., Chironi, G., Callebert, J., Peoc'h, K., Megnien, J.L., Mallet, J., Simon, A., Rendu, F., 2009. Smoking induces long-lasting effects through a monoamine-oxidase epigenetic regulation. PLoS ONE 4, e7959.
Lehto, S.M., Niskanen, L., Miettola, J., Tolmunen, T., Viinamäki, H., Mäntyselkä, P., 2010a. Serum anti-inflammatory markers in general population subjects with elevated depressive symptoms. Neurosci. Lett. 484, 201–205.
Lehto, S.M., Niskanen, L., Herzig, K.-H., Tolmunen, T., Huotari, A., Viinamäki, H., Koivumaa-Honkanen, H., Honkalampi, K., Ruotsalainen, H., Hintikka, J., 2010b. Serum chemokine levels in major depressive disorder. Psychoneuroendocrinology 35, 226–232.
Leonard, B., Myint, K., 2006. Inflammation and depression: is there a causal connection with dementia? Neurotox Res. 10 (2), 149–160.
Leonard, B., Maes, M., 2012. Mechanistic explanations how cell-mediated immune activation, inflammation and oxidative and nitrosative stress pathways and their sequels and concomitants play a role in the pathophysiology of unipolar depression. Neurosci. Biobehav. Rev. 36 (12), 764–785.
Leventhal, A.M., Kahler, C.W., Ray, L.A., Zimmerman, M., 2009. Refining the depression-nicotine dependence link: patterns of depressive symptoms in psychiatric outpatients with current, past, and no history of nicotine dependence. Addict. Behav. 34 (3), 297–303.
Liukkonen, T., Silvennoinen-Kassinen, S., Jokelainen, J., Räsänen, P., Leinonen, M., Meyer-Rochow, V.B., Timonen, M., 2006. The association between C-reactive protein levels and depression: results from the northern Finland 1966 birth cohort study. Biol. Psychiatry 60, 825–830.
Maes, M., Meltzer, H., Jacobs, J., Suy, E., Calabrese, J., Minner, B., Raus, J., 1993. Autoimmunity in depression: increased antiphospholipid autoantibodies. Acta Psychiatr. Scand. 87 (3), 160–166.
Maes, M., Yirmyia, R., Noraberg, J., Brene, S., Hibbeln, J., Perini, G., Kubera, M., Bob, P., Lerer, B., Maj, M., 2009. The inflammatory & neurodegenerative (I&ND) hypothesis of depression: leads for future research and new drug developments in depression. Metab. Brain Dis. 24, 27–53.
Maes, M., Leonard, B., Fernandez, A., Kubera, M., Nowak, G., Veerhuis, R., Gardner, A., Ruckoanich, P., Geffard, M., Altamura, C., Galecki, P., Berk, M., 2011. (Neuro)inflammation and neuroprogression as new pathways and drug targets in depression: from antioxidants to kinase inhibitors. Prog. Neuropsychopharmacol. Biol. Psychiatry 35 (3), 659–663.
Maes, M., Ringel, K., Kubera, M., Berk, M., Rybakowski, J., 2012a. Increased autoimmune activity against 5-HT: a key component of depression that is associated with inflammation and activation of cell-mediated immunity, and with severity and staging of depression. J. Affect. Disord. 136, 386–392.
Maes, M., Fišar, Z., Medina, M., Scapagnini, G., Nowak, G., Berk, M., 2012b. New drug targets in depression: inflammatory, cell-mediated immune, oxidative and nitrosative stress, mitochondrial, antioxidant, and neuroprogressive pathways. And new drug candidates-Nrf2 activators and GSK-3 inhibitors. Inflammopharmacology 20 (3), 127–150.

Maes, M., Berk, M., Goehler, L., Song, C., Anderson, G., Gaecki, P., Leonard, B., 2012c. Depression and sickness behavior are Janus-faced responses to shared inflammatory pathways. BMC Med. 10, 66.
Malhi, G.S., Berk, M., 2007. Does dopamine dysfunction drive depression? Acta Psychiatr. Scand. Suppl. 433, 116–124.
Malone, K.M., Waternaux, C., Haas, G.L., Cooper, T.B., Li, S., Mann, J.J., 2003. Cigarette smoking, suicidal behavior, and serotonin function in major psychiatric disorders. Am. J. Psychiatry 160, 773–779.
Manji, H., Kato, T.D., Prospero, N.A., Ness, S., Beal, M.F., Krams, M., Chen, G., 2012. Impaired mitochondrial function in psychiatric disorders. Nat. Rev. Neurosci. 13 (5), 293–307.
McNally, L., Bhagwagar, Z., Hannestad, J., 2008. Inflammation, glutamate, and glia in depression: a literature review. CNS Spectr. 13, 501–510.
Miller, A.H., Maletic, V., Raison, C.L., 2009. Inflammation and its discontents: the role of cytokines in the pathophysiology of major depression. Biol. Psychiatry 65, 732–741.
Miller, G.E., Cole, S.W., 2012. Clustering of depression and inflammation in adolescents previously exposed to childhood adversity. Biol. Psychiatry 72 (1), 34–40.
Miró, O., Alonso, J.R., Jarretra, D., Casademont, J., Urbano-Marquez, A., Cardellach, F., 1999. Smoking disturbs mitochondrial respiratory chain function and enhances lipid peroxidation on human circulating lymphocytes. Carcinogenesis 20 (7), 1331–1336.
Monji, A., Kato, T., Kanba, S., 2009. Cytokines and schizophrenia: microglia hypothesis of schizophrenia. Psychiatry Clin. Neurosci. 63, 257–265.
Moylan, S., Maes, M., Wray, N.R., Berk, M., 2012. The neuroprogressive nature of major depressive disorder: pathways to disease evolution and resistance, and therapeutic implications. Mol. Psychiatry, http://dx.doi.org/10.1038/mp.2012.33.
Müller, N., Schwarz, M.J., 2008. A psychoneuroimmunological perspective to Emil Kraepelins dichotomy: schizophrenia and major depression as inflammatory CNS disorders. Eur. Arch. Psychiatry Clin. Neurosci. 258 (Suppl. 2), 97–106.
Muñoz, P., Huenchuguala, S., Paris, I., Villa, M., Cuevas, C., Caviede, P., Segura-Aguilar, J., Tizabi, Y., 2012. Protective effects of nicotine against aminochrome-induced toxicity in substantia nigra derived cells: implications for Parkinson's disease. Neurotox Res. 22, 177–180.
Myint, A.-M., Kim, Y.K., Verkerk, R., Scharpa, S., Steinbusch, H., Leonard, B., 2007. Kynurenine pathway in major depression: evidence of impaired neuroprotection. J. Affect. Disord. 98, 143–151.
Nakamura, T., Lipton, S.A., 2011. Redox modulation by S-nitrosylation contributes to protein misfolding, mitochondrial dynamics, and neuronal synaptic damage in neurodegenerative diseases. Cell Death Differ. 18 (9), 1478–1486.
Nemeroff, C.B., Simon, J.S., Haggerty J.J.Jr. Evans, D.L., 1985. Antithyroid antibodies in depressed patients. Am. J. Psychiatry 142, 840–843.
Nemeroff, C.B., Vale, W.W., 2005. The neurobiology of depression: in roads to treatment and new drug discovery. J. Clin. Psychiatry 66 (Suppl. 7), 5–13.
Nunes, S.O.V., Watanabe, M.A.E., Morimoto, H.K., Moriya, R., Reiche, E.M.V., 2010. The impact of childhood sexual abuse on activation of immunological and neuroendocrine response. Aggress. Violent Behav. 15 (6), 440–445.
Nunes, S.O.V., Vargas, H.O., Brum, J., Prado, E., Vargas, M.M., de Castro, M.R.P., Dodd, S., Berk, M., 2012. A comparison of inflammatory markers in depressed and nondepressed smokers. Nicotine Tob. Res. 14, 540–546.
Nunnari, J., Suomalainen, A., 2012. Mitochondria: in sickness and in health. Cell 148, 1145–1159.
Pariante, C.M., Miller, A.H., 2001. Glucocorticoid receptors in major depression: relevance to pathophysiology and treatment. Biol. Psychiatry 49, 391–404.
Patton, G.C., Carlin, J.B., Coffey, C., Wolfe, R., Hibbert, M., Bowes, G., 1998. Depression, anxiety, and smoking initiation: a prospective study over 3 years. Am. J. Public Health 88 (10), 1518–1522.
Pasco, J.A., Williams, L.J., Jacka, F.N., Ng, F., Henry, M.J., Nicholson, G.C., Kotowicz, M.A., Berk, M., 2008. Tobacco smoking as a risk factor for major depressive disorder: population-based study. Br. J. Psychiatry 193, 322–326.
Pasco, J.A., Nicholson, G.C., Williams, L.J., Jacka, F.N., Henry, M.J., Kotowicz, M.A., Schneider, H.G., Leonard, B.E., Berk, M., 2010. Association of high-sensitivity C-reactive protein with de novo major depression. Br. J. Psychiatry 197, 372–377.
Pedersen, W., von Soest, T., 2009. Smoking, nicotine dependence and mental health among young adults: a 13-year population-based longitudinal study. Addiction 104 (1), 129–137.
Pike, J.L., Irwin, M.R., 2006. Dissociation of inflammatory markers and natural killer cell activity in major depressive disorder. Brain Behav. Immun. 20, 169–174.
Quaak, M., van Schayck, C.P., Knaapen, A.M., van Schooten, F.J., 2009. Genetic variation as a predictor of smoking cessation success. A promising preventive and intervention tool for chronic respiratory diseases? Eur. Respir. J. 33 (3), 468–480.
Queiroga, C.S.F., Almeida, A.S., Vieira, H.L.A., 2012. Carbon monoxide targeting mitochondria. Biochem. Res. Int., 1–9.
Raison, C.L., Miller, A.H., 2003. When not enough is too much: the role of insufficient glucocorticoid signaling in the pathophysiology of stress-related disorders. Am. J. Psychiatry 160, 1554–1565.
Raison, C.L., Capuron, L., Miller, A.H., 2006. Cytokines sing the blues: inflammation and the pathogenesis of depression. Trends Immunol. 27, 24–31.
Rohleder, N., Kirschbaum, C., 2006. The hypothalamic–pituitary–adrenal (HPA) axis in habitual smokers. Int. J. Psychophysiol. 59, 236–243.
Rueff-Barroso, C.R., Trajano, E.T., Alves, J.N., Paiva, R.O., Lanzetti, M., 2010. Organ-related cigarette smoke-induced oxidative stress is strain-dependent. Med. Sci. Monit. 16 (7), BR218–BR226.
Rytilä, P., Rehn, T., Ilumets, H., Rouhos, A., Sovijärvi, A., Myllärniemi, M., Kinnula, V.L., 2006. Increased oxidative stress in asymptomatic current chronic smokers and GOLD stage 0 COPD. Respir. Res. 7, 69.
Salim, S., Chugh, G., Asghar, M., 2012. Inflammation in anxiety. Adv. Protein Chem. Struct. Biol. 88, 1–25, http://dx.doi.org/10.1016/B978-0-12-398314-5.00001-5.
Saveanu, R.V., Nemeroff, C.B., 2012. Etiology of depression: genetic and environmental factors. Psychiatr. Clin. N. Am. 35 (1), 51–71.
Savitz, J., Drevets, W.C., 2009. Bipolar and major depressive disorder: neuroimaging the developmental-degenerative divide. Neurosci. Biobehav. Rev. 33, 699–771.
Schroeder, S.A., 2011. Depression, smoking, and heart disease: how can psychiatrists be effective? Am. J. Psychiatry 168 (9), 876–878.
Seppet, E., Gruno, M., Peetsalu, A., Gizatullina, Z., Nguyen, H.P., Vielhaber, S., Wussling, M.H.P., Trumbeckaite, S., Arandarcikaite, O., Jerzembeck, D., Sonnabend, M., Jegorov, K., Zierz, S., Striggow, F., Gellerich, F.N., 2009. Mitochondria and energetic depression in cell pathophysiology. Int. J. Mol. Sci. 10, 2252–2303.
Sies, H., 1991. Oxidative stress: from basic research to clinical application. J. Med. 91 (3), S31–S35.
Sies, H., 1997. Oxidative stress: oxidants and antioxidants. Exp. Physiol. 82, 291–295.
Sieminska, A., Buczkowski, K., Jassem, E., Tkacz, E., 2008. Lack of association between serotonin transporter gene polymorphism 5-HTTLPR and smoking among Polish population: a case–control study. BMC Med. Genet. 9, 76, http://dx.doi.org/10.1186/1471-2350-9-76.
Smith, R.S., 1991. The macrophage theory of depression. Med. Hypotheses 35, 298–306.
Stockmeier, C.A., 2003. Involvement of serotonin in depression: evidence from postmortem and imaging studies of serotonin receptors and the serotonin transporter. J. Psychiatr. Res. 37, 357–373.
Su, S., Miller, A.H., Snieder, H., Bremner, J., Ritchie, J., Maisano, C., Jones, L., Murrah, N.V., Goldberg, J., Vaccarino, V., 2009. Common genetic contributions to depressive symptoms and inflammatory markers in middle-aged men: the Twins Heart Study. Psychosom. Med. 71, 152–158.
Sublette, M.E., Galfalvy, H.C., Fuchs, D., Lapidus, M., Grunebaum, M.F., Oquendo, M.A., Mann, J.J., Postolache, T.T., 2011. Plasma kynurenine levels are elevated in suicide attempters with major depressive disorder. Brain Behav. Immun. 25, 1272–1278.
Swan, G., Lessov-Schlaggar, C., 2007. The effects of tobacco smoke and nicotine on cognition and the brain. Neuropsychol. Rev. 17, 259–273.
Talhout, R., Opperhuizen, A., van Amsterdam, J.G., 2007. Role of acetaldehyde in tobacco smoke addiction. Eur. Neuropsychopharmacol. 17 (10), 627–636.
Uher, R., 2009. The role of genetic variation in the causation of mental illness: an evolution-informed framework. Mol. Psychiatry 14, 1072–1082.
van der Vaart, H., Postma, D.S., Timens, W., ten Hacken, N.H.T., 2004. Acute effects of cigarette smoke on inflammation and oxidative stress: a review. Thorax 59, 713–721.
Vassalle, C., Novembrino, C., Maffei, S., Sciarrino, R., De Giuseppe, R., Vigna, L., de Liso, F., Mercuri, A., Bamonti, F., 2011. Determinants of oxidative stress related to gender: relevance of age and smoking habit. Clin. Chem. Lab. Med. 49 (9), 1509–1513.
Watanabe, M.A.E., Nunes, S.O.V., Amarante, M.K., Guembarovski, R.L., Oda, J.M.M., Lima, K.W.A.D., Fungaro, M.H.P., 2011. Genetic polymorphism of serotonin transporter 5-HTTLPR: involvement in smoking behaviour. J. Genet. 90, 179–185.
Yager, S., Forlenza, M.J., Miller, G.E., 2010. Depression and oxidative damage to lipids. Psychoneuroendocrinology 35, 1356–1362.
Yanbaeva, D.G., Dentener, M.A., Creutzberg, E.C., Wesseling, G., Wouters, E.F.M., 2007. Systemic effects of smoking. Chest 131, 1557–1566.
Zhang, X., Salmeron, B.J., Ross, T.J., Geng, X., Yang, Y., Stein, E.A., 2011. Factors underlying prefrontal and insula structural alterations in smokers. Neuroimage 54, 42–48.
Ziedonis, D., Hitsman, B., Beckham, J.C., Zvolensky, M., Adler, L.E., Audrain-McGovern, J., Breslau, N., Brown, R.A., George, T.P., Williams, J., Calhoun, P.S., Riley, W.T., 2008. Tobacco use and cessation in psychiatric disorders: National Institute of Mental Health report. Nicotine Tob. Res. 10 (12), 1691–1715.

