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NEUROBIOLOGIA DA DEPRESSÃO E DO COMPORTAMENTO SUICIDA: MARCADORES INFLAMATÓRIOS E IMPACTO DA CETAMINA

Porto Alegre

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Tese apresentada ao Programa de Pós-Graduação em Ciências Biológicas: Bioquímica do Instituto de Ciências Básicas da Saúde da Universidade Federal do Rio Grande do Sul como requisito parcial para a obtenção do título de Doutora em Bioquímica.

Orientadora: Profª. Drª. Márcia Kauer Sant'Anna

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Aprovado em:_____de _______de_____.

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Dedico este trabalho a todos os pacientes cuja coragem e participação nesta pesquisa não apenas auxiliaram na construção do conhecimento, mas acenderam uma luz de esperança. Que este estudo sirva como um farol para aqueles sob o diagnóstico de depressão e ideação suicida, guiando-os em direção a uma vida com a maior qualidade e dignidade possíveis.

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Na tese de doutorado, apesar do processo solitário a que qualquer pesquisador está destinado, reúne contributos de várias pessoas. Desde o início, contei com a confiança e o apoio de inúmeras pessoas e instituições. Sem aqueles contributos, esta pesquisa não teria sido possível.

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determinação em me “segurar”/suportar durante as crises de ansiedade, medo e insegurança, bem como por acreditar em mim quando nem eu mesma acreditava. Finalmente, agradeço a ele por sua paciência ao longo dos últimos meses enquanto escrevia está tese e pelo apoio incondicional em todos os momentos, principalmente nos de incerteza.

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I do not know what I may appear to the world, but to myself I seem to have been only like a boy playing on the seashore, and diverting myself in now and then finding a smoother pebble or a prettier shell than ordinary, whilst the great ocean of truth lay all undiscovered before me.

[Tenho a impressão de ter sido uma criança brincando à beira-mar, divertindo-me em descobrir uma pedrinha mais lisa ou uma concha mais bonita que as outras, enquanto o imenso oceano da verdade continua misterioso diante de meus olhos].

(Isaac Newton)

APRESENTAÇÃO

Essa tese tem como propósito estudar os mecanismos da depressão e seus potenciais tratamentos, investigando a neurobiologia e os possíveis alvos para intervenções. De forma específica, a tese avaliou biomarcadores inflamatórios no curso do transtorno depressivo e o efeito potencial da cetamina em episódios depressivos e ideação suicída, bem como e a importância da padronização de um protocolo terapêutico.

1) Biomarcadores Inflamatórios: O foco foi na exploração da existência de um perfil inflamatório característico, diferenciando um subgrupo de pacientes com depressão de início precoce (conhecida como "*early onset*") e de início tardio ("*late onset*").

2) Padronização do Protocolo da Cetamina: A possibilidade do uso da Cetamina endovenosa e subcutânea, trouxe heterogeneidade para o campo. Assim, a padronização do método, além de contribuir para a eficácia terapêutica, pode facilitar a implementação de estudos controlados de larga escala, os quais são indispensáveis para a validação da cetamina como um tratamento seguro e eficaz para a depressão e a prevenção do suicídio.

3) Efeito Clínico da Cetamina: Com seu efeito de rápida ação, a cetamina tem sido apontada como uma medicação com potencial para tratamento de pacientes com risco de suicídio. Um estudo clínico com desfecho primário a avaliação do risco de suicídio pode contribuir para validar esta intervenção. Dessa forma, em um ensaio clínico naturalístico, com protocolo estabelecido na etapa anterior, analisamos as mudanças clínicas, incluindo a potencial redução dos sintomas depressivos e do risco suicida, aplicando-se escalas validadas em pacientes com episódios depressivos de humor. Essa padronização e avaliação do risco de suicídio com escalas específicas, além de contribuir para o entendimento da eficácia terapêutica, pode facilitar a implementação de mais estudos com menor heterogeneidade e resultados mais significativos.

Com o propósito de proporcionar uma estrutura coerente e lógica, esta tese de doutorado foi organizada em três partes principais, cada uma correspondendo a uma das perspectivas mencionadas acima.

Parte I: Resumo, Resumo em inglês (abstract), Lista de ilustração, Lista de tabelas, Lista de abreviações, Introdução, Justificativa e Objetivos.

Parte II: Metodologia e Resultados

A metodologia empregada nesta tese é meticulosamente desenhada para explorar e elucidar aspectos complexos da depressão e ideação suicida. Os resultados são articulados em seis artigos científicos distintos, cada um iluminando uma dimensão específica e crítica da pesquisa. Unindo inovação e rigor científico, esses artigos não apenas aprofundam nossa compreensão dos mecanismos subjacentes e biomarcadores associados à depressão e ideação suicida, mas também abrem novos caminhos para tratamentos e intervenções baseados em evidências. A contribuição de cada artigo é única, e juntos, eles formam um mosaico de *insights* que enriquecem e expandem o campo da pesquisa em saúde mental.