Artigo 3 – Oxidative stress and lowered total antioxidant status are associated with a history of suicide attempts

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Research report

Oxidative stress and lowered total antioxidant status are associated with a history of suicide attempts ☆

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ABSTRACT

Background: There is evidence that depression is accompanied by inflammation, oxidative and nitrosative stress (O&NS) and metabolic disorders. However links between oxidative stress and suicide attempts in depressed patients are poorly understood. This study examines whether a history of suicide attempts is associated with inflammation, O&NS and metabolic disorders.

Methods: Blood specimens were collected from study participants aged 18–60 ($N=342$) recruited at the State University of Londrina, Brazil, and measured for oxidative stress biomarkers: nitric oxide metabolites (NO*x*), lipid hydroperoxides, malondialdehyde, advanced oxidation protein products and plasma total antioxidant potential (TRAP); inflammatory biomarkers: fibrinogen, high-sensitivity C-reactive protein, erythrocyte sedimentation rate, interleukin-6 and tumor necrosis factor-α; and metabolic variables. Subjects were divided into those with ($n=141$) and without ($n=201$) a history of suicidal attempts.

Results: Individuals with a history of suicide attempts had significantly higher levels of NO*x* and lipid hydroperoxides and lowered TRAP as compared to individuals without suicide attempts. There were no significant associations between a history of suicide attempts and inflammatory and metabolic biomarkers and metabolic syndrome. Logistic regression showed that both unipolar and bipolar disorder, female gender, smoking behavior and lipid hydroperoxides were significantly associated with a history of suicide attempts. The combined effects of oxidative stress, smoking, depression, female gender were independent from classical risk factors, including marital status, years of education and anxiety.

Conclusions: O&NS as well as lowered antioxidant levels may play a role in the pathophysiology of suicidal behavior independently from the effects of depression and smoking, both of which are associated with increased O&NS, and classical suicide predictors, such as years of education and marital status.

1. Introduction

Activation of inflammatory pathways and oxidative and nitrosative stress (O&NS), O&NS damage to fatty acids, proteins, DNA, mitochondria and consequent autoimmune reactions frequently occur in depressive disorders (Berk et al., 2011; Maes et al., 2011). Major depressive disorder is accompanied by a decrease in antioxidant status and an increase in O&NS, which may cause illness neuroprogression, from neurodegeneration and apoptosis, and lowered neurogenesis and neuroplasticity, all playing an important role in the pathophysiology of depressive disorders (Forlenza and

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Miller 2006; Berk et al., 2011; Maes et al., 2011; Leonard and Maes, 2012; Moylan et al., 2012; Talarowska et al., 2012).

Depression is accompanied by higher levels of acute phase proteins and pro-inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α), and interleukin-6 (IL-6) (Nunes et al., 2012). There are many studies confirming that elevated levels of C-reactive protein (CRP) predict a higher risk for depression (Almeida et al., 2007; Hamer et al., 2009; Pasco et al., 2008) even when other variables are adjusted for, e.g. gender, age, smoking status, physical activity weight, as well as medication use and medical conditions (Danner et al., 2003; Elovainio et al., 2009; Ford and Erlinger, 2004; Liukkonen et al., 2006). These alterations in oxidative stress and inflammatory pathways may lead to alterations in neurotransmitters, including serotonin and dopamine, which in turn may be associated with suicidal behavior (Miller et al., 2009; Raison et al., 2006). To the best of our knowledge there are no data linking O&NS to suicide attempts, although one report showed lower levels of carotenoids in subjects with a history of suicide attempts (Li and Zhang, 2007). There are also some data that activation of immune-inflammatory pathways may occur in association with suicidal acts. Higher concentrations of plasma soluble interleukin-2 receptor (S-IL-2R) were detected in suicide attempters who were free of medication compared to healthy controls, suggesting T cell activation (Nässberger and Träskman-Bendz, 1993). Mendlovic et al. (1999) reported a Th1-like response in suicide attempters. Kim et al. (2007) on the other hand found lowered IL-2 and IL-6 production in depressed suicidal patients. To the best of our knowledge there are no data relating suicide attempts with acute phase proteins, such as CRP and fibrinogen and pro-inflammatory cytokines, such as TNFα.

Previous studies have reported that smoking is associated with a risk of suicidal behavior and depressive disorders (Malone et al., 2003; Breslau et al., 2005; McGee et al., 2005; Hughes, 2008; Goodwin et al., 2013). Other studies have found associations between prior smoking or nicotine dependence and subsequent suicidal behavior, which were independent of depression (Bolden et al., 2008; Bronisch et al., 2008). There is also some evidence that smoking is associated with activated inflammatory pathways (Nunes et al., 2012) and that nicotine dependence is similarly associated with oxidative stress (Rytilä et al., 2006). In addition, the smoking-suicide nexus may be explained, in part by depletion of serotonin (Malone et al., 2003; Sublette et al., 2011).

There is also some evidence that metabolic biomarkers of depression, e.g. lowered levels of cholesterol and in particular HDL-cholesterol, may be associated with suicidal behavior (Maes et al., 1997). The metabolic syndrome is more frequent in heavy smokers (more than 20 pack-years) (Chen et al., 2008). Long term smokers are more insulin resistant and hyperinsulinemic and also have higher concentrations of plasma triglycerides (TG) and lower high-density lipoprotein (HDL) cholesterol concentrations than nonsmokers (Facchini et al., 1992). Smoking is also associated with hypercholesterolemia and may be linked to oxidative stress by reducing isoprostanes (Burke and Fitzgerald, 2003).

The purpose of this study was to elucidate the association of different biomarkers in study participants with a history of suicide attempts compared to those without a history of suicide attempts: O&NS biomarkers, including nitric oxide metabolites (NO*x*), lipid hydroperoxides, malondialdehyde (MDA), advanced oxidation protein products (AOPP), plasma total antioxidant potential (TRAP); inflammatory biomarkers, including CRP, fibrinogen, IL-6, TNFα and erythrocyte sedimentation rate (ESR); and metabolic biomarkers, including total cholesterol, LDL and HDL-cholesterol, TG, insulin, glucose, homocysteine, body mass index (BMI) and metabolic syndrome.

2. Methods

2.1. Study population

Subjects with a history of suicide attempts ($n=150$) and without suicide attempts ($n=201$) were recruited from outpatients at the Centre of Approach and Treatment for Smokers and from staff, at Londrina State University (UEL), Paraná, Brazil. All participants were men and women aged 18–60 and all ethnicities were accepted for this study. The study was conducted from March 2011 to July 2012.

Exclusion criteria were presence of medical comorbidities, such as chronic obstructive pulmonary disease, rheumatoid arthritis, systemic lupus erythematosus, inflammatory bowel disease, HIV infection, neurodegenerative and neuroinflammatory disorders, such as Alzheimer's, Huntington's and Parkinson's disorder, multiple sclerosis and stroke, and conditions such as hemodialysis and use of interferon-α-based immunotherapy. All these conditions are known to share peripheral inflammation and cell-mediated immune activation (Leonard and Maes, 2012).

All study participants had normal blood values on the following laboratory tests: hemogram, aspartate transaminase (AST), alanine transaminase (ALT), urea and creatinine.

All subjects had given written informed consent to participate in the study after the approval of this research by the Ethics Research Committee at Londrina State University (UEL), number 250/2010.

2.2. Instruments

2.2.1. Questionnaire

A self-reported questionnaire was used to gather information on socio-demographic, clinical characteristics, smoking status and life time suicidal behavior.

2.2.2. Major depressive disorder and nicotine dependence

The diagnoses of major depressive disorder and nicotine dependence were made at interview by a trained clinician using the semistructured DSM-IV interview (SCID), that was translated into Portuguese and validated (Del Ben et al., 2001).

2.2.3. The alcohol, smoking and substance involvement screening test (ASSIST)

The ASSIST is a questionnaire to screen for levels of risk for alcohol, smoking and substance use in adults (World Health Organization (WHO), 2002). ASSIST scores were calculated for all participants. A risk score for alcohol was calculated as low risk (score 0–3), moderate risk (score 4–26) or high risk (score ≥27) (World Health Organization (WHO), 2002).

2.3. Metabolic syndrome

The International Diabetes Federation had generated the previous definitions of metabolic syndrome (visceral obesity, dyslipidemia, hyperglycemia, and hypertension), To assess the presence of metabolic syndrome, an accordance to these criteria, metabolic syndrome is defined by central obesity (waist circumference for ethnic-specific cutoffs) and plus any two or more of the following characteristics: (1) hypertriglyceridemia, serum triglyceride level≥1.69 mmol/L (≥150 mg/dL) or on hypolipidemic agent; (2) low serum high-density lipoprotein cholesterol (HDL-C) level <03 mmol/L (<40 mg/dL) in men or $<.29$ mmol (<50 mg/dL) in women or on hypolipidemic agent; (3) average blood pressure ≥130/85 mm Hg or currently taking antihypertensive medication; (4) high fasting glucose ≥100 mg/dL or on oral antidiabetic

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medication. The waist circumference in Europids origen of ≥94.0 cm for men and of ≥80.0 cm for women as a diagnostic criterion of abdominal obesity (Alberti et al.,2005, 2009). Waist circumference cut-offs for different ethnicity: waist circumference in South Asian and South Americans of ≥90 cm for men and ≥80 cm for women (Alberti et al., 2005; Lear et al., 2010).

2.4. Laboratory assessments

Peripheral blood samples were collected from all participants after 12–14 h overnight fasting. We measured serum concentration of hs-CRP by immunonephelometry system on a BNII analyzer (Siemens ® System BNTMII, Deerfield, IL, USA). ESR was performed by an automatic analyzer (MicroTest1X - Sire Analytical Systems, Udine, Italy). The quantitative determination of fibrinogen in plasma was based on the Clauss method, measuring the rate of fibrinogen to fibrin conversion in the presence of excess thrombin coagulation analyzer, Destiny Plus - Trinity Biotech GmbH, Lemgo, Germany). Evaluation of lipid hydroperoxides was carried out by spectrophotometry using an adaptation of the technique (FOX) described by Jiang et al. (1991). The method is based on oxidation of ferrous to ferric ions by peroxides under acidic conditions, which react with the indicator dye (xylenol orange) and produce a colored complex. The reading was performed in a spectrophotometer (Helios α ThermoSpectronic ®, Waltham, MA, USA) at a wavelength of 560 nm. The levels of nitric oxide metabolites (NO*x*) were assessed by measuring the plasma concentration of nitrite and nitrate, using an adaptation of the technique described by (Navarro-Gonzálvez et al., 1998). The method is based on the reduction of nitrate to nitrite, mediated by oxidation–reduction reactions occurring between the nitrate present in the sample and the system cadmium-copper reagent with subsequent diazotization and colorimetric detection of compound formed by complexation with the Griess reagent at a wavelength of 550 nm in a spectrophotometer (Helios α ThermoSpectronic ®, Waltham, MA, USA). Total plasma antioxidant capacity (TRAP) was measured by chemiluminescence as described by Repetto et al. (1996). The 2,2' azo-bis generates peroxyl radicals rapidly via interaction with carbon-centered radicals and molecular oxygen. These free radicals react with luminol (which acts as an amplifier), producing chemiluminescence. The addition of plasma reduces the chemiluminescence at baseline levels for a period (induction time) proportional to the concentration of plasma antioxidants (TRAP) until free radicals are regenerated, returning to initial levels of chemiluminescence. This experiment was conducted in a Beckman β counter, model LS 6000 (Fullerton, CA, USA) in a non-coincident counting mode for 25 min and with a response range between 300 and 620 nm. The quantification of advanced oxidation protein products (AOPP) in plasma used the method described by Witko-Sarsat et al. (1996). The method is based on the reaction of oxidized proteins with potassium iodide in acid conditions. The absorbance of the reaction was read in a spectrophotometer (Helios α ThermoSpectronic ®, Waltham, MA, USA) at 340 nm. Malondialdehyde (MDA) is a secondary product of lipid peroxidation and was determined using the method described by Jentzch et al. (1996). The method is based on the reaction of thiobarbituric acid (TBA) with MDA forming TBA-MDA and the colorimetric complex formed was measured by a spectrophotometer (Helios α ThermoSpectronic ®, Waltham, MA, USA) at wavelenghts 535 and 572 nm.

The measurement of total cholesterol (TC), HDL-C, triglycerides (TGs) and glucose were performed in an automated biochemical system (Dimension ® RXL, Deerfield, IL, USA). LDL-C was calculated by Friedewald's equation. Plasma insulin levels were determined by MEIA (AXSYM, Abbott® Laboratory, Germany). Total plasma homocysteine levels were measured by chemiluminescent immunoassay (Immulite 2000 Homocysteine, Diagnostic Procedures Corporation, Los Angeles, CA, USA). The inter-assay coefficients of variability for all analytes were less than 10%. Assays with a coefficient of variation $>10\%$ were reanalyzed.

Serum IL-6, and TNF-α levels were measured using commercially available enzyme-linked immunosorbent assay (ELISA) reagents (Becton Dickinson OptEIA, BD Biosciences Pharmingen, San Diego, CA, USA).

2.5. Statistical analyses

Contrasts between groups, i.e. subjects with a history of suicide attempts versus those without, were ascertained by independent sample *t*-tests or Mann–Whitney U tests in case of heterogeneity of variance. Analysis of covariance (ANCOVA) were used to compare the biomarkers between the groups divided according to suicide attempts while adjusting for relevant covariates. Analysis of contingence Tables (Chi-square test) or Fisher's exact probablilty test were used to ascertain the distribution of sociodemographic and clinical measurements in individuals with suicide attempts versus those without.

Univariate comparisons were initially conducted and then variables that were statistically significant were included in multivariate analyses. A binary logistic regression analysis was carried out using patients with suicide attempts compared to those without suicide attempts as the dependent variable.

All analyses were performed using SPSS (Version 20). All tests were 2-tailed and a *p*-value of 0.05 was used for statistical significance.

3. Results

3.1. Socio-demographic and clinical characteristics

Table 1 shows the demographic data and the differences between subjects with and without a history of suicide attempts. Analysis of contingency tables showed that there was a significant association between suicide attempts and female gender. There was no significant association between suicide attempts and either age or ethnicity. Individuals without stable relationships and divorced and widowed individuals showed higher rates of suicide attempts. There was also a significant association between years of education and suicide attempts. Smokers showed a significantly higher prevalence of suicide attempts than non-smokers. There was no significant association between a history of suicide attempts and use of alcohol or metabolic syndrome. Subjects with bipolar depression and anxiety showed higher rates of a history of suicide attempts.

3.2. Metabolic markers

Table 2 shows the differences in metabolic markers between individuals with and without a history of suicide attempts. BMI and waist circumference were significantly higher in subjects with suicide attempts than in those without. There were no significant differences in any of the other variables, including glucose, insulin, cholesterol, either LDL or HDL, and triglycerides.

3.3. Measurements of the inflammatory and O and NS biomarkers

Table 3 shows the measurements of the inflammatory and O&NS biomarkers between individuals with and without a history of suicide attempts. There were no significant differences between both groups in CRP, fibrinogen, ERS, IL-6 and TNFα. Individuals with suicide attempts had significantly higher levels of NO*x* and

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lipid hydroxyperoxides than those without suicidal attempts, whereas TRAP was significantly lower in suicide attempters. Using depression versus non-depression as a covariate in ANCOVAS did not change these results. There were no significant differences in AOPP or MDA between both groups.

3.4. Suicide attempt, clinical characteristics and laboratory measures

We also examined whether there were differences in any of the variables between subjects with 1 or subjects with more than 1 suicide attempt. The results showed that there were no significant differences in any of the demographic data, including mood disorders, anxiety, use of psychopharmacologic drugs, positive family history or smoking, and inflammatory and O&NS biomarkers (Table 4).

3.5. Logistic regression

Table 5 shows the outcome of a binary logistic regression analysis with the groups according to suicide attempts as dependent variable and gender, smoking, unipolar and bipolar depression and inflammatory, O&NS and metabolic biomarkers as

Table 1
Socio-demographic and clinical characteristics by suicide attempt.

Characteristics	Suicide attempt						*p*-value[a]
	No (n=315)		Yes (n=27)		Total		
	n	%	N	%	*n*	%	
Gender							**0.009**
Female	202	(64.1)	24	(88.9)	226	(66.1)	
Male	113	(35.9)	3	(11.1)	116	(33.9)	
Age group							0.610
18–29	15	(4.8)	2	(7.4)	17	(5.0)	
30–39	41	(13.0)	5	(18.5)	46	(13.5)	
40–49	142	(45.1)	13	(48.1)	155	(45.3)	
50–60	117	(37.1)	7	(25.9)	124	(36.3)	
Race							0.373
Caucasian	213	(67.6)	21	(77.8)	234	(68.4)	
Black	35	(11.1)	1	(3.7)	36	(10.5)	
Yellow	16	(5.1)	0	(.0)	16	(4.7)	
Mixed	51	(16.2)	5	(18.5)	56	(16.4)	
Marital status							**0.000**
Single	45	(14.3)	6	(22.2)	51	(14.9)	
Stable relationship	217	(68.9)	9	(33.3)	226	(66.1)	
divorced/separated	48	(15.2)	9	(33.3)	57	(16.7)	
Widow	5	(1.6)	3	(11.1)	8	(2.3)	
Years of education							**0.007**
< =12 years	129	(42.0)	18	(69.2)	147	(44.1)	
> =13 years	178	(58.0)	8	(30.8)	186	(55.9)	
Smoking							**0.000**
No	188	(59.7)	3	(11.1)	191	(55.8)	
Yes	127	(40.3)	24	(88.9)	151	(44.2)	
Alcohol (ASSIST)							0.142
Low risk	292	(93.0)	23	(85.2)	315	(92.4)	
Moderate risk	22	(7.0)	4	(14.8)	26	(7.6)	
Mood disorder							**0.000**
No depression	199	(63.2)	2	(7.4)	201	(58.8)	
Unipolar depression	85	(27.0)	7	(25.9)	92	(26.9)	
Bipolar depression	31	(9.8)	18	(66.7)	49	(14.3)	
Metabolic syndrome							0.183
No	225	(71.4)	16	(59.3)	241	(70.5)	
Yes	90	(28.6)	11	(40.7)	101	(29.5)	
Anxiety							**0.000**
No	191	(60.6)	2	(7.4)	193	(56.4)	
Yes	124	(39.4)	25	(92.6)	149	(43.6)	

[a] The *p*-value is based on the Chi-square statistic.

Table 3
Laboratory measures by suicide attempt.

Laboratory measures	Suicide attempts						*p*-value[b]
	No		Yes		Total		
	Mean	SD	Mean	SD	Mean	SD	
CRP	3.2	(4.4)	3.9	(3.4)	3.3	(4.3)	0.059
Fibrinogen	343.0	(71.0)	356.6	(60.2)	344.1	(70.3)	0.219
ERS	13.5	(11.2)	18.1	(13.5)	13.8	(11.4)	0.109
IL-6[a]	4.8	(12.3)	2.6	(3.7)	4.4	(11.4)	0.152
TNF-alpha[a]	9.3	(15.4)	8.3	(12.7)	9.1	(15.0)	0.131
NO*x*	3.9	(2.3)	5.0	(1.8)	4.0	(2.3)	**0.001**
Lipid hydroperoxides	0.9	(.4)	1.3	(.7)	1.0	(.4)	**0.001**
AOPP	105.1	(43.7)	115.8	(42.2)	106.0	(43.6)	0.111
TRAP	829.8	(135.1)	755.8	(120.6)	824.0	(135.3)	**0.005**
MDA	15.8	(5.8)	15.9	(5.2)	15.8	(5.8)	0.713

Note: All variables except TRAP were not normally distributed.

[a] The mean and SD for IL-6 and TNF-alpha are calculated ***only*** for the smokers and for the other laboratory measurements the full sample (i.e. smokers and non-smokers) was used.

[b] The *p*-value for all the variables except TRAP is based on the Mann–Whitney test and for TRAP it is based on the independent sample *t*-test.

Table 2
Metabolic characteristics by suicide attempt.

Metabolic characteristics	Suicide attempts						*p*-value[a]
	No		Yes		Total		
	Mean	SD	Mean	SD	Mean	SD	
BMI	26.8	(5.0)	29.2	(5.3)	27.0	(5.1)	**0.033**
Systolic	122.2	(19.2)	123.7	(15.5)	122.3	(19.0)	0.659
Diastolic	78.6	(12.7)	82.2	(13.4)	78.9	(12.8)	0.233
Waist circumference	90.3	(12.7)	95.7	(7.4)	90.6	(12.5)	**0.015**
Hip circumference	105.6	(11.5)	109.1	(8.5)	105.8	(11.3)	0.133
Plasma glucose	90.3	(16.9)	90.8	(9.6)	90.3	(16.4)	0.804
Total cholesterol	195.1	(39.7)	199.0	(49.7)	195.4	(40.5)	0.694
HDL	47.6	(14.8)	46.9	(9.7)	47.5	(14.4)	0.719
LDL	121.6	(35.7)	124.9	(44.6)	121.8	(36.4)	0.709
Ratio of total cholesterol/HDL	4.4	(1.5)	4.4	(1.6)	4.4	(1.5)	0.959
Ratio of LDL/HDL	2.8	(1.2)	2.8	(1.3)	2.8	(1.2)	0.980
Triglycerides	128.7	(80.0)	134.0	(63.4)	129.1	(78.8)	0.685
Insulin	9.4	(5.6)	9.6	(3.6)	9.4	(5.5)	0.764
Homocysteine	13.2	(4.8)	13.2	(4.2)	13.2	(4.7)	0.989

[a] The *p*-value is based on the independent sample *t*-test.

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explanatory variables. This regression analysis showed that unipolar and bipolar depression, gender, smoking behavior and lipid hydroperoxides increased the odds of suicide attempts. The combined effects of oxidative stress, smoking, depression, female gender are independent from classical risk factors, including marital status, years of education and anxiety. These factors were not significant in this analysis. Other O&NS biomarkers were marginally significant, e.g. NO*x*, AOPP, and MDA. TRAP, the inflammatory and metabolic biomarkers were not significant in this analysis.

4. Discussion

The first major finding of this study is that O&NS, but not inflammatory and metabolic biomarkers, are related to a history of suicide attempts. The current study demonstrated that patients with suicidal behavior had significantly higher levels of NO*x* (products of nitrates and nitrites), lipid hydroperoxides (a biomarker of oxidative damage to lipids or lipid peroxidation) and lower levels of TRAP (a biomarker of total anti-oxidant defenses) than subjects without a history of suicide attempts. Studies examining the association of O&NS markers with suicidal behavior are limited. One study found that higher concentration of plasma nitric oxide in patients with recurrent depressive disorder was associated with the severity of depressive symptom and cognitive impairment (Talarowska et al., 2012). Our findings on lower levels of antioxidant defenses extent those of a previous study showing lower carotenoid levels in subjects with a history of suicide attempts (Li and Zhang, 2007).

Many pathways may explain the association between O&NS and suicidal behavior. First, O&NS and lowered levels of antioxidants may cause neuroprogression. Data were recently published linking neuroprogression to suicide (Underwood and Arango, 2011). Second, O&NS may cause secondary autoimmune responses damaging neurotransmitters and mounting an autoimmune response to the damaged epitopes (Maes et al., (1996)). There are data linking suicide to central autoimmune reactions directed against dopamine (Bergquist et al., 2002). Third, oxidative stress is a mechanism activating IDO, which in turn may increase the production of tryptophan catabolites (TRYCATs), e.g kynurenine (Maes et al., 2011). Recent data show increased plasma kynurenine levels in suicide attempters (Sublette et al., 2011). Activation of the TRYCAT pathway also causes decreased levels of serotonin (Maes et al., 2011), which is related to suicidal behavior (Malone et al. 2003).

The second major findings is that smoking and bipolar depression are associated with a history of suicide attempts. Our data linking smoking with suicide are consistent with previous reports that have shown that current smoking and depressive disorders are associated with subsequent suicidal behavior (Malone et al., 2003; Breslau et al., 2005; McGee et al., 2005; Hughes et al., 2008; Goodwin et al., (2013)). Person with mental disorders and depression have increased rates of smoking than persons without mental disorders or depression (Lasser et al., 2000). Therefore, it is necessary to consider the cumulative effects of depression and smoking on suicide rates. Doing so, we found that depression, either unipolar or bipolar, and smoking have cumulative effects on suicide attempt rates. Patients with affective disorders, either unipolar or bipolar, have a high risk of subsequent suicide (Lester, 1993). Lester (1993) reported that bipolar disorder is associated with an increased number of suicide attempts (Lester, 1993). Disalver (2006) similarly found a significant correlation

Table 4
Number of suicide attempts by mood disorder, anxiety, drug and smoking history and laboratory measures.

Characteristics	Number of suicide attempts				*p*-value[a]
	1 (*n*=14)		≥2 (*n*=13)		
	n	%	*n*	%	
Bipolar depression	8	(57.1)	10	(76.9)	0.420
Anxiety	13	(92.9)	12	(92.3)	1.000
Psychopharmacologic drugs	5	(35.7)	6	(46.2)	0.704
Family psychiatric history	8	(61.5)	7	(53.8)	0.999
Smoking	12	(85.7)	12	(92.3)	1.000
Laboratory measures	**Mean**	**SD**	**Mean**	**SD**	***p*-value[c]**
CRP	3.8	(4.3)	4.1	(2.2)	0.374
IL-6[b]	3.9	(4.8)	1.2	(.6)	0.251
TNF-alpha[b]	8.7	(15.9)	7.9	(8.9)	0.809
NOx	5.1	(1.9)	4.8	(1.8)	0.560
Lipid hydroperoxides	1.2	(.5)	1.5	(.9)	0.430
AOPP	110.6	(31.8)	120.9	(51.5)	0.960
TRAP	746.6	(124.3)	765.6	(120.7)	0.685
MDA	15.6	(4.2)	16.2	(6.3)	0.519
Fibrinogen	353.8	(60.3)	359.7	(62.5)	0.999
ERS	16.2	(11.2)	20.7	(16.2)	0.483

[a] The *p*-value is based on the Fisher Exact Probability test and tests for the Chi-square test between the listed groups the number of suicide attempts and the other characteristics.

[b] The mean and SD for IL-6 and TNF-alpha are calculated ***only*** for the smokers and for the other laboratory measurements the full sample (i.e. smokers and non-smokers) was used.

[c] The *p*-value for the laboratory measures is based on the Mann–Whitney test.

Table 5
Logistic regression analysis for odds of suicide attempt.

	Variables	OR	95% CI		*p*-value
			Lower	Upper	
Gender[a]	Female	6.66	1.09	40.72	**0.040**
Smoking[a]	Smoker	6.59	1.40	31.12	**0.017**
Mood disorder[a]	Unipolar depression	9.79	1.52	63.01	**0.016**
	Bipolar depression	45.68	7.18	290.46	**0.000**
Oxidative stress markers	CRP	0.96	0.82	1.11	0.566
	Nox	1.25	0.97	1.60	0.083
	Lipid hydroperoxides	5.19	1.68	15.98	**0.004**
	AOPP	1.01	1.00	1.03	0.054
	TRAP	1.00	0.99	1.00	0.240
	MDA	0.93	0.84	1.01	0.099
	Fibrinogen	1.00	0.98	1.01	0.429

Note: Other variables like metabolic syndrome, anxiety, BMI, years of education, marital status, etc. were initially introduced into the model but they were not statistically.

[a] Reference groups: Gender—male; smoking—non-smoker; mood disorder—no depression.

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between bipolarity and suicidal ideation among a sample of 311 adolescents (Disalver, 2006).

A third important finding is that unipolar and bipolar depression, gender, smoking behavior and lipid hydroperoxides have cumulative effects predicting a history of suicide attempts. Moreover, the combined effects of oxidative stress, smoking, depression, female gender are independent of the effects of classical risk factors, including marital status, years of education and alcohol abuse. The latter well-known predictors of suicide risk were not significant or no longer significant after considering the effects of depression, oxidative stress and smoking. This could indicate that the effects of classical social predictors are mediated via depression, smoking or oxidative stress. Indeed, psychological stress and psychosocial stress may cause increased reactive oxygen species, O&NS and damage to lipids and DNA (Maes et al., 2011).

The fourth major finding of this study is that a lifetime history of suicide attempts is not related to the presence of metabolic syndrome. On the other hand, we found significantly higher values of BMI and abdominal circumference in individuals with suicide attempts. Since most persons with metabolic syndrome have abdominal obesity, these data could suggest a relationship between a history of suicide attempts and metabolic syndrome (Alberti et al., 2009). However, after introducing other variables, including O&NS biomarkers, in multivariate analyses these associations disappeared. It is important to analyze these data with mutivariate analyses because the variables may be interrelated, e.g. depression, smoking, suicide, inflammation and O&NS. For example, metabolic syndrome is more frequent in current smokers than in those who have never smoked (Chen et al., 2008). There was a weak association between depression and low high-density lipoprotein cholesterol but not with other component criteria of the metabolic syndrome (Foley et al., 2010). In addition, there are some studies regarding the relationship between cholesterol and depression and suicide. Some studies found that subjects with both obesity and the metabolic syndrome had more depressive symptoms than those without (Capuron et al., 2008; Williams et al., 2009). In both men and women, the metabolic syndrome was associated with an increased prevalence of depression (Skilton et al., (2007)) and the prevalence of the metabolic syndrome in patients with depression is high (Kozumplik and Uzun, 2011). There was a weak association between depression and low high-density lipoprotein cholesterol but not with other component criteria of the metabolic syndrome (Foley et al., 2010).