Eles estão divididos da seguinte maneira:

a) "Earlier age of onset is associated with a pro-inflammatory state in major depressive disorder": Este artigo avalia a associação entre o início precoce do TDM e alterações nos marcadores inflamatórios sistêmicos.
b) "Ketamine study: Protocol for naturalistic prospective multicenter study on subcutaneous ketamine infusion in depressed patients with active suicidal ideation": Publicamos o protocolo do estudo principal desta tese, para avaliar os benefícios da cetamina em pacientes com TDM e risco suicida, bem como suas alterações bioquímicas.
c) "Repeated doses of subcutaneous esketamine in patients with treatment-resistant depression: case series in a general hospital in Southern Brazil": Uma série de casos do uso da cetamina, seguindo o mesmo protocolo de tratamento publicado no artigo 2, em pacientes com depressão resistente ao tratamento.
d) "Allergic reaction induced by subcutaneous administration of ketamine: a case report": Um relato pioneiro de uma reação alérgica à cetamina, compatível com ativação de mastócitos e liberação de mediadores pré-formados (urticária na face e no tronco).
e) "Efficacy of ketamine for depressive symptoms and suicidal ideation: 6-month naturalistic study": Este artigo, prestes a ser submetido, apresenta os resultados detalhados do protocolo de tratamento com cetamina descrito no item b.

Parte III: Discussão, Conclusões, Referências citadas na Parte I e na Parte III, e Anexos.

A seção de Anexos compreende: a) Carta de Aprovação do Comitê de Ética em Pesquisa-HCPA (Anexo A) e Parecer consubstanciado do Comitê de Ética em Pesquisa-HMV (Anexo B); b) artigos científicos publicados em autoria ou coautoria durante o período do doutorado, os quais não estão diretamente associados ao tema da tese (Anexo B).

Os trabalhos que compõem esta tese foram desenvolvidos entre os anos de 2020 e 2023 no Laboratório de Psiquiatria Molecular, localizado no Centro de Pesquisas Experimentais do Hospital de Clínicas de Porto Alegre (HCPA), sob orientação da Profª. Drª. Márcia Kauer Sant'Anna. Este estudo foi apoiado pelos fundos brasileiros CAPES, INCT-TM (465458/2014-9), FAPERGS e FIPE-HCPA.

RESUMO

A depressão, uma patologia psiquiátrica de alta prevalência, também causa prejuízossubstanciaisa saúde física e a funcionalidade dos indivíduos afetados. Esta condição, limitante para a atividade diária e o bem-estar, tem contribuído para um aumento da demanda por serviços de saúde. O Transtorno Depressivo Maior (TDM) é particularmente complexo, caracterizado por ser uma condição multifatorial que envolve a interação entre fatores ambientais estressantes e susceptibilidade genética, incluindo padrões de resposta de citocinas pró-inflamatórias na sua neurobiologia. O desfecho mais grave na psiquiatria, o suicídio é altamente associado a presença de depressão. Dessa forma, esta tese tem como objetivo explorar novas abordagens para o tratamento da depressão e ideação suicida, enfatizando a identificação de biomarcadores e a avaliação do impacto de novos tratamentos como a cetamina em adultos com episódios depressivos. No Capítulo I os dados revelaram uma associação significativa entre níveis séricos elevados de TNFα e IL-1β e o início precoce de TDM, sustentando a hipótese de que alterações nestes biomarcadores podem identificar um subgrupo específico de pacientes, marcado por um início precoce da doença.Diante da complexidade do tratamento da depressão e da resistência de muitos pacientes às terapias convencionais, a busca por intervenções rápidas e eficazes torna-se crucial. Neste contexto, a cetamina emergiu como uma alternativa terapêutica promissora. O protocolo, detalhado no Capítulo II, avalia os benefícios da cetamina em pacientes com TDM e risco suicida, além de suas alterações bioquímicas.No Capítulo III uma análise preliminar de pacientes submetidos ao protocolo de tratamento com cetamina subcutânea (SC) mostrou uma melhoria notável nos sintomas depressivos e uma redução significativa na ideação suicida. No entanto, destaca-se a importância de considerar os potenciais efeitos adversos associados ao uso da cetamina. O Capítulo IV relata que a cetamina pode desencadear reações alérgicas como urticária, sendo a intervenção imediata com prednisolona eficaz na mitigação desses sintomas. No Capítulo V um estudo naturalístico e prospectivo associou significativamente o potencial terapêutico da cetamina com melhoras clínicas. Os dados indicam uma relação dose-resposta, caracterizada por um aumento progressivo nas concentrações administradas e melhorias correspondentes em depressão, ideação suicida e funcionamento global. Em suma, a utilização de biomarcadores séricos para identificar subgrupos de pacientes, que possam futuramente ser avaliados na resposta a tratamentos específicos, como no caso daqueles pacientes com início precoce do TDM e alterações nos marcadores inflamatórios,pode contribuir para melhorar as taxas de resposta as intervenções. Ainda, ampliar a compreender a cetamina administrada SC

emerge como uma terapia inovadora para episódios depressivos.
Palavras-chave: episódio depressivo; ideação suicida; cetamina; biomarcadores; neuroinflamação

ABSTRACT

Depression, a highly prevalent psychiatric pathology, is also associated with a decline in physical health and functionality of affected individuals. This condition limits daily functionality, well being and has contributed to high rates of healthcare services utilization. Major Depressive Disorder (MDD) is particularly complex, characterized as a multifactorial condition involving the interaction between environmental stress factors and genetic susceptibility, including patterns of pro-inflammatory cytokine responses in its neurobiology. This thesis aims to explore the neurobiology of depression, suicidal ideation and potential new approaches for treatment. For instance, we looked for inflammatory biomarkers in patients with a depressive episode, and we examined the impact of ketamine in adults with depressive episodes. Chapter I data revealed a significant association between elevated serum levels of TNFα and IL-1β and early onset of MDD, supporting the hypothesis that changes in these biomarkers may be useful to identify a specific patient subgroup, marked by an early onset of the disease. Given the complexity of treating depression and the resistance of many patients to conventional therapies, the search for rapid and effective interventions is warranted. In this context, ketamine has emerged as a promising therapeutic alternative. The protocol, detailed in Chapter II, assesses the benefits of ketamine in patients with MDD and suicidal risk, along with its biochemical alterations. In Chapter III, a preliminary analysis of patients subjected to the subcutaneous (SC) ketamine treatment protocol showed remarkable improvement in depressive symptoms and a significant reduction in suicidal ideation. However, the importance of considering the potential adverse effects associated with the use of ketamine is highlighted. Chapter IV reports that ketamine can trigger allergic reactions such as urticaria, with immediate intervention with prednisolone effective in mitigating these symptoms. In Chapter V, a naturalistic and prospective study of ketamine showed significant clinical improvement. The data indicate a dose-response relationship, characterized by a progressive increase in administered concentrations and corresponding improvements in depression, suicidal ideation, and overall functioning. In summary, the use of serum biomarkers may be useful to identify a subset of patients with early onset MDD with systemic inflammatory markers changes. In addition, SC-administered ketamine emerges as an

innovative option, with greater feasibility, for treating depressive episodes.
Keywords: depressive episode; suicidal ideation; ketamine; biomarkers; neuroinflammation

LISTA DE FIGURAS

LISTA DE ABREVIATURAS E SIGLAS

5-HIAA	Ácido 5-hidroxi-indolacético
5-HT	5-hidroxitriptamina
ABNT	Associação Brasileira de Normas Técnicas.
AMPA	ácido alfa-amino-3-hidroxi-5-metil-4-isoxazolpropiônico
APA	*American Psychiatric Association*
BPRS	*Brief Psychiatric Rating Scale*
CC	Circunferência da cintura
C-CASA	Classification Algorithm of Suicide Assessment
CDC	*Centers for Disease Control and Prevention*
CID	Classificação Internacional de Doenças
CPDA	Centro de Pronto Diagnóstico Ambulatorial do HCPA
C-SSRS	*Columbia-Severity Suicide Rating Scale*
CTQ-SF	*Childhood Trauma Questionnaire-Short Form*
DSM-5	*Diagnostic and Statistical Manual of Mental Disorders Fifth Edition*
ECA	*Epidemiologic Catchment Area Study*
EV	Endovenosa (via)
FAST	*Functioning Assessment Short Test*
FDA	*Food and Drug Administration*
GABA	Ácido Gama-Aminobutírico
HAM-D17	Hamilton Depression 17
HCPA	Hospital das Clínicas de Porto Alegre
HPA	Eixo hipotálamo-hipófise-adrenal
IL-6	Interleucina-6
IMC	Índice de Massa Corporal
KA	Cainato
LepRb	*Involvement of Leptin Receptor Long Isoform*
MADRS	*Montgomery-Asberg Depression Rating Scale*
MINI	*International Neuropsychiatric Interview*
m-RNA	Ácido Ribonucleico Mensageiro
MS	Ministério da Saúde
NAD	*Nicotinamide adenine nucleotide*

NMDA	N-metil-D-aspartato
OMS	Organização Mundial da Saúde
PCR-us	Proteína C-reativa ultrassensível
REBEC	Registro Brasileiro de Ensaios Clínicos
RNA	*Ribonucleic acid* – ácido ribonucleico
SC	Subcutânea (via)
SM	Síndrome metabólica
SNC	Sistema Nervoso Central
TCLE	Termo de Consentimento Livre e Esclarecido
TNF-alfa	Fator de Necrose Tumoral alfa
WHO	*World Health Organization*
YMRS	*Yong Mania Rating Scale*

SUMÁRIO

PARTE I

Introdução, Justificativa e Objetivos

1 INTRODUÇÃO

A Depressão é uma patologia psiquiátrica de alta prevalência, com impactos significativos sobre a saúde física e a funcionalidade dos indivíduos afetados (Juruena *et al*., 2015). A depressão limita a atividade diária e o bem-estar, ocasionando uma demanda aumentada por serviços de saúde (Kessler *et al*., 2003). De acordo com o Ministério da Saúde (2022) a prevalência de depressão ao longo da vida no Brasil está em torno de 15,5%. Segundo a OMS, a prevalência de depressão na rede de atenção primária de saúde é 10,4%, isoladamente ou associada a um transtorno físico. De acordo com a OMS, a depressão situa-se em 4º lugar entre as principais causas de ônus, respondendo por 4,4% dos ônus acarretados por todas as doenças durante a vida. Ocupando o 1º lugar quando considerado o tempo vivido com incapacitação ao longo da vida (11,9%).