Previous studies found that serum HDL-C levels were significantly lower in depressed men who had at some time made serious suicidal attempts than in those without such suicidal behavior (Maes et al., 1997). There are also studies showing that suicidal ideation is associated with lower serum cholesterol and HDL-cholesterol levels (Kim and Myint, 2004; Rabe-Jabłońska and Poprawska (2000)). There was also a weak association between depression and low HDL-cholesterol (Foley et al., 2010). Nevertheless, other studies have not found significant differences in serum cholesterol, HDL-cholesterol, LDL-cholesterol and triglycerides between patients who had attempted suicide and those who had not. Low serum cholesterol levels did not predict subsequent suicide (Fiedorowicz and Coryell, 2007). Given these conflicting data between low serum cholesterol levels and suicide, researchers have postulated that there may be a relationship between altered lipid metabolism and changes in serotonin functioning (Sansone, 2008).

Finally, our findings that suicidal attempts are not related to inflammation contrasts some previous findings. Those people with major depressive disorders who were suicidal had significantly lower IL-2 compared with non-suicidal patients and normal controls Non-suicidal with major depressive disorders participants had significantly higher IL-6 production than suicidal with major depressive disorders patients and normal controls (Kim et al., 2008). Levels of high-sensitivity C-reactive protein (CRP), interleukin -6 (IL-6), and soluble receptor tumor-necrosis-factor alpha (TNF-α) have been reported to be significantly higher in twins with metabolic syndrome, free of symptomatic cardiovascular disease. Adjusting for these associations is also important for inflammatory biomarkers. For example, after adjusting for the effects of metabolic syndrome, the relationships between severity of depression and inflammatory biomarkers, including CRP and IL-6, remained significant even after adjusting for age, education, and current smoking (Capuron et al., 2008).

There are several strengths in our study: (a) many potential confounders were included in our study, such as classical risk factors for suicide and putative risk factors, such as metabolic syndrome, BMI, age, and sex; and (b) by using multivariate statistical analyses we have controlled for the cumulative effects of important risk factors, such as smoking, bipolar depression and oxidative stress. Limitations of this study are: (a) all participants were recruited from the center for smoking cessation treatment, while it is known that women tend to seek more assistance for smoking cessation than men. (b) The inflammatory, O&NS and metabolic biomarkers were assessed at baseline and correlated with a history of attempted suicide. Obviously, in such analyses only trait markers may come out significantly, whereas state markers may not be significant in the analyses. This may perhaps explain the lack of correlation between suicide attempts and the inflammatory biomarkers, which are more state-related than O&NS damage biomarkers. For example, Suchankova et al., (2012) reported a significant association between suicide attempts and a polymorphism located in the CRP gene, i.e. +1444C allele. (c) Finally, many factors undoubtedly contribute to the development of suicidal behavior including inherited and environmental factors. Not all of these factors are known. Therefore, our results can only delineate associations and not causality.

In conclusion, these results show that there is a positive association between O&NS biomarkers, i.e. lipid peroxidation, nitrosative stress and lowered total antioxidant levels, and a history of suicide attempts. In addition, we found that smoking behavior, bipolar depression and O&NS independently from each other are associated with a history of suicidal attempts. No associations were found between the latter and inflammatory or metabolic biomarkers.

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Conflict of interest

Seetal Dodd

Grant/Research Support: Stanley Medical Research Institute, NHMRC, Beyond Blue, ARHRF, Simons Foundation, Geelong Medical Research Foundation, Eli Lilly, Glaxo SmithKline, Organon, Mayne Pharma, Servier.

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References

Alberti, K.G., Zimmet, P., Shaw, J., 2005. The metabolic syndrome—a new worldwide definition. Lancet 366 (9491), 1059–1062.

Alberti, K.G., Eckel, R.H., Grundy, S.M., Zimmet, P.Z., Cleeman, J.I., Donato, K.A., Fruchart, J.C., James, W.P., Loria, C.M., Smith Jr., S.C., 2009. Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. Circulation 120 (16), 1640–1645.

Almeida, O.P., Norman, P., Hankey, G.J., Jamrozik, K., Flicker, L., 2007. The association between C-reactive protein concentration and depression in later life is due to poor physical health: results from the Health in Men Study (HIMS). Psychological Medicine 37, 1775–1786.

Bergquist, J., Träskman-Bendz, L., Lindström, M.B., Ekman, R., 2002. Suicide-attempters having immunoglobulin G with affinity for dopamine in cerebrospinal fluid. European Neuropsychopharmacology 12, 153–158.

Berk, M., Kapczinski, F., Andreazza, A.C., Dean, O.M., Giorlando, F., Maes, M., Yucel, M., Gama, C.S., Dodd, S., Dean, B., Magalhães, P.V.S., Amminger, P., McGorry, P., Malhi, G.S., 2011. Pathways underlying neuroprogression in bipolar disorder: focus on inflammation, oxidative stress and neurotrophic factors. Neuroscience and Biobehavioral Reviews 35 (3), 804–817.

Breslau, N., Schultz, L.R., Johnson, E.O., Peterson, E.L., Davis, G.C., 2005. Smoking and the risk of suicidal behavior: a prospective study of a community sample. Archives of General Psychiatry 62 (3), 328–334.

Bronisch, T., Höfler, M., Lieb, R., 2008. Smoking predicts suicidality: findings from a prospective community study. Journal of Affective Disorders 108, 135–145.

Burke, A., Fitzgerald, G.A., 2003. Oxidative stress and smoking-induced vascular injury. Progress in Cardiovascular Diseases 46 (1), 79–90.

Capuron, L., Su, S., Miller, A.H., Bremner, J.D., Goldberg, J., Vogt, G.J., Maisano, C., Jones, L., Murrah, N.V., Vaccarino, V., 2008. Depressive symptoms and metabolic syndrome: is inflammation the underlying link? Biological Psychiatry 64 (10), 896–900.

Chen, C-C., Li, T.C., Chang, P-C., Liu, C.S., Lin, W-Y., Wu, M-T., Li, C-I., Lai, M-M., Lin, C-C., 2008. Association among cigarette smoking, metabolic syndrome, and its individual components: the metabolic syndrome study in Taiwan. Metabolism 57, 544–548.

Danner, M., Kasl, S.V., Abramson, J.L., Vaccarino, V., 2003. Association between depression and elevated C-reactive protein. Psychosomatic Medicine 65, 347–356.

Del Ben, C.M., Vilela, J.A.A, Crippa, J.A.S., Hallak, J.E.C., Labate, C.M., Zuardi, A.W., 2001. Confiabilidade da "Entrevista Clínica Estruturada para o D.S.M.–IV"—versão clínica traduzida para o português. Revista Brasileira de Psiquiatria 23 (3), 156–159.

Elovainio, M., Aalto, A.M., Kivimäki, M., Pirkola, S., Sundvall, J., Lönnqvist, J., Reunanen, A., 2009. Depression and C-reactive protein: population-based Health 2000 study. Psychosomatic Medicine 71, 423–430.

Facchini, F.S., Hollenbeck, C.B., Jeppesen, J., Chen, Y.D., Reaven, G.M., 1992. Insulin resistance and cigarette smoking. Lancet 339 1128–30.

Fiedorowicz, J.G., Coryell, W.H., 2007. Cholesterol and suicide attempts: a prospective study of depressed inpatients. Psychiatric Research 152 (1), 11–20.

Foley, D.L., Morley, K.I., Madden, P.A., Heath, A.C., Whitfield, J.B., Martin, NG., 2010. Major depression and the metabolic syndrome. Twin Research and Human Genetics 13 (4) 347–58.

Ford, D.E., Erlinger, T.P., 2004. Depression and C-reactive protein in US adults: data from the Third National Health and Nutrition Examination Survey. Archives of Internal Medicine 164, 1010–1014.

Forlenza, M.J., Miller, G.E., 2006. Increased serum levels of 8-hydroxy-2'-deoxyguanosine in clinical depression. Psychosomatic Medicine 68 (1), 1–7.

Goodwin, R.D, Prescott, M.R., Tamburrino, M., Calabrese, J.R., Liberzon, I., Galea, S., 2013. Cigarette smoking and subsequent risk of suicidal ideation among National Guard Soldiers. Journal of Affective Disorders 145, 111–114.

Hamer, M., Molloy, G.J., de Oliveira, Demakakos, P., 2009. Persistent depressive symptomatology and inflammation: to what extent do health behaviours and weight control mediate this relationship? Brain Behav. Immun. 23, 413–418.

Hughes, J.R., 2008. Smoking and suicide: a brief overview. Drug and Alcohol Dependence 98, 169–178.

Jentzch, A.M., Bachman, H., Fürst, P., Biesalski, H.K., 1996. Improved analysis of malondialdehyde in human body fluids. Free Radical Biology and Medicine 20, 251–256.

Jiang, Z., Woollard, A.C.S., Wolff, S.P., 1991. Lipid hydroperoxide measurement by oxidation of Fe^{2+} in the presence of xylenol orange. Comparison with the TBA assay and an iodometric method. Lipids 26 (10), 353–356.

Kim, Y.K., Myint, A.M., 2004. Clinical application of low serum cholesterol as an indicator for suicide risk in major depression. Journal of Affective Disorders 81, 161–166.

Kim, Y.K., Lee, S.W., Kim, S.H., Shim, S.H., Han, S.W., Choi, S.H., Li, B.H., 2007. Differences in cytokines between non-suicidal patients and suicidal patients in major depression. Progress in Neuro-Psychopharmacology and Biological Psychiatry 32, 356–361.

Kim Y.K., Lee S-W., Kim S-H., Shim S-H., Han S-W., Choi S-H., Lee B-H., 2008. Differences in cytokines between non-suicidal patients and suicidal patients in major depression. Progress in Neuro-Psychopharmacology & Biological Psychiatry 32, 356–361.

Kozumplik, O., Uzun, S., 2011. Metabolic syndrome in patients with depressive disorder-features of comorbidity. Psychiatria Danubina 23 (1), 84–88.

Lasser, K., Boyd, J.W., Woolhandler, S., Himmelstein, D.U., McCormick, D., Bor, D.H., 2000. Smoking and mental illness: a population-based prevalence study. JAMA 284 (20), 2606–2610.

Lear, S.A., James, P.T., Ko, G.T., Kumanyik, S., 2010. Appropriateness of waist circumference and waist-to-hip ratio cutoffs for different ethnic groups. European Journal of Clinical Nutrition 64, 42–61.

Leonard, L., Maes, M., 2012. Mechanistic explanations how cell-mediated immune activation, inflammation and oxidative and nitrosative stress pathways and their sequels and concomitants play a role in the pathophsyology of unipolar depression. Neuroscience and Biobehavioral Reviews 36 (12), 764–785.

Lester, D., 1993. Suicidal behavior in bipolar and bipolar affective disorders: a meta-analysis. Journal of Affective Disorders 27, 117–121.

Li, Y., Zhang, J., 2007. Serum concentrations of antioxidant vitamins and carotenoids are low in individuals with a history of attempted suicide. Nutritional Neuroscience 10 (1–2), 51–58.

Liukkonen, T., Silvennoinen-Kassinen, S., Jokelainen, J., Räsänen, P., Leinonen, M., Meyer-Rochow, V.B., Timonen, M., 2006. The association between C-reactive protein levels and depression: results from the northern Finland 1966 birth cohort study. Biological Psychiatry 60, 825–830.

Maes, M., Bosmans, E., Ranjan, R., Vandoolaeghe, E., Meltzer, H.Y., De Ley, M., Berghmans, R., Stans, G., Desnyder, R., 1996. Lower plasma CC16, a natural anti-inflammatory protein, and increased plasma interleukin-1 receptor antagonist in schizophrenia: effects of antipsychotic drugs. Schizophrenia Research 21 (1), 39–50.

Maes, M., Smith, R., Christophe, A., Vandoolaeghe, E., Van Gastel, A., Neels, H., Demedts, P., Wauters, A., Meltzer, H.Y., 1997. Lower serum high-density lipoprotein cholesterol (HDL-C) in major depression and in depressed men with serious suicidal attempts: relationship with immune-inflammatory markers. Acta Psychiatrica Scandinavica 95 (3), 212–221.

Maes, M., Chang, Y.S., Galecki, P., Berk, M., 2011. A review on the oxidative and nitrosative stress (O&NS) pathways in major depression and their possible contribution to the (neuro)degenerative processes in that illness. Progress in Neuro-Psychopharmacology and Biological Psychiatry 35, 676–692.

Malone, K.M., Waternaux, C., Haas, G.L., Cooper, T.B., Li, S., Mann, J.J., 2003. Cigarette smoking, suicidal behavior, and serotonin function in major psychiatric disorders. American Journal of Psychiatry 160, 773–779.

McGee, R., Williams, S., Nada-Raja, S., 2005. Is cigarette smoking associated with suicidal ideation among young people? American Journal of Psychiatry 162 (3) 619–20.

Mendlovic, S., Mozes, E., Eilat, E., Doron, A., Lereya, J., Zakuth, V., Spirer, Z., 1999. Immune activation in non-treated suicidal major depression. Immunology Letters 67 (2), 105–108.

Miller, A.H., Maletic, V., Raison, C.L., 2009. Inflammation and its discontents: the role of cytokines in the pathophysiology of major depression. Biological Psychiatry 65, 732–741.

Moylan, S., Maes, M., Wray, N.R., Berk, M., 2012. The neuroprogressive nature of major depressive disorder: pathways to disease evolution and resistance, and therapeutic implications. Molecular Psychiatry.

Nässberger, L., Träskman-Bendz, L., 1993. Increased soluble interleukin-2 receptor concentrations in suicide attempters. Acta Psychiatrica Scandinavica 88 (1), 48–52.

Navarro-Gonzálvez, J.A., García-Benayas, C., Arenas, J., 1998. Semiautomated measurement of nitrate in biological fluids. Clinical Chemistry 44, 679–681.

Nunes, S.O.V., Vargas, H.O., Brum, J., Prado, E., Vargas, M.M., de Castro, M.R.P., Dodd, S., Berk, M., 2012. A comparison of inflammatory markers in depressed and non-depressed smokers. Nicotine and Tobacco Research 14, 540–546.

Pasco, J.A., Williams, L.J., Jacka, F.N., Ng, F., Henry, M.J., Nicholson, G.C., Kotowicz, M. A., Berk, M., 2008. Tobacco smoking as a risk factor for major depressive disorder: population-based study. British Journal of Psychiatry 193, 322–326.