O Transtorno Depressivo Maior (TDM) é uma condição multifatorial, com etiologia associada à interação entre exposições ambientais estressantes - como consumo de álcool, dependência de drogas, traumas precoces e eventos adversos significativos - e suscetibilidade endógena. Esta última pode incluir o padrão deresposta de citocinas pró-inflamatórias como Interleucina (IL)-6, IL-10, IL-1β e Fator de Necrose Tumoral alfa (TNFα) (Janelidze *et al*., 2011) e a presença e intensidade dos sintomas depressivos (Alesci *et al*., 2005; Zorrilla *et al*., 2001).

A associação entre depressão e comportamento suicida é muito forte. A (*World Health Organization*, 2002), aponta que mais de 90% dos casos de suicídio estão associados a transtornos psiquiátricos, especialmente a depressão (Cutcliffe, 2003; Kessler *et al*., 1999; Lee *et al*., 2007; Moscicki, *et al*., 1998). Pesquisas realizadas em diversos contextos geográficos indicam a depressão como principal patologia associada a tentativas de suicídio, ideação suicida e planos de suicídio (Roza *et al*., 2023). É importante salientar que a mais recente revisão do Manual Diagnóstico e Estatístico de Transtornos Mentais, 5ª edição Texto Revisado (DSM-5-TR, APA 2022) incluiu um avanço significativo na nosologia psiquiátrica, especialmente no que se refere ao reconhecimento e abordagem do comportamento suicida. Este comportamento passou a ser classificado como um transtorno independente, podendo estar presente em comorbidade com qualquer diagnóstico e não mais exclusivamente como um especificador dentro dos transtornos do humor.

O DSM-5-TR introduz uma abordagem mais matizada para a avaliação do risco de suicídio, reconhecendo a complexidade e a multifatorialidade deste fenômeno. Diferentemente das edições anteriores, ele enfatiza a importância de considerar fatores de

risco específicos e sinais de alerta, como histórico de tentativas anteriores, presença de transtornos mentais, uso de substâncias, e situações de vida estressantes. Além disso, o manual propõe uma categorização mais detalhada dos comportamentos suicidas, distinguindo entre ideação suicida, planejamento, tentativas e automutilação sem intenção suicida. Esses avanços reforçam a possibilidade de incluir a avaliação do risco de suicídio como desfecho primário dos estudos,enfatizando uma abordagem mais sistemática e padronizada para sua avaliação, que gerará no futuro mais dados sobre tratamentos que atuam diretamente na redução do risco.

Contudo, os tratamentos atuais para transtornosdo humor apresentam limitações, como as respostas,muitas vezes,lentas e insuficientes com antidepressivos, especialmente em pacientes idosos com depressão maior e risco de suicídio elevado (Szanto *et al*., 2003). Recentemente, a cetamina emergiu como uma potencial alternativa terapêutica para a depressão e a ideação suicida, mas os resultados dos estudos ainda são conflitantes. Domany *et al*. (2020) demonstraram que a cetamina é segura e eficaz para a rápida redução da ideação suicida em indivíduos deprimidos com alto risco de suicídio. Por outro lado, Ionescu *et al*., (2019) encontraram resultados opostos, nos quais doses repetidas e não escalonadas de cetamina não superaram o placebo. Assim, a necessidade de uma padronização de protocolo terapêutico e a identificação de biomarcadores periféricos específicos para o diagnóstico e monitoramento da resposta ao tratamento se torna fundamental.

Portanto, dados os desafios para um novo tratamento para a depressão e a ideação suicida aguda, bem como a importância da descoberta de novos biomarcadores, estudamos o impacto da cetamina na ideação suicida em uma amostra de adultos com depressão. Nossa hipótese é que a cetamina possa desempenhar um papel significativo no tratamento da depressão em pacientes com ideação suicida e/ou comportamento suicida, quando os tratamentos convencionais se mostram ineficazes. A cetamina, através de seus mecanismos de ação sobre receptor N-metil-D-aspartato (NMDA) e neuroplasticidade, poderia reduzir os sintomas depressivos e a ideação e/ou comportamento suicida em pacientes em episódio agudo, e podemos medir estes desfechos através de critérios das escalas de humor e suicidabilidade como a Escala de Avaliação da Depressão de Hamilton com 17 itens (HAM-D17), Escala de Avaliação da Depressão de Montgomery-Åsberg (MADRS) e Escala de Avaliação do Risco de Suicídio de Columbia (C-SSRS).

1.1 EPIDEMIOLOGIA DA DEPRESSÃO

Estima-se que aproximadamente 280 milhões de indivíduos globalmente são afetados pela depressão, representando cerca de 3,8% da população mundial, conforme dados (*World Health Organization*, 2023). Esta prevalência inclui 5% da população adulta, com uma distribuição de 4% entre os homens e 6% entre as mulheres, e eleva-se para 5,7% entre indivíduos com idade superior a 60 anos.

Um estudo da OMS (2004) estimou que em torno de 6% da população adulta dos EUA apresentaram um episódio de transtorno depressivo durante os doze meses anteriores à publicação da pesquisa, sendo portanto uma doença altamente prevalente na população (*World Health Organization*, 2004). No Brasil, a OMS estima que este transtorno mental afeta cerca de 5,8% da população do país (*World Health Organization,* 2017). Essa condição atinge mais mulheres (cerca de duas vezes mais), devido a alterações hormonais, estressores psicossociais e à maternidade (Kessler *et al*., 2003; Lam, 2012; Schuch *et al*., 2014). No que diz respeito às comorbidades, também há uma diferença entre os gêneros: as mulheres com depressão apresentam níveis significativamente maiores de ansiedade generalizada, bulimia e somatização, enquanto os homens normalmente são mais afetados por transtorno relacionado ao uso de substâncias (Schuch *et al*., 2014). A idade média do primeiro episódio depressivo maior é de 27 anos, podendo variar de acordo com o gênero do sujeito e a exposição a estressores (Lam, 2012). Além disso, pacientes que sofrem depressão grave apresentam altas taxas de morbidade e de mortalidade, com profundas consequências econômicas e sociais (Nemeroff; Owens, 2002; Carvalho, 2017).

Já o TB é uma condição psiquiátrica que afeta cerca de 60 milhões de pessoas globalmente, independente de etnia, nacionalidade ou status socioeconômico (*World Health Organization,* 2020), implicando uma diminuição significativa na qualidade de vida, funcionamento e expectativa de vida, reduzindo-a em 8 a 12 anos (Wollenhaupt-Aguiar *et al*., 2021). Dados mundiais indicam variações na prevalência do TB, com 0,6% para o TB tipo I e 0,4% para o TB tipo 2 em 11 países estudados (Merikangas *et al*., 2011). No Brasil, Moreno *et al*. (2005) reportaram prevalências de 1% para TB tipo I, 1,1% para TB tipo 2 e 6,6% para o espectro bipolar. Rowland & Marwaha (2018) mostram prevalência de cerca de 1% para o TB tipo 1, com variações regionais significativas. Nos Estados Unidos, a prevalência é aproximadamente 1%, enquanto uma pesquisa na Inglaterra indicou uma prevalência de 2%. Uma meta-análise revelou prevalências de 1,06% para TB tipo 1 e 1,57% para TB tipo 2, principalmente nas Américas (Clemente *et al*., 2015).

Na década de 1990, o TB foi responsável por causar a incapacitação de 6,6 milhões de pessoas ao redor do mundo e, em 2013, esse número subiu para cerca de 9 milhões. Passou de 76ª para a 19ª principal causa de doenças incapacitantes e, de 54ª para 16ª principal causa de mortalidade e de morbidade prematura durante os anos em que o paciente se encontra incapacitado. Entre 1990 e 2013, o número de casos subiu de 32,7 milhões para 48,8 milhões de pessoas afetadas, o que equivale a um aumento de 49,1% na prevalência da doença (Ferrari *et al*., 2016), sendo estimado que hoje cerca de 1 - 4 % da população é afetada pela doença (Saunders; Geddes, 2016).

1.2 DEPRESSÃO UNIPOLAR

O termo depressão unipolar, apesar de não ser um diagnóstico específico do DSM-V-TR, será utilizado aqui didaticamente para se referir ao diagnóstico de episódio depressivo maior quando ele ocorrer no curso longitudinal de um TDM. O mesmo é uma doença mental frequente e tratável, tendo como sintomas mais comuns um humor deprimido de longa duração, sentimentos de culpa, ansiedade e pensamentos recorrentes sobre morte e suicídio. O diagnóstico de episódio depressivo maior é caracterizado por uma diminuição do humor ou uma incapacidade de experimentar prazer, ou ambos, combinada com sintomas cognitivos e vegetativos e ocorrência de sofrimento ou prejuízo ao indivíduo (*American Psychiatric Association, 2022*). É caracterizado pela presença de um ou mais episódios de depressão maior, sem história de mania ou hipomania. A taxa de suicídio por TDM pode atingir os 15%, sendo superior nos pacientes com idade superior a 55 anos. Por vezes, oTDM é precedido pelo Transtorno Distímico (10 a 25% dos casos). Os pacientes com estados físicos gerais crônicos ou graves têm maior risco de desenvolver TDM e o seu prognóstico é também afetado por este transtorno. O Transtorno Distímico é diferenciado do TDM pela cronicidade, pela leveza e pela persistência dos sintomas, isto é, pelo menos dois anos de humor depressivo, durante mais de metade dos dias, acompanhado ou não por sintomas depressivos adicionais que não preenchem os critérios para TDM.

A etiologia da depressão é multifatorial e não totalmente compreendida, sendo explicado por meio do efeito cumulativo de fatores: genéticos (diversos genes que podem conferir suscetibilidade ao desenvolvimento de TDM, porém de baixo efeito (Sullivan; Neale; Kendler, 2000); ocorrência de eventos adversos na infância ou eventos estressores relacionados a adversidades do dia a dia (abuso sexual ou psicológico, baixo suporte social,

divórcio (Hasler, 2010) e biológicoscomo alterações em sistemas de neurotransmissão, no ritmo circadiano, em fatores neurotróficos e em vias relacionadas ao estresse oxidativo e nitrosativo, bem como desregulação do eixo Hipotálamo-Hipófise-Adrenal (HHA) e disfunção mitocondrial (Hasler, 2010; Moylan *et al*., 2013).