Rabe-Jabłońska, J., Poprawska, I., 2000. Levels of serum total cholesterol and LDL-cholesterol in patients with major depression in acute period and remission. Medical Science Monitor 6 (3), 539–547.

Raison, C.L., Capuron, L., Miller, A.H., 2006. Cytokines sing the blues: inflammation and the pathogenesis of depression. Trends in Immunology 27, 24–31.

Repetto, M., Reides, C., Gomez Carretero, M.L., Costa, M., Griemberg, G., Llesuy, S., 1996. Oxidative stress in blood of HIV infected patients. Clinica Chimica Acta 255, 107–117.

Rytilä, P., Rehn, T., Ilumets, H., Rouhos, A., Sovijärvi, A., Myllärniemi, M., Kinnula, V.I., 2006. Increased oxidative stress in asymptomatic current chronic smokers and GOLD stage 0 COPD. Respiratory Research 7, 69.

Sansone, R.A., 2008. Cholesterol quandaries. Psychiatry 5 (3), 22–34.

Skilton, M.R., Moulin, P., Terra, J.L., Bonnet, F., 2007. Associations between anxiety, depression, and the metabolic syndrome. Biological Psychiatry 62 (11), 1251–1257.

Sublette, M.E., Galfalvy, H.C., Fuchs, D., Lapidus, M., Grunebaum, M.F., Oquendo, M. A., John Mann, J., Postolache, T.T., 2011. Plasma kynurenine levels are elevated

in suicide attempters with major depressive disorder. Brain, Behavior, and Immunity 25, 1272–1278.
Talarowska, M., Gałecki, P., Maes, M., Orzechowska, A., Chamielec, M., Bartosz, G., Kowalczyk, E., 2012. Nitric oxide plasma concentration associated with cognitive impairment inpatients with recurrent depressive disorder. Neuroscience Letters 510, 127–131.
Underwood, M.D., Arango, V., 2011. Evidence for neurodegeneration and neuroplasticity as part of the neurobiology of suicide. Biological Psychiatry 70, 306–307.
Williams, L.J., Pasco, J.A., Henry, M.J., Jacka, F.N., Dodd, S., Nicholson, G.C., Kotowicz, M.A., Berk, M., 2009. Lifetime psychiatric disorders and body composition: a population-based study. Journal of Affective Disorders 118 (1–3) 173–9.
Witko-Sarsat, V., Friedlander, M., Capeillere-Blandin, C, Nguyen-Khoa, C., Nguyen, T., Zingraff, A.T., Jungers, J., Deschamps-Latscha B., P., 1996. Advanced oxidation protein products as a novel marker of oxidative stress in uremia. Kidney International 4, 1304–1313.
World Health Organization (WHO), 2002. The alcohol, smoking and substance involvement screening test (ASSIST): development, reliability and feasibility. Addiction 97 (9), 1183–1194.

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6 CONCLUSÕES E CONSIDERAÇÕES FINAIS

Os resultados dos artigos demonstraram que fumantes depressivos apresentavam níveis maiores de NOx, fibrinogênio, PCR, AOPP, VHS e menores níveis de TRAP, quando comparados ao grupo de indivíduos que não apresentavam transtornos depressivos e nunca fumaram. Foram verificadas, ainda, alterações significativas nas concentrações do NOx, lipídios hidroperóxidos, AOPP, TRAP e fibrinogênio nos fumantes depressivos quando comparados aos outros grupos. Foi demonstrado também que os indivíduos com transtornos depressivos e transtornos por uso do tabaco apresentavam maior incapacitação para o trabalho, com maior gravidade dos sintomas depressivos, assim como maior risco para tentativas de suicídios, quando comparados aos demais grupos. As alterações no estresse oxidativo e nos marcadores inflamatórios permaneceram significativas, após os dados serem ajustados pelo gênero, idade, anos de educação, incapacidade para o trabalho e pelas medidas laboratoriais. Indivíduos com histórico de tentativas de suicídio tinham níveis significativamente mais elevados de NOx e de hidroperóxidos lipídicos e baixo TRAP, em comparação com outros sem tentativas de suicídio. Não houve associação significativa entre a história de tentativas de suicídio e biomarcadores inflamatórios e metabólicos e síndrome metabólica. A regressão logística mostrou que tanto o transtorno depressivo unipolar como o bipolar, no sexo feminino, o comportamento de fumar e hidroperóxidos lipídicos foram significativamente associados com um histórico de tentativas de suicídio. Os efeitos combinados do estresse oxidativo, tabagismo e depressão, no sexo feminino, eram independentes de fatores de risco clássicos, incluindo estado civil, anos de escolaridade e ansiedade.

Pacientes com comportamento suicida tiveram níveis significativamente mais elevados de NOx, hidroperóxidos lipídicos e menores níveis de TRAP do que pacientes sem um histórico de tentativas de suicídio.

Nossos dados estão de acordo com outros estudos que demonstram a associação dos marcadores de estresse oxidativo e da atividade inflamatória nos processos depressivos e que podem ser agravados pela comorbidade com o transtorno por uso de tabaco. Estes achados ajudam a entender melhor a associação de enfermidades comuns em grande parte dos pacientes e abre caminho para o desenvolvimento de novas terapêuticas efetivas e com melhor resolutibilidade

para o transtorno depressivo e para o transtorno por uso de tabaco, reduzindo a morbidade e a mortalidade.

Para nosso sistema de saúde é importante ressaltar que fumantes depressivos são muito comuns na prática clínica, e o desconhecimento de que ambas as enfermidades interagem pode piorar o prognóstico e contribuir para uma desordem neurodegenerativa. Desta forma, é indispensável para o clinico tratar ambas as comorbidades precocemente.

REFERÊNCIAS

1. Ezzati M, Lopez AD. Estimates of global mortality attributable to smoking 2000. Lancet. 2003 Sept;362(9387):847-52.

2. Centers for Disease Control and Prevention (CDC). Cigarette smoking among adults -United States, 2007. MMWR Morb Mortal Wkly Rep. 2008;57(45):1221-26.

3. World Health Organization. WHO report on the global tobacco epidemic, 2008: the MPOWER package. Geneva: World Health Organization; 2008.

4. American Psychiatric Association. Diagnostic and statical manual of mental disorders: DSM-5. 5th ed. Washington: American Psychiatric Pushing; 2013.

5. Kessler RC, Birnbaum HG, Shahly V, Bromet E, Hwang I, McLaghlin KA, et al. Age differences in the prevalence and comorbidity of DSM-IV major depressive episodes: results from the WHO World Mental Health Survey Initiative. Depress Anxiety. 2010;27(4):351-64.

6. Lopez AD, Mathers CD, EzzatI M, Jamison DT, Murray CJL. Global and regional burden of disease and risk factors, 2001: systematic analysis of population health data. Lancet. 2006;367(9524):1747-57.

7. Maes M, Yirmyia R, Noraberg J, Brene S, Hibbeln J, Perini G. The inflammatory & neurodegenerative (I&ND) hypothesis of depression: leads for future research and new drug developments in depression. Metab Brain Dis. 2009;24(1):27-53.

8. Maes M, Galecki P, Chang YS, Berk M. A review on the oxidative and nitrosative stress (O&NS) pathways in major depression and their possible contribution to the neurodegenerative process in the illness. Prog Neuropsychopharmacol Biol Psychiatry. 2011;35(3):676-92.

9. Berk M, Kapczinski F, Andreazza AC, Dean OM, Giorlando F, Maes M, et al. Pathways underlying neuroprogression in bipolar disorder: focus on inflammation, oxidative stress and neurotrophic factors. Neurosci Biobehav Rev. 2011 Jan;35(3):804-17.

10. Pasco JA, Nicholson GC, Ng F, Henry MJ, Williams LJ, Kotowicz MA, et al. Oxidative stress may be a common mechanism linking major depression and osteoporosis. Acta Psychiatr. 2008 June;20(3):112-16.

11. Stahl SM. Stahl`s essential psychopharmacology. Neuroscientific basis and practical applications. 4th ed. New York: Cambridge University Press; 2013.

12. Jamal M, Willem Van der Does AJ, Cuijpers P, Penninx BWJH. Association of smoking and nicotine dependence with severity and course of symptoms in patients with depressive or anxiety disorder. Drug Alcohol Depend. 2012; 126(1-2):138-46.

13. Pasco JA, Williams LJ, Jacka FN, Ng F, Henry MJ, Nicholson GC, et al. Tobacco smoking as a risk factor for major depressive disorder: population-based study. Br J Psychiatry. 2008;193(4):322-26.

14. Leventhal AM, Kahler CW, Ray LA, Zimmerman M. Refining the depression-nicotine dependence link: patterns of depressive symptoms in psychiatric outpatients with current, past, and no history of nicotine dependence. Addict Behav. 2009;34(3):297-03.

15. Dodd S, Brnabic AJ, Berk L, Fitzgerald PB, Decastella AR, Filia S, et al. A prospective study of the impact of smoking on outcomes in bipolar and schizoaffective disorder. Compr Psychiatry. 2010;51(5):504-9.

16. Castro MRP, Matsuo T, Nunes SOV. Características clínicas e qualidade de vida de fumantes em um centro de referência de abordagem e tratamento do tabagismo. J Bras Pneumol. 2010;36(1):67-74.

17. Sies H. Oxidative stress: from basic research to clinical application. Am J Med. 1991;91(3 Suppl 3):S31-5.

18. Kapczinski F, Dias VV, Kauer-Sant'Anna M, Frey BN, Grassi-Oliveira R, Colom F, et al. Clinical implications of staging model for bipolar disorders. Expert Rev Neurother. 2009;9(7):957-66.

19. Halliwell B. Oxidative stress and neurodegeneration: where are we now? J. Neurochem. 2006;97(6):1634-58.

20. Yanbaeva D, Dentener M, Creutzberg E, Wessling G, Wounters E. Systemic effects of smoking. Chest. 2007;135(5):1557-66.

21. Vargas HO, Nunes SOV, Castro MRP, Vargas MM, Barbosa DS, Bortolasci CC, et al. Oxidative stress and inflammatory markers are associated with depression and nicotine dependence. Neurosci Lett. 2013 June;544:136-40.

22. Organização Mundial de Saude. Classificação de Transtornos Mentais e de Comportamento da CID-10. Porto Alegre: Artes Médicas; 1993.

23. Centers for Disease Control and Prevention (CDC). Smoking attributable mortality, years of potential life lost and productivity losses - United States, 2000-2004. MMWR Morb Mortal Wkly Rep. 2008;57(45):1226-28.

24. Murphy JM, Monson RR, Olivier DC, Sobol AM, Leighton AH. Affective disorders and mortality: a general population study. Arch Gen Psychiatry. 1987 May;44(5):473–80.

25. Murphy J, Horton NJ, Monson RR, Laird N, Leighton AH. Historical trends from the stirling county study. Am J Psychiatry. 2003;160:1663–69

26. Hanna EZ, Yi HY, Dufour MC, Whitmore CC. The relationship of early-onset regular smoking to alcohol use, depression, illicit drug use, and other risky behaviors during early adolescence:results from the Youth Supplement to the

Third National Health and Nutrition Examination Survey. J Subst Abuse. 2001 Oct.;13(3):265–82.

27. Allgower AN, Wardle J, Steptoe A. Depressive symptoms, socialsupport, and personal health behaviors in young men andwomen. Health Psychol. 2001 May;20(3):223–27.

28. Nunes SOV, Vargas HO, Prado E, Barbosa DS, Melo LP, Moylan S, et al. The shared role of oxidative stress and inflammation in major depressive disorder and nicotine dependence. Neurosc Biobehav Rev. 2013 Sept;37(8):1336-345.

29. Rytilä P, Rehn T, Ilumets H, Rouhos A, Sovijärvi A, Myllärniemi M, et al. Increased oxidative stress in asymptomatic current chronic smokers and GOLD stage 0 COPD. Respir Res. 2006 Apr;7:69.

30. Lasser K, Boyd JW, Woolhandler S, Himmelstein DU, McCormick D, Bor DH. Smoking and mental illness: a population-based prevalence study. JAMA. 2000;284(20):2606-10.

31. Berlin I, Covey L, Glassman A. Smoking and depression: a co-morbidity. J Dual Diagnosis. 2009;5(2):149-58.

32. Boden JM, Fergusson DM, Horwood J. Cigarette smoking and depression: tests of causal linkages using a longitudinal birth cohort. Br J Psychiatr. 2010;196:440–46.

33. Ziedonis D, Hitsman B, Beckham JC, Zvolensky M, Adler LE, Audrain-McGovern J, et al. Tobacco use and cessation in psychiatric disorders: National Institute of Mental Health report. Nicotine Tob Res. 2008;10(12):1691-715.

34. Klungsoyr O, Nygar JF, Sorense T, Sandanger I. Cigarette smoking and incidence of first depressive episode: an 11-Year, population-based follow-up Study. Am J Epidemiol. 2006 Mar;163(5):421-32.

35. Glassman AH, Helzer JE, Covey LS, Cottler LB, Stetner F, Tipp JE, et al. Smoking, smoking cessation, and major depression. JAMA 1990 Sept; 264(12):1546–549.

36. Khantzian EJ. The self-medication hypothesis of substance use disorders: a reconsideration and recent applications. Harv Rev Psychiatry. 1997;4(5):231–44.

37. Kendler KS, Neale MC, Maclean CJ, Heath AC, Eaves LJ, Kessler C. Smoking and major depression: a causal analysis. Arch Gen Psychiatry. 1993 Jan;50(1):36–43.

38. Tsuang MT, Francis T, Minor K, Thomas A, Stone WS. Genetics of smoking and depression. Hum Genet. 2012 June;131(6):905-15.

39. Benowitz NL. Nicotine addiction. N Engl J Med. 2010;362(24):2295-303.

40. Malhi GS, Berk M. Does dopamine dysfunction drive depression? Acta Psychiatr Scand Suppl. 2007;(433):116-24.

41. Berk M, Plein H, Ferreira D. Platelet glutamate receptor supersensitivity in major depressive disorder. Clin Neuropharmacol. 2001;24(3):129-32.

42. Benowitz NL. Clinical pharmacology of nicotine: implications for understanding, preventing, and treating tobacco addiction. Clin Pharmacol Ther. 2008 Apr;83(4):531-41.

43. Dani JA, Bertrand D. Nicotinic acetylcholine receptors and nicotinic cholinergic mechanisms of the central nervous system. Annu Rev Pharmacol Toxicol. 2007;47:699-729.

44. Lambert G, Johansson M, Agren H, Friberg P. Reduced brain norepinephrine and dopamine release in treatment-refractory depressive illness: evidence in support of the catecholamine hypothesis of mood disorders. Arch Gen Psychiatry. 2000 Aug;57(8):787-93.

45. Danovitch I. The clinical assessment and treatment of nicotine dependence. Focus. 2011;9(1):15-24.

46. Koob GF, Volkow ND. Neurocircuitry of Addiction. Neuropsychopharmacol. 2010;35:217–38.

47. Brady K, Sinha R. Co-occurring Mental and substance use disorders: the neurobiological effects of chronic stress. Am J Psychiatry. 2005;162(8):1483-493.