1.3 DEPRESSÃO BIPOLAR

O termo depressão bipolar, apesar de não ser um diagnóstico específico do DSM-5-TR, será utilizado aqui didaticamente para se referir ao diagnóstico de episódio depressivo maior quando ele ocorrer no curso longitudinal do TB. O TB é uma doença psiquiátrica crônica caracterizada por alterações frequentes e recorrentes de humor, que variam entre episódios maníacos,hipomaníacos e depressivos (Anderson *et al.,* 2012). Entre a ocorrência dos episódios maníacos ou hipomaníacos e depressivos, o indivíduo com TB passa por períodos de eutimia, caracterizados por serem períodos em que o paciente apresenta remissão dos sintomas (Souza, 2005).

O episódio maníaco consiste em um estado de humor persistentemente elevado, expansivo ou irritável e aumento da energia ou da atividade dirigida a objetivos, com duração mínima de uma semana ou se houver necessidade de hospitalização (*American Psychiatric Association*, 2022). Em geral, o paciente em episódio maníaco apresenta sintomas como desorganização e impulsividade comportamentais, energia em alta, agitação psicomotora, redução significativa da necessidade de sono, autoestima inflada ou grandiosidade, fuga de ideias e envolvimento excessivo em atividades com elevado potencial para consequências dolorosas (*American Psychiatric Association, 2022*). A hipomania, por sua vez, consiste em um estado de euforia com sintomas semelhantes à mania, porém com alterações leves e duração mínima de quatro dias, geralmente não envolvendo um estado a ponto de causar prejuízos funcionais acentuados no âmbito familiar, social e ocupacional ou haver necessidade de internação (*American Psychiatric Association*, 2022; Belmaker, 2004).

No episódio depressivo maior, o paciente entra em estado de tristeza profunda, lentidão psicomotora, inibição do pensamento, exclui-se do convívio social e pode apresentar ideias suicidas ou cometer o suicídio. Dessa forma, o TB apresenta uma alta taxa de mortalidade decorrente dos episódios depressivos. A taxa de suicídios entre casos com TB é vinte vezes maior, quando comparada com a população em geral, com taxas de suicídio de 390/100.000 por ano e com risco de 23,4% ao longo da vida (Porto *et al*., 2005; Nery-Fernandes *et al*., 2012).

No Episódio com Características Mistas, os sintomas maníacos e depressivos co-ocorrem e pelo menos 3 sintomas do polo oposto em cada fase especifica são necessários para o diagnóstico. No DSM-5-TR, os episódios mistos deixaram de ser uma classificação independente e passaram a ser considerados especificador de curso da doença tanto no episódio depressivo quanto do episódio de mania, i.e., episódio depressivo com características mistas. Para o diagnóstico, é necessário preencher os critérios para o episódio de humor predominante, depressivo ou de mania, e ao mesmo tempo apresentar 3 sintomas do polo opostopelo mesmo período de tempo. É comum que o paciente apresente rápida alternância de humor e sintomas como hiperatividade, desregulação do apetite, alteração do sono, e comportamento suicida (*American Psychiatric Association, 2022).*

Quanto à fisiopatologia, o TB tem como contribuintes tanto fatores herdados como baseados em experiências, sendo que os últimos, em que se incluem trauma psicológico no início da vida, insultos fisiológicos (infecções no Sistema Nervoso Central (SNC), uso ou abuso de drogas e trauma cerebral ou vascular) são melhores compreendidos do que os primeiros (Bowden *et al*., 2008). Alterações também têm sido relatadas em vias biológicas envolvidas com sistemas de neurotransmissores, neurotrofinas, inflamação, sinalização de cálcio, estresse oxidativo e nitrosativo, desregulação do eixo HHA, transporte e bioenergética celular, cronobiologia, entre outras (Sigitova *et al*., 2017). Esta tese tem foco no estudo das alterações inflamatórias que serão descritas em item específico adiante. No entanto, assim como para a maioria das doenças psiquiátricas, diversos genes de suscetibilidade de baixo efeito são identificados, porém até o momento não são conhecidos marcadores biológicos específicos para o TB (Harrison; Geddes; Tunbridge, 2018).

1.4 SUICÍDIO

O termo "suicídio" é definido desde o século XVI como o ato de tirar a própria vida (Werlang; Botega, 2004). No entanto, essa definição pode abranger uma gama de atos, incluindo negligência em face da doença e *overdose* de drogas. Silva Filho (2009) propõe uma definição mais específica, Na qual, suicídio é um ato executado com a intenção clara de morrer, através de um método que o indivíduo acredita ser fatal. Este conceito exclui situações em que a intenção de morrer não é clara. Portanto, o suicídio é definido como um ato voluntário e consciente que leva à morte. No entanto, a voluntariedade pode ser questionada, uma vez que, muitas vezes, indivíduos que tentam o suicídio expressam simultaneamente desejos de viver e de morrer. Além disso, o termo "consciente" também

pode ser ambíguo, pois o ato suicida pode ocorrer em estados de confusão ou alterações de consciência (Cassorla, 1998). Para reduzir a ambiguidade, Werlang e Botega (2004), propõem a definição de suicídio como um dano fatal autoinfligido de maneira intencional e consciente, mesmo que de forma vaga ou ambígua. Os comportamentos que indicam risco de suicídio, conhecidos como "continuum dos comportamentos suicidas", englobam uma ampla gama de ações que aumentam em gravidade, exigindo investigação minuciosa.

O "continuum dos comportamentos suicidas" é uma classificação que considera a intencionalidade e letalidade dos atos suicidas. A intencionalidade refere-se ao desejo do indivíduo de terminar com a própria vida, enquanto a letalidade é uma conotação clínica que se refere à gravidade médica das consequências do ato (Werlang; Botega, 2004). A letalidade pode ser classificada em sete níveis, variando desde a expressão verbal de desejo de suicídio até o suicídio propriamente dito. No entanto, este continuum não é uma regra fixa, já que é possível que ocorram tentativas de suicídio ou suicídios sem passar por todas essas "etapas" (Coelho; Mello-Santos; Wang, 2011). As tentativas de suicídio não planejadas são comuns, especialmente entre jovens, indivíduos com transtornos de personalidade, usuários de substâncias e após eventos estressantes.

Destaca-se a indispensabilidade das ferramentas de avaliação quantitativa, sobretudo no âmbito do comportamento suicida, uma temática de elevada complexidade e gravidade.A *Columbia-Suicide Severity Rating Scale* (C-SSRS), desenvolvida em 2007 por Kelly Posner e colaboradores, se destaca como padrão ouro no campo de estudo do suicídio. Endossada por diversas agências internacionais de acreditação, como o Centro de Controle e Prevenção de Doenças (CDC) e a *Food and Drug Administration* (FDA), a C-SSRS é um instrumento valioso para a análise precisa de questões relacionadas ao comportamento suicida (Posner *et al*., 2011).

Em 2022 a APA, publicou uma revisão do DSM-5 para o DSM-5-TR, na qual trouxe mudanças significativas na maneira como os profissionais de saúde mental abordam e entendem o suicídio. Estas alterações refletem um avanço na pesquisa e na compreensão clínica deste fenômeno complexo e multifacetado:

a) *Avaliação Quantitativa e Qualitativa do Risco de Suicídio:* foi introduzida uma abordagem mais rigorosa para a avaliação do risco de suicídio, enfatizando a necessidade de uma análise quantitativa e qualitativa. Isso envolve a utilização de escalas validadas, combinadas com uma avaliação clínica detalhada dos fatores de risco, como histórico psiquiátrico, comorbidades e fatores psicossociais;
b) *Classificação Detalhada dos Comportamentos Suicidas:* adota uma taxonomia

mais refinada para classificar os comportamentos suicidas. A diferenciação entre ideação, planejamento e tentativas de suicídio é delineada com critérios específicos, baseados em evidências empíricas. Isso permite uma categorização mais precisa, essencial para estudos epidemiológicos e para o desenvolvimento de intervenções clínicas baseadas em evidências;

c) *Especificação da Ideação Suicida:* alinhada com as diretrizes de pesquisa contemporâneas, que enfatizam a importância de entender a ideação como um espectro, variando em intensidade e frequência. A avaliação clínica detalhada da ideação suicida é crucial para identificar indivíduos em alto risco e para o desenvolvimento de intervenções preventivas;
d) *Avaliação de Planejamento e Tentativas de Suicídio:* fornece critérios mais rigorosos para avaliar o planejamento e as tentativas de suicídio, incluindo a consideração da letalidade do método, a determinação da intencionalidade e a presença de um plano concreto. Essa abordagem é alinhada com as práticas de avaliação de risco de suicídio baseadas em evidências, que são fundamentais para o manejo clínico eficaz;
e) *Distinção entre Automutilação e Comportamento Suicida:* a distinção entre automutilação sem intenção suicida e comportamentos suicidas no DSM-5-TR é um avanço importante, refletindo a necessidade de abordagens terapêuticas diferenciadas. Esta distinção é apoiada por pesquisas que demonstram diferenças neurobiológicas e psicopatológicas entre esses comportamentos;
f) *Implicações para Pesquisa e Prática Clínica:* as mudanças têm implicações diretas para a pesquisa em saúde mental e para a prática clínica. Elas facilitam a realização de estudos epidemiológicos mais precisos e o desenvolvimento de intervenções clínicas baseadas em protocolos específicos para diferentes tipos de comportamentos suicidas. Além disso, essas mudanças promovem uma maior precisão no diagnóstico e no planejamento terapêutico, essenciais para o manejo eficaz do risco de suicídio.

1.4.1 Epidemiologia do suicídio

O suicídio, caracterizado como um problema de saúde pública de notável gravidade, é uma ação complexa interligada a uma variedade de fatores. O estudo *Global Burden of Disease* classifica o suicídio como a 15ª causa de morte, ressaltando que o suicídio é de duas a

vinte vezes mais prevalente que o homicídio na maioria dos países. Em 2019, o suicídio representou cerca de 1,3% do total de mortes, com taxas variando de 0,5 a 1% no Brasil (Roth *et al*., 2018). É crucial reconhecer que, embora as estatísticas globais de suicídio tenham se mantido relativamente estáveis, é consensual entre especialistas que muitas mortes por suicídio são ocultadas ou subnotificadas devido a fatores socioculturais e religiosos. De acordo com a OMS, mortes por suicídio são frequentemente categorizadas de maneira errônea como "mortes de intenção indeterminada" ou "acidentes". Ademais, a falta de registros vitais confiáveis em muitos países e a precisão questionável dos dados fornecidos pelos Estados-Membros à OMS complicam ainda mais a quantificação precisa do suicídio. Dentre os 172 Estados-Membros da OMS, apenas 60 possuem dados de registro vital de qualidade adequada para estimar as taxas de suicídio.