48. Nemeroff CB, Vale WW. The neurobiology of depression: in roads to treatment and new drug discovery. J Clin Psychiatr. 2005;66(Suppl 7):5-13.

49. Bruijnzeel AW. Tobacco addiction and the dysregulation of brain stress systems. Neurosci Biobehav Rev. 2012 May;36(5):1418-441.

50. Rendu F, Peoc'h K, Berlin I, Thomas D, Launay JM. Smoking related diseases: the central role of monoamine oxidase. Int J Environ Res Public Health. 2011;8(1):136-47.

51. Talhout R, Opperhuizen A, van Amsterdam JG. Role of acetaldehyde in tobacco smoke addiction. Eur Neuropsychopharmacol. 2007 Oct;17(10):627-36.

52. Caspi A, Sugden K, Moffitt TE, Taylor A, Craig IW, Harrington H, et al. Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. Science. 2003 July;301(5631):386-89.

53. Watanabe MAE, Nunes SOV, Amarante MK, Guembarovski RL, Oda JM, Lima KWAD, et al. Genetic polymorphism of serotonin transporter 5-HTTLPR: involvement in smoking behaviour. J Genet. 2011 Apr;90(1):179-85.

54. Malone KM, Waternaux C, Haas GL, Cooper TB, Li S, Mann JJ. Cigarette smoking, suicidal behavior, and serotonin function in major psychiatric disorders. Am J Psychiatry. 2003;160(4):773-79.

55. Breslau N, Novak SP, Kessler RC. Psychiatric disorders and stages of smoking. Biol Psychiatry. 2004 Jan;55(1):69–76.

56. Nunes SOV, Castro MRP, Vargas HO, Vargas MM, Bueno RRM, Fonseca ICB, et al. Clinical Characteristics and Smoking Cessation: An Analysis of Sex and Depressive Disorders Differences. Addict Disord Their Treat. 2013b;12(3):158-65.

57. Nunes SOV, Vargas HO, Brum J, Prado E, Vargas MM, Castro MRP, et al. A comparison of inflammatory markers in depressed and nondepressed smokers. Nicotine Tob Res. 2012 maY;14(5):540-46.

58. Danner M, Kasl SV, Abramson JL, Vaccarino V. Association between depression and elevated C-reactive protein. Psychosom Med. 2003 May/June; 65(3):347-56.

59. Elovainio M, Aalto AM, Kivimäki M, Pirkola S, Sundvall J, Lönnqvist J, et al. Depression and C-reactive protein: population-based Health 2000 Study. Psychosom Med. 2009;71(4):423-30.

60. Liukkonen T, Silvennoinen-Kassinen S, Jokelainen J, Räsänen P, Leinonen M, Meyer-Rochow VB, et al. The association between C-reactive protein levels and depression: results from the northern Finland 1966 birth cohort study. Biol Psychiatry. 2006 Oct;60(8):825-30.

61. Almeida OP, Norman P, Hankey GJ, Jamrozik K, Flicker L. The association between C-reactive protein concentration and depression in later life is due to poor physical health: results from the Health in Men Study (HIMS). Psychol Med. 2007;379(12):1775-86.

62. Hamer M, Molloy GJ, de Oliveira C, Demakakos P. Leisure time physical activity, risk of depressive symptoms, and inflammatory mediators: the English Longitudinal Study of Ageing. Psychoneuroendocrinology. 2009;34(7):1050-55.

63. Pasco JA, Nicholson GC, Williams LJ, Jacka FN, Henry MJ, Kotowicz MA, et al. Association of highsensitivity C- reactive protein with de novo major depression. Br J Psychiatry. 2010;197(5):372-77.

64. Su S, Miller AH, Snieder H, Bremner J, Ritchie J, Maisano C, et al. Common genetic contributions to depressive symptoms and inflammatory markers in middleaged men: the Twins Heart Study. Psychosom Med. 2009;71(2):152-58.

65. Asthana A, Johnson HM, Piper ME, Fiore MC, Baker TB, Stein JH. Effects of smoking intensity and cessation on inflammatory markers in a large cohort of active smokers. Am Heart J. 2010;160(3):458-63.

66. Almeida OP, McCaul K, Hankey GJ, Norman P, Jamrozik K, & Flicker L. Homocysteine and depression in later life. Arch Gen Psychiatr. 2008;65(11):1286-94.

67. Pike JL, Irwin MR. Dissociation of inflammatory markers and natural killer cell activity in major depressive disorder. Brain Behav Immun. 2006 Mar; 20(2):169-74.

68. Danese A, Moffitt TE, Pariante CM, Ambler A, Poulton R, Caspi A. Elevated inflammation levels in depressed adults with a history of childhood maltreatment. Arch Gen Psychiatry. 2008 Apr;65(4):409-15.

69. Miller GE, Cole SW. Clustering of depression and inflammation in adolescents previously exposed to childhood adversity. Biol Psychiatr. 2012; 72(1):34-40.

70. Heim C, Newport DJ, Heit S, Graham YP, Wilcox M, Bonsall R, et al. Pituitary-adrenal and autonomic responses to stress in women after sexual and physical abuse in childhood. JAMA. 2000 Aug;284(5):592-97.

71. Nunes SOV, Watanabe MAE, Morimoto HK, Moriya R, Reiche EMV. The impact of childhood sexual abuse on activation of immunological and neuroendocrine response. Aggress Violent Beh. 2010 Nov./Dec;15(6):440-45.

72. Pariante CM, Miller AH. Glucocorticoid receptors in major depression: relevance to pathophysiology and treatment. Biol Psychiatr. 2001 Mar;49(5):391-404.

73. Rohleder N, Kirschbaum C. The hypothalamic-pituitary-adrenal (HPA) axis in habitual smokers. Int J Psychophysiol. 2006 Mar;59(3):236-43.

74. Kapczinski F, Vieta E, Andreazza AC, Frey BN, Gomes FA, Tramontina J, et al. Allostatic load in bipolar disorder: implications for pathophysiology and treatment. Neurosci Biobehav Rev. 2008;32(4):675-92.

75. Müller N, Schwarz MJ. A psychoneuroimmunological perspective to Emil Kraepelins dichotomy: schizophrenia and major depression as inflammatory CNS disorders. Eur Arch Psychiatry Clin Neurosci. 2008 June;258(Suppl 2):97-106.

76. Miller AH, Maletic V, Raison CL. Inflammation and its discontents: the role of cytokines in the pathophysiology of major depression. Biol Psychiatry. 2009 May;65(9):732-41.

77. Schroeder SA. Depression, smoking, and heart disease: how can psychiatrists be effective? Am J Psychiatry. 2011;168(9):876-78.

78. Leonard B, Maes M. Mechanistic explanations how cell-mediated immune activation, inflammation and oxidative and nitrosative stress pathways and their sequels and concomitants play a role in the pathophysiology of unipolar depression, Neurosci Biobehav Rev. 2012;36(12):764-85.

79. Moylan S, Maes M, Wray NR, Berk M. The neuroprogressive nature of major depressive disorder: pathways to disease evolution and resistance, and therapeutic implications. Mol Psychiatry. 2013 May;18(5):595-06.

80. Turcatel E, Funchal CS, Gomez R. Alterações comportamentais e de estresse oxidativo no sistema nervoso central pelo uso de álcool e tabaco. Rev Neurocienc. 2012;20(3):444-54.

81. Berk M. Should we be targeting smoking as a routine intervention? Acta Neuropsychiatr. 2007;19(2):131-32.

82. Andreazza AC, Kapczinski F, Kauer-Sant'Anna M, Walz JC, Bond DJ, Gonçalves CA, et al. 3-Nitrotyrosine and glutathione antioxidant system in patients in the early and late stages of bipolar disorder. J Psychiatry Neurosci. 2009;34(4):263-71.

83. Manji H, Kato TD, Prospero NA, Ness S, Beal MF, Krams M, et al. Impaired mitochondrial function in psychiatric disorders. Nat Rev Neurosci. 2012;13(5):293-307.

84. Queiroga CSF, Almeida AS, Vieira HLA. Carbon monoxide targeting mitochondria. Biochem Research Int. 2012:1-9.

85. Kobrosly R, van Wijngaarden E. Associations between immunologic, inflammatory, and oxidative stress markers with severity of depressive symptoms: an analysis of the 2005-2006 National Health and Nutrition Examination Survey. Neurotoxicology. 2010 jan;31(1):126-33.

86. Forlenza MJ, Miller GE. Increased serum levels of 8-hydroxy-2'-deoxyguanosine in clinical depression. Psychosom Med. 2006 jan./Feb;68(1):1-7.

87. Yager S, Forlenza MJ, Miller GE. Depression and oxidative damage to lipids. Psychoneuroendocrinology. 2010 Oct;35(9):1356-62.

88. Gama CS, Kuns M, Magalhães PVS, Kapczinski F. Staging and neuroprogression in bipolar disorder: a sistematic review of the literature. Rev Bras Psiquiatr. 2013 mar;35(1):70-4.

89. Maes M, Fišar Z, Medina M, Scapagnini G, Nowak G, Berk M. New drug targets in depression: inflammatory, cell-mediated immune, oxidative and nitrosative stress, mitochondrial, antioxidant, and neuroprogressive pathways. And new drug candidates-Nrf2 activators and GSK-3 inhibitors. Inflammopharmacology. 2012 June;20(3):127-50.

90. Raison CL, Capuron L, Miller AH. Cytokines sing the blues: inflammation and the pathogenesis of depression. Trends Immunol. 2006 Jan;27(1):24-31.

91. Pedersen W, von Soest T. Smoking, nicotine dependence and mental health among young adults: a 13-year population-based longitudinal study. Addiction. 2009 jan;104(1):129-37.

92. Berlin I, Covey LS, Donohue MC, Agostiv V. Duration of smoking abstinence and suicide-related outcomes. Nicotine Tob Res. 2011 Oct;13(10):887-93.

93. Goodwin RD, Prescott MR, Tamburrino M, Calabrese JR., Liberzon I, Galea S. Cigarette smoking and subsequent risk of suicidal ideation among National Guard Soldiers. J Affect Disord. 2013 Feb 15;145(1):111-4

94. Courtet P, Baud P, Abbar M, Boulenger JP, Castelnau D, Mouthon D, et al. Association between violent suicidal behavior and the low activity allele of the serotonin transporter gene. Mol Psychiatry. 2001May;6(3):338-41.

95. Boden JM, Fergusson DM, Horwood LJ. Cigarette smoking and suicidal behaviour: results from a 25year longitudinal study. Psychol Med. 2008 Mar; 38(3):433–39.

96. Bronisch T, Höfler M, Lieb R. Smoking predicts suicidality: findings from a prospective community study. J Affect Disord. 2008 May;108(1-2):135-45.

97. Mann J, Huang YY, Underwood D, Kassir SA, Oppenheim S, Kelly TM, et al. A serotonin transporter gene promoter polymorphism (5-HTTLPR) and prefrontal cortical binding in major depression and suicide. Arch Gen Psychiatry. 2000 Aug;57(8):729-38.

98. Benwell ME, Balfour DJ, Anderson JM. Smoking-associated changes in the serotonergic systems of discrete regions of human brain. Psychopharmacology 1990;102(1):68–72.

99. Mann JJ, Waternaux C, Haas GL, Malone KM. Towards a clinical model of suicidal behavior in psychiatric patients. Am J Psychiatry.1999;156(2):181-89.

100. Breslau N, Schultz L, Johnson E, Peterson E, Davis G. Smoking and the risk of suicidal behavior: a prospective study of a community sample. Arch Gen Psychiatry. 2005 Mar;62(3):328-34.

101. Vargas HO, Nunes SOV, Castro MP, Bortolasci CC, Barbosa DS, Morimoto HK, et al. Oxidative stress and lowered total antioxidant status are associated with history of suicide attempts. J Affect Disord. 2013 Sept;150(3):923-30.

102. Ministério da Saúde (Brasil), Secretaria de Atenção à Saúde. Portaria SAS/MS 442/04 de 13 de agosto de 2004. Aprova o plano de implantação da abordagem e tratamento do tabagismo no SUS. Brasília: Ministério da saúde; 2004.

103. Nunes SOV, Vargas HO, Castro MRP, Machado RCBR, Carmo DR. Abordagem intensiva. In: Nunes SOV, Castro MRP. Tabagismo: abordagem, prevenção e tratamento. Londrina: Eduel; 2011. 97-116.

104. Del Ben CM, Vilela JAA, Crippa JAS, Hallak JEC, Labate CM, Zuardi AW, Confiabilidade da “entrevista clinica estruturada para o DSM-IV” – versão clínica traduzida para o português. Rev Bras Psiquiatr. 2001;23(3):156-59.

105. Moreno RA, Moreno DH, Hamilton and Montgomery & Asberg depression rating scales. Rev Psiquiatr Clin. 1998;25(5):262-72.

106. World Health Organization (WHO). The ASSIST Working Group. The Alcohol, Smoking and Substance Involvement Screening Test (ASSIST): development, reliability and feasibility. Addiction. 2002;97(9);1183-94.

107. Henrique IFS, de Micheli D, Lacerda RB, Lacerda LA, Formigoni MLOS. Validação da Versão do Teste de Triagem do Envolvimento com Álcool, Cigarro e outras Substâncias (ASSIST). Rev Assoc Med Bras. 2004;50(2):1-8.

108. Fargerström KO. Measuring degree of physical dependence to tobacco smoking with reference to individualization of treatment. Addict Behav. 1978; 3(3-4):235-41.

109. Carmo JT, Pueyo AA. A adaptação ao português do Fagerström Test for Nicotine Dependence (FTND) para avaliar a dependência e tolerância à nicotina em fumantes. Rev Bras Med. 2002;59(1-2):73-80.

110. Menezes-Gaya IC, Zuardi AW, Loureiro SR, Crippa JAS. As propriedades psicométricas do teste de Fagerström para dependência de nicotina. J Bras Pneumol. 2009;35(1):73-82.

111. Storr CL, Reboussin BA, Anthony JC. The Fagerström test for nicotine dependence: a comparison of standard scoring and latent class analysis approaches. Drug Alcohol Depend. 2005;80(2):241-50.

112. Vasconcelos SML, Goulart MOF, Moura JBF, Benfato VMMS, Kubota LT. Espécies reativas de oxigênio e de nitrogênio, antioxidantes e marcadores de dano oxidativo em sangue humano: principais métodos para sua determinação. Quim Nova. 2007;30(5):1323-38.

113. Jentzch AM, Bachman H, Furst P, Biesalski HK. Improved analysis of malondialdehyde in human body fluids. Free Radic Biol Med. 1996;20(2):251-56.

114. Jiang Z, Woollard ACS, Wolff SP. Lipid hydroperoxide measurement by oxidation of Fe^{2+} in the presence of Xylenol Orange. Comparison with the TBA assay and an Iodometric method. Lipids. 1991 Oct;26(10):853-56.

115. Navarro-Gonzálvez JA, Garcia-Benayas C, Arenas J. Semiautomated measurement of nitrate in biological fluids. Clin Chem. 1998;44(3):679-81.

116. Repetto M, Reides C, Carretero MLG, Costa M, Griemberg G, Llesuy S. Oxidative stress in blood of HIV infected patients. Clin Chim Acta. 1996; 255(2):107-17.

117. Witko-Sarsat V, Frielander M, Capeillere-Blandin C, Nguyen-Khoa T, Nguyen AT. Zingraff J, et al. Advanced oxidation protein products as a novel marker of oxidative stress in uremia. Kid Int. 1996;49(5):1304-13.

ANEXOS

ANEXO A

Parecer de Aprovação do Comitê de Ética

Universidade Estadual de Londrina
COMITÊ DE ÉTICA EM PESQUISA

COMITÊ DE ÉTICA EM PESQUISA ENVOLVENDO SERES HUMANOS
Universidade Estadual de Londrina/ Hospital Universitário Regional Norte do Paraná
Registro CONEP 268

Parecer de Aprovação Nº 250/10 **CAAE Nº 0230.0.268.000-10** **FOLHA DE ROSTO Nº 376220**	Londrina, 19 de outubro de 2010.

PESQUISADOR: **SANDRA ODEBRECHT VARGAS NUNES**

CCS/DEPARTAMENTO DE CLÍNICA MÉDICA

Prezada Senhora:

O "**Comitê de Ética em Pesquisa Envolvendo Seres Humanos da Universidade Estadual de Londrina/ Hospital Universitário Regional Norte do Paraná**" (Registro CONEP 268)– de acordo com as orientações da Resolução 196/96 do Conselho Nacional de Saúde/MS e Resoluções Complementares, avaliou o projeto:

"MARCADORES BIOLÓGICOS EM FUMANTES DE UM CENTRO DE REFERÊNCIA PARA TRATAMENTO DO TABAGISMO"

Situação do Projeto: **APROVADO**

Informamos que deverá ser comunicada, por escrito, qualquer modificação que ocorra no desenvolvimento da pesquisa, bem como deverá apresentar ao CEP/UEL relatório final da pesquisa.

Atenciosamente,

Profª. Dra. Alexandrina Aparecida Maciel
Coordenadora
Comitê de Ética em Pesquisa - CEP/UEL

Campus Universitário: Rodovia Celso Garcia Cid (PR 445), km 380 - Fone (043) 371-4000 PABX - Fax 328-4440 - Caixa Postal 6.001 - CEP 86051-990 - Internet http://www.uel.br
Hospital Universitário/Centro de Ciências da Saúde: Av. Robert Koch, 60 - Vila Operária - Fone (043) 301-2000 PABX – Fax 337-4041 e 337-7495 - Caixa Postal 791 - CEP 86038-440
LONDRINA - PARANA - BRASIL

ANEXO B

Questionário

AMBULATÓRIO DE TABAGISMO – AVALIAÇÃO CLÍNICA

Instrumento Número: |___|___|___|. Data da primeira avaliação: _____/_____/_____

Etiqueta de Identificação

1. População:

01.Tabagista Depressivo

02. Tabagista Não Depressivo

03. Depressivo Não Tabagista

04. Controle Saudável

I - Caracterização Sócio-demográfica da clientela

Nome/Apelido:_____

2. Data de Nascimento: __/___/____. **3.** Idade (em anos): ____.

4. Naturalidade: _____**5.** Gênero: 1. Masculino 2. Feminino

6. Situação conjugal:

1. Solteiro 2. União estável 3. Separado/Divorciado 4. Viúvo

7. Cor da pele:

1. Branca 2. Negra 3. Amarela 4. Mulata 5. Parda 6. Indígena

8. Anos de estudo:

9. Nível de Escolaridade: 01. Analfabeto 02. Alfabetizado 03. Fundamental incompleto

04. Fundamental Completo 05. Médio Incompleto 06. Médio Completo

07. Superior Incompleto 08. Superior Completo 09. Pós-graduação lato sensu

10. Pós-graduação stricto sensu

10. Reside:

1. Sozinho 2. Parceiro 3. Família 4. Familiares 5. Asilo 6. Outros

_____

Endereço:

_____

Município: _____ CEP: _____ Estado:

_____

Telefone Contato: _____ Celular: _____

Ramal:_____

II – Situação de Trabalho 1

13. Local de Trabalho: _____

Endereço: _____

Município: _____ CEP: _____ Estado: _____

14. Formação: _____

15. Profissão: _____

16. Ocupação:_____

17. Relação com o trabalho:

1. Formal 2. Informal 3. Autônomo 4. Servidor Público ☐

18. Situação trabalhista:

1. Desempregado 2. Auxílio-desemprego 3. Atividade não Remunerada 4. Atividade Remunerada 5. Auxílio-doença 6. Estudante 7. Aposentado 8. Outro _____ ☐

19. Possui doença que o afaste do trabalho: 1. sim 2. não ☐

20. Qual é a doença? _____

21. Esta doença torna-o incapaz para o trabalho? 1. sim 2. não ☐

22. No último mês, quantos dias ficou afastado das suas atividades laborais? ☐

23. Qual foi o motivo/doença? _____

24. No último ano, quantos dias ficou afastado das suas atividades laborais? _____ ☐☐☐

25. Qual foi o motivo/doença? _____

26. Esta doença o incapacitou para as atividades domésticas? 1. sim 2. não ☐

27. Teve alguma internação geral recente:1. sim 2. não

28. Por quantas vezes foi internado? _____ ☐☐

29. Quantos dias duraram cada internação? _____ ☐☐

III – DADOS DE ENCAMINHAMENTO

32. A procura deu-se**:**

1. Voluntariamente 2. Por encaminhamento médico ou clínica 3. Sugestão familiar 4. Sugestão amigo 5. Sugestão colega de trabalho 6. Outro _____ ☐

IV – ABORDAGEM E TRATAMENTO DO TABAGISTA

História Pregressa da Doença

33. (01) Você tem ou teve frequentemente aftas, lesões (feridas) e/ou sangramento na boca? ☐

1. Sim 2. Não..

33.1. Está em tratamento? 1. Sim 2. Não ☐

34.(02) Você tem diabetes mellitus?

1. Sim 2. Não ☐

34.1. Está em tratamento? 1. Sim 2. Não ☐

35.(03) Você tem hipertensão arterial?

1. Sim 2. Não ☐

35.1. Está em tratamento? 1. Sim 2. Não ☐

36. (04) Você tem ou teve algum problema cardíaco?

1. Sim 2. Não ☐

36.1. Qual? _____

36.2. Está em tratamento? 1. Sim 2. Não ☐

37.(05) Você tem ou teve frequentemente queimação, azia, dor no estômago, úlcera ou gastrite? ☐

1. Sim 2. Não

37.1. Está em tratamento? 1. Sim 2. Não ☐

38.(06) Você tem ou teve algum problema pulmonar?

1. Sim 2. Não ☐

38.1. Qual? _____

38.2. Está em tratamento? 1. Sim 2. Não ☐

39.(07) Você tem alergia respiratória?

1. Sim 2. Não ☐

39.1. Está em tratamento? 1. Sim 2. Não ☐

40.(08) Você tem alergia cutânea?

1. Sim 2. Não ☐

40.1. Está em tratamento? 1. Sim 2. Não ☐

41.(09) Você tem ou teve alguma lesão ou tumor maligno?

1. Sim 2. Não ☐

41.1. Onde (local)? _____

41.2. Está em tratamento? 1. Sim 2. Não ☐

42. (10) Você tem ou teve crise convulsiva, convulsão febril na infância ou epilepsia?

1. Sim 2. Não ☐

42.1. Está em tratamento? 1. Sim 2. Não ☐

43. (11) Você tem anorexia nervosa ou bulimia?

1. Sim 2. Não

43.1. Está em tratamento? 1. Sim 2. Não

44. (12) Você costuma ter crises de depressão ou ansiedade?

1. Sim 2. Não

44. 1. Está em tratamento? 1. Sim 2. Não

45. (13) Você faz ou fez algum tratamento psicológico ou psiquiátrico?

1. Sim 2. Não

45. 1. Está em tratamento? 1. Sim 2. Não

45.2. Qual a medicação? _____

45.3. Você já tentou suicídio? 1. Sim 2. Não

45.4 Quantas vezes?

45.5 Métodos de tentativa de suicídio

1. Ingestão de medicamento
2. Ingestão de organofosforado
3. Enforcamento
4. Arma branca
5. Arma de fogo
6. Gás
7. Precipitar-se de alturas
8. Precipitar-se de carro em movimento
9. Outros_____

46. (a) Já fez uso de alguma medicação, mesmo que não prescrita por médico, para dormir ou se acalmar?

1. Sim 2. Não .

46.1. Qual? _____

50.3. SCID - Transtorno de Humor

0- Sem alteração de Humor 1- Transtorno Bipolar, tipo Maníaco 2- Transtorno Bipolar, tipo Hipomaníaco 3- Transtorno Bipolar, tipo Depressivo 4- Transtorno Bipolar, tipo Misto 5- Transtorno Depressivo Maior, Unipolar 6- Transtorno Depressivo Maior, em Remissão 7- Transtorno Distímico 8- Transtorno de Humor, devido a uma Condição Médica Geral 9- Transtorno de Humor, Induzido por Substância 10- Transtorno Bipolar em Remissão

57. (15) Você tem ou teve algum outro problema sério de saúde que não foi citado?

1. Sim 2. Não **57.1.** Qual? _____

57.2. Está em tratamento? 1. Sim 2. Não_____

57.3. Qual? _____

58. (16) Algum medicamento em uso atual?

1. Sim 2. Não ☐

58.1. Qual? _____

As perguntas 60 e 61 deverão ser respondidas por todos os pacientes do sexo feminino. Se NÃO ir para a questão 62.

60. (18) Está grávida? ☐

1. Sim 2. Não

60.1. Quantos meses? _____

60.2. Número gestações _____

61. (19) Está amamentando? ☐

1. Sim 2. Não

História Tabagística

62. (01) Com quantos anos você começou a fumar ? ☐☐

62. (02) Quantos anos fuma: ☐☐

62.(03) Quantos cigarros fuma por dia? ☐☐

62.(04) Anos/Maço. (nºcigarros x anos fumando/20)

65. (04) Quantas vezes você tentou parar de fumar?

1. De 1 a 3 vezes 2. Mais de 3 vezes 3. Nunca tentou *(seguir para a questão 69)* ☐

66. (05) Quantas vezes você ficou sem fumar por pelo menos um dia? ☐

1. Uma vez 2. Duas vezes 3. Três vezes 4. Mais de três vezes 5. Nenhuma vez

67. (06) Quais foram os motivos que levaram você a voltar a fumar? (Múltipla escolha)

. Bebida ☐ .Estressor de Perda ☐ .Briga – Raiva ☐ .Festa ☐ .Tensão ☐

.Alegria ☐ .Influência ☐ .Condicionamento ☐ **.**Medo ganhar peso ☐ .Ansiedade ☐

.Sem motivo aparente ☐ .Outro ☐

68. (07) Alguma vez na vida utilizou algum recurso para deixar de fumar?

1. Nenhum 2. Apoio de profissional de saúde 3. Leitura em folhetos, revistas, jornais e outros ☐

4. Medicamento

4.1. Qual? _____

5. Outros_____

69. (08) Você participou de algum grupo de apoio para abordagem e tratamento do tabagismo em

algum lugar? ☐

1. Sim 2. Não

70. Fez uso de tratamento para parar de fumar (pode escolher várias) :

☐ ☐

. bupropiona ☐ **.** reposição com adesivo .goma .acupuntura ☐
. homeopatia ☐ **.** grupo terapêutico ☐ **.** apoio de profissionais de saúde ☐ **.** outros ☐ medicamentos ☐
. Qual? _____

71. A última vez que ficou abstinente foi por quanto tempo (em meses) ☐☐

72. (09) **Por que você quer deixar de fumar agora? (Pode assinalar várias alternativas)**

. Por que está afetando minha saúde ☐
. Outras pessoas estão me pressionando ☐
. Pelo bem-estar de minha família ☐
. Estou preocupado com minha saúde no futuro ☐
. Porque meus filhos pedem ☐
. Porque não gosto de ser dependente ☐
. Fumar é anti-social ☐
. Porque gasto muito dinheiro com cigarro ☐
. Fumar é um mal exemplo para as crianças ☐
. Por conta das restrições de fumar em ambientes fechados ☐
. Outros ☐

73. (10) Você convive com fumantes na sua casa?
1. Sim 2. Não ☐

73.1. Qual o grau de parentesco? _____

74. (11) Você se preocupa em ganhar peso ao deixar de fumar?
1. Sim 2. Não ☐

Escala de Tolerância de Fagerstrõm – Gravidade à Dependência de Nicotina

75. (01) Quanto tempo depois de acordar fuma o primeiro cigarro?
0. Após 60 minutos 1. Entre 31 a 60 minutos 2. Entre 06 a 30 minutos
3. Nos primeiros 5 minutos ☐

76. (02) Você acha difícil não fumar em lugares onde é proibido, como em igrejas, bibliotecas, local de trabalho, shoppings, etc?
1. Sim 0. não ☐

77. (03) Qual o cigarro do dia traz mais satisfação?
1.O primeiro da manhã 0. Outros ☐

78. (04) Quantos cigarros você fuma por dia?
0. Menos de 10 1. De 11 a 20 2. De 21a 30 3. Mais de 31 ☐

79. (05) Você fuma mais pela manhã?

1. Sim 2. Não ☐

80. (06) Você fuma mesmo doente quando precisa ficar na cama a maior parte do tempo?

1.Sim 0. Não ☐

80.1. Pontuação ☐☐

História Familiar de Tabagismo em Primeiro Grau

81. Seu pai fuma ou já fumou? 1. Sim 2. Não ☐

82. Sua mãe fuma ou já fumou? 1. Sim 2. não ☐

83. Número de irmãos? _____ **84.** Quantos irmãos fumam? ☐☐

85. Número de filhos? _____ **86.** Quantos filhos fumam? ☐☐

87. História familial: 1. Positiva 2. Negativa 3. Desconhece ☐

88. História familiar de transtorno mental: 1.Sim 2. Não ☐

88.1. Qual familiar? _____

88.2. Qual transtorno mental? _____

Exame Físico – Fase 0

90. Altura do paciente (m): ☐ m ☐☐ cm **91.** Peso (k): ☐☐☐ k ☐☐☐ g

92. IMC – Índice de Massa Corpórea (peso/ altura²): ☐☐☐☐

93. PA: ☐☐☐ x ☐☐☐ **94.** FC: ☐☐☐ **95.1** Circunferência Abdominal: ☐☐☐

95.2 Circunferência Quadril: ☐☐

100. Glicemia: ☐☐☐

102.1- Colesterol Total: ☐☐☐ **102.2-** Colesterol HDL: ☐☐☐

102.3- Colesterol LDL: ☐☐☐ **102.4-** Triglicerídeos: ☐☐☐☐

ASSIST 103. (01) Na sua vida qual dessas substâncias você já usou (Somente uso não médico)

	Não	Sim
103.1. Derivados do tabaco (cigarro, charuto, cachimbo, fumo de corda ...)	0	3
103.2. Bebidas alcoólicas (cerveja, vinho, destilados – pinga, uísque ...)	0	3
103.3. Maconha (baseado, erva, haxixe ...)	0	3
103.4. Cocaína, crack (pó, pedra, branquinha, nuvem ...)	0	3
103.5. Estimulantes como anfetaminas ou ecstasy (bolinhas, rebites ...)	0	3
103.6. Inalantes (cola de sapateiro, cheirinho-da-loló, tinta, gasolina, éter ...)	0	3
103.7. Hipnóticos e sedativos (remédios para dormir, diazepan, lorax ...)	0	3
103.8. Drogas Alucinógenas (como LSD, ácido, chá-de-lírio, cogumelos...)	0	3

103.9. Opióides (heroína, morfina, metadona, coldeína ...)	0	3
103.10. Outros, Especificar : _____	0	3

Se NÃO em todos os itens questionar “Nem mesmo quando você estava na escola?”.