No que diz respeito à evolução das taxas de suicídio globais, os dados apresentam uma variação considerável. Muitos países apresentaram redução das taxas desde 1990, enquanto outros, incluindo alguns na América do Sul, como Uruguai e Paraguai, registraram um aumento. Especificamente no Brasil, houve um crescimento leve, mas perceptível, no número de mortes por suicídio entre 2015 e 2019. O Uruguai, por outro lado, registrou um aumento significativo durante o período de 2013 a 2019.

1.4.2 Comportamento Suicida em Pacientes Psiquiátricos

Os transtornos de humor são extensivamente reconhecidos entre os principais fatores de risco para o suicídio. Mortensen *et al*., (2000), após estudo realizado na Dinamarca, observaram que a hospitalização psiquiátrica recente era o fator mais fortemente associado a suicídio. Esse achado reforça a ideia de que o transtorno mental grave é um dos principais indicadores de risco de suicídio. Em 2002, Bertolote e Fleischmann realizaram uma revisão sistemática, na qual observaram que 98% dos que morreram por suicídio tinham um transtorno mental. A figura 1, retirada do artigo mencionado, fornece detalhes sobre os tipos de transtornos mentais e os transtornos de humor, representam a maior parcela dos transtornos diagnosticados em casos de suicídio, notadamente para aqueles indivíduos que foram diagnosticados sem internação hospitalar. Os autores também observaram que as as “abordagens gerais” provavelmente não são sólidas e cada transtorno mental listado precisa de uma abordagem terapêutica diferente.

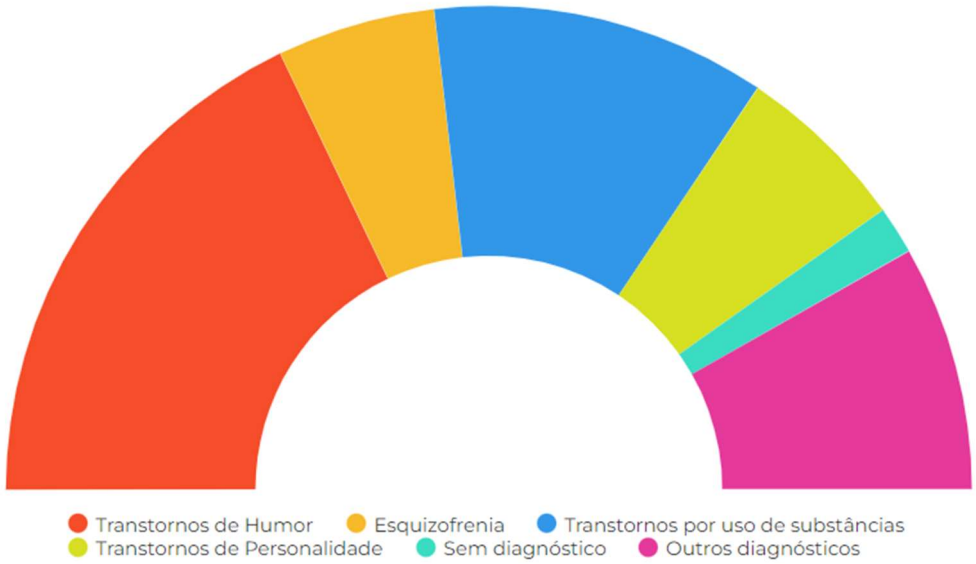

Figura 1 - Suicídio e transtornos mentais: distribuição dos diagnósticos na população em geral.

Dados originados de uma revisão sistemática que incluiu 31 artigos (15629 casos de suicídio) na população em geral, publicados entre 1959 e 2001 em todo o mundo.

Fonte: Adaptado de Bertolote e Fleischmann (2002).

Posteriormente, em 2010, foi visto que os transtornos mentais e por uso de substâncias foram responsáveis por dois terços dos suicídios e, quando considerado o déficit pelo suicídio, passaram da quinta para a terceira principal causa de prejuízo por doenças (Ferrari *et al*., 2014).

O risco de suicídio aumenta mais de vinte vezes em indivíduos com TDM, e é ainda maior em sujeitos com comorbidade com outros transtornos psiquiátricos ou doenças clínicas (Lönnqvist, 2000). Dados de autópsia mostram que, aproximadamente a metade dos indivíduos que faleceram por suicídio estava sofrendo de depressão (Rich; Young; Fowler, 1966). Lee *et al*. (2007) observaram que, comparado aos transtornos de ansiedade generalizada, o diagnóstico de TDM esteve associado a uma razão de chances cerca de dez vezes maiores.

Também é observado que diversos fatores de risco para suicídio no TB se sobrepõem àqueles para a população em geral. A tentativa prévia e a história familiar de suicídio parecem estar entre as variáveis preditivas mais relevantes para a realização desse ato. Entre os pacientes com TB, os homens, no início do curso do TB do tipo I, apresentam o maior risco. Não estar em tratamento e ter alta hospitalar há menos de três meses também aparecem como fatores de risco (Pompili *et al*., 2013). No entanto, ainda que compartilhem fatores de risco, as pessoas com TB apresentam risco de suicídio de 20 a 30 vezes maiores que