Se NÃO em todos os itens, páre a entrevista e vá para a questão 111.

Se SIM para alguma droga, prossiga para a questão 104 para cada droga usada.

104. (02) Durante os três últimos meses, com que frequência você utilizou essa(s) substância(s)
que mencionou?

	Nunca	1 a 2 vezes	Mensalmente	Semanalmente	Diariamente ou quase todo dia
104.1. Derivados do tabaco (cigarro, charuto, cachimbo, fumo de corda ...)	0	2	3	4	6
104.2. Bebidas alcoólicas (cerveja, vinho, destilados – pinga, uísque ...)	0	2	3	4	6
104.3. Maconha (baseado, erva, haxixe ...)	0	2	3	4	6
104.4. Cocaína, crack (pó, pedra, branquinha, nuvem ...)	0	2	3	4	6
104.5. Estimulantes como anfetaminas ou ecstasy (bolinhas, rebites ...)	0	2	3	4	6
104.6. Inalantes (cola de sapateiro, cheirinho-da-loló, tinta, gasolina, éter ...)	0	2	3	4	6
104.7. Hipnóticos e sedativos (remédios para dormir, diazepan, lorax ...)	0	2	3	4	6
104.8. Drogas alucinógenas (como LSD, ácido, chá-de-lírio, cogumelos...)	0	2	3	4	6
104.9. Opióides (heroína, morfina, metadona, coldeína ...)	0	2	3	4	6
104.10. Outras, Especifica _____	0	2	3	4	6

Se NUNCA em todos os itens da Questão 104, vá para a questão 111.

Se SIM para alguns destes itens prossiga respondendo as questões 105 a 110.

105. (03) Durante os três últimos meses, com que frequência você teve um forte desejo ou urgência em consumir? (Primeira droga, depois a segunda droga, etc)

	Nunca	1 a 2 vezes	Mensalmente	Semanalmente	Diariamente ou quase todo dia
105.1. Derivados do tabaco (cigarro, charuto, cachimbo, fumo de corda ...)	0	3	4	5	6
105.2. Bebidas alcoólicas (cerveja, vinho, destilados – pinga, uísque ...)	0	3	4	5	6
105.3. Maconha (baseado, erva, haxixe ...)	0	3	4	5	6

105.4. Cocaína, crack (pó, pedra, branquinha, nuvem ...)	0	3	4	5	6
105.5. Estimulantes como anfetaminas ou ecstasy (bolinhas, rebites ...)	0	3	4	5	6
105.6. Inalantes (cola de sapateiro, cheirinho-da-loló, tinta, gasolina, éter ...)	0	3	4	5	6
105.7. Hipnóticos e sedativos (remédios para dormir, diazepan, lorax ...)	0	3	4	5	6
105.8. Drogas alucinógenas (como LSD, ácido, chá-de-lírio, cogumelos...)	0	3	4	5	6
105.9. Opióides (heroína, morfina, metadona, coldeína ...)	0	3	4	5	6
105.10. Outras, Especifica _____	0	3	4	5	6

106. (04) Durante os últimos três meses com que frequência o seu consumo de (Primeira droga,

depois a segunda droga, etc) resultou em problemas de saúde, social, legal ou financeiro?

	Nunca	1 a 2 vezes	Mensalmente	Semanalmente	Diariamente ou quase todo dia
106.1. Derivados do tabaco (cigarro, charuto, cachimbo, fumo de corda ...)	0	4	5	6	7
106.2. Bebidas alcoólicas (cerveja, vinho, destilados – pinga, uísque ...)	0	4	5	6	7
106.3. Maconha (baseado, erva, haxixe ...)	0	4	5	6	7
106.4. Cocaína, crack (pó, pedra, branquinha, nuvem ...)	0	4	5	6	7
106.5. Estimulantes como anfetaminas ou ecstasy (bolinhas, rebites ...)	0	4	5	6	7
106.6. Inalantes (cola de sapateiro, cheirinho-da-loló, tinta, gasolina, éter ...)	0	4	5	6	7
106.7. Hipnóticos e sedativos (remédios para dormir, diazepan, lorax ...)	0	4	5	6	7
106.8. Drogas alucinógenas (como LSD, ácido, chá-de-lírio, cogumelos...)	0	4	5	6	7
106.9. Opióides (heroína, morfina, metadona, coldeína ...)	0	4	5	6	7
106.10. Outras, Especifica _____	0	4	5	6	7

107. (05) (Durante os três últimos meses, com que frequência por causa do seu uso você deixou de (Primeira droga, depois a segunda droga,etc) você deixou de fazer coisas que eram normalmente esperadas por você?

	Nunca	1 a 2 vezes	Mensalmente	Semanalmente	Diariamente ou quase todo dia
107.1. Derivados do tabaco (cigarro, charuto, cachimbo, fumo de corda ...)	0	5	6	7	8
107.2. Bebidas alcoólicas (cerveja, vinho, destilados – pinga, uísque ...)	0	5	6	7	8
107.3. Maconha (baseado, erva, haxixe ...)	0	5	6	7	8
107.4. Cocaína, crack (pó, pedra, branquinha, nuvem ...)	0	5	6	7	8
107.5. Estimulantes como anfetaminas ou ecstasy (bolinhas, rebites ...)	0	5	6	7	8
107.6. Inalantes (cola de sapateiro, cheirinho-da-loló, tinta, gasolina, éter ...)	0	5	6	7	8
107.7. Hipnóticos e sedativos (remédios para dormir, diazepan, lorax ...)	0	5	6	7	8
107.8. Drogas alucinógenas (como LSD, ácido, chá-de-lírio, cogumelos...)	0	5	6	7	8
107.9. Opióides (heroína, morfina, metadona, coldeína ...)	0	5	6	7	8
107.10. Outras, Especifica _____	0	5	6	7	8

108.(06) Há amigos, parentes ou outras pessoas que tenha demonstrado preocupação com seu uso de (Primeira Droga, depois a segunda droga, etc)?

	NÃO, Nunca	SIM, mas não nos últimos 3 meses	SIM, nos últimos 3 meses
108.1. Derivados do tabaco (cigarro, charuto, cachimbo, fumo de corda ...)	0	3	6
108.2. Bebidas alcoólicas (cerveja, vinho, destilados – pinga, uísque ...)	0	3	6
108.3. Maconha (baseado, erva, haxixe ...)	0	3	6
108.4. Cocaína, crack (pó, pedra, branquinha, nuvem ...)	0	3	6
108.5. Estimulantes como anfetaminas ou ecstasy (bolinhas, rebites ...)	0	3	6
108.6. Inalantes (cola de sapateiro, cheirinho-da-loló, tinta, gasolina, éter ...)	0	3	6
108.7. Hipnóticos e sedativos (remédios para dormir, diazepan, lorax ...)	0	3	6
108.8. Drogas alucinógenas (como LSD, ácido, chá-de-lírio, cogumelos...)	0	3	6

108.9. Opióides (heroína, morfina, metadona, coldeína ...)	0	3	6
108.10. Outras, Especifica _____	0	3	6

109. (07) Alguma vez você já tentou controlar, diminuir ou parar o uso de (Primeira droga, depois a segunda droga, etc)?

	NÃO, Nunca	SIM, mas não nos últimos 3 meses	SIM, nos últimos 3 meses
109.1. Derivados do tabaco (cigarro, charuto, cachimbo, fumo de corda ...)	0	3	6
109.2. Bebidas alcoólicas (cerveja, vinho, destilados – pinga, uísque ...)	0	3	6
109.3. Maconha (baseado, erva, haxixe ...)	0	3	6
109.4. Cocaína, crack (pó, pedra, branquinha, nuvem ...)	0	3	6
109.5. Estimulantes como anfetaminas ou ecstasy (bolinhas, rebites ...)	0	3	6
109.6. Inalantes (cola de sapateiro, cheirinho-da-loló, tinta, gasolina, éter ...)	0	3	6
109.7. Hipnóticos e sedativos (remédios para dormir, diazepan, lorax ...)	0	3	6
109.8. Drogas alucinógenas (como LSD, ácido, chá-de-lírio, cogumelos...)	0	3	6
109.9. Opióides (heroína, morfina, metadona, coldeína ...)	0	3	6
109.10. Outras, Especifica _____	0	3	6

110. (08) Alguma vez você já usou drogas por injeção? (Apenas uso não médico)

0. NÃO, Nunca ☐
3. SIM, mas não nos últimos 3 meses
2. SIM, nos últimos 3 meses

110.1 Pontuação – Hamilton ☐☐

110.2 Pontuação - Tabaco ☐

1. 0-3 2. 4-26 3. 27 ou mais

110.3 Pontuação – Bebidas alcoólicas ☐

1. 0-10 2. 11-26 3. 27 ou mais

110.4 Pontuação - Maconha ☐

1. 0-3 2. 4-26 3. 27 ou mais

110.5 Pontuação – Cocaína, crack ☐

1. 0-3 2. 4-26 3. 27 ou mais

110.6 Pontuação – Estimulantes como anfetaminas ou ecstasy ☐

1. 0-3 2. 4-26 3. 27 ou mais

110.7 Pontuação - Inalantes ☐

1. 0-3 2. 4-26 3. 27 ou mais

110.8 Pontuação – Hipnóticos e sedativos ☐

1. 0-3 2. 4-26 3. 27 ou mais

110.9 Pontuação – Drogas alucinógenas ☐

1. 0-3 2. 4-26 3. 27 ou mais

110.10 Pontuação – Opióides ☐

1. 0-3 2. 4-26 3. 27 ou mais

110.11 Pontuação – Outras ☐

1. 0-3 2. 4-26 3. 27 ou mais

137. Sessões Terapêuticas

Situação Paciente (Sit Pac.) ☐

1. Fumante 2. Não fumante 3. Não compareceu
4. Lapso recaída 5. Lapso abstinência 6. Abandono

Tratamento (Tratam.) ☐

01. Grupo 02. Grupo+adesivo 03. Grupo+goma
04. Grupo+adesivo+goma 05. Grupo+bupropriona 06. Grupo+bupropriona+adesivo
07. Grupo+bupropriona+adesivo+goma 08. Grupo+bupropriona+goma
09. Grupo+ISRS 10. Grupo+ISRS+adesivo 11. Grupo+ISRS+goma
12. Grupo+ISRS+goma+adesivo 13. Grupo+nortriptilina 14. Grupo+nortriptilina+goma
15. Grupo+nortriptilina+adesivo 16. Grupo+nortriptilina+adesivo+goma
17. Nenhum 18. Grupo + outro 19. Outro _____

Monóxido de Carbono exalado (CO exal) - PPM e %

Sit.Pac	.	Tratam.	CO exal %	CO exal ppm	Sit. Pac	Tratam	CO exal %	CO exal ppm

Avaliação			.	**9Sessão**			.
1 Sessão			.	**10 Sess.**			.
2 Sessão			.	**11 Sess.**			.
3 Sessão			.	**12 Sess.**			.
4 Sessão			.	**13 Sess.**			.
5 Sessão			.	**14 Sess.**			.
6 Sessão			.	**15 Sess.**			.
7 Sessão			.	**16 Sess.**			.
8 Sessão			.	**17 Sess.**			.

139.1 Dosagem IL-6 – **Fase 0**:

139.2 Dosagem da PCR - **Fase 0**:

139.3. 5 HTT – Polimorfismo **– Fase 0**:

1. 12-12 2. 12-10 3. 10-10 4. 9-12 5. 9-10 6. 9-9

139.4 TNF α – **Fase 0:**

139.5 IL- 1 - **Fase 0**: ,

139.6 IL- 4 - **Fase 0**: ,

139.7 IL- 10 - **Fase 0**: ,

139.8 Potencial Antioxidante Total Plasmático – (TRAP) - **Fase 0**: ,

139.9 Dialdeído Malônico (MDA) - **Fase 0**: ,

139.10 Óxido Nítrico (NO) - **Fase 0**: ,

139.11 Hidroperóxidos Lipídicos (FOX) - **Fase 0:** ,

139.12 Produtos Avançados de Proteínas Oxidadas (AOPP) **- Fase 0**: ☐☐☐,☐☐

139.13 Hb A1c - **Fase 0**: ☐☐☐☐☐

139.14 α1 glicoproteína ácida - **Fase 0:** ☐☐☐☐☐

139.15 Insulina - **Fase 0**: ☐☐☐☐☐

139.16 Gama GT - **Fase 0**: ☐☐☐☐

139.17 Ácido úrico - **Fase 0:** ☐☐☐☐☐

139.18 Fibrinogênio - **Fase 0:** ☐☐☐☐☐

139.19 Homocisteína – **Fase 0:** ☐☐☐☐☐

139.20 Eletroforese Proteína – **Fase 0**: ☐, ☐☐ g/dl

☐☐, ☐ %

139.21 Hemograma Anemia – **Fase 0**: 1- Normal 2- Alterado ☐

139.22 Hemograma Infecção – **Fase 0:** 1- Normal 2- Alterado ☐

139.23 VHS **- Fase 0**: ☐☐☐

139.24 Hepatite B – **Fase 0**: 1- Reagente 2- Não Reagente ☐

139.25 Hepatite C **– Fase 0**: 1- Reagente 2- Não Reagente ☐

139.26 HIV – **Fase 0**: 1- Reagente 2- Não Reagente ☐

139.27 TGO – **Fase 0**: ☐☐☐☐

139.28 TGP – **Fase 0**: ☐☐☐☐

139.29 Creatinina – **Fase 0:** ☐☐☐

139.30 Função Pulmonar - fase 0:

CVF: ☐☐ I CVF%: ☐☐☐ VEF_1: ☐☐, VEF_1%: ☐☐☐ VEF_1/CVF: ☐☐☐

CV: ☐☐ ☐☐☐ ☐☐☐ ☐☐☐ ☐☐☐

CV%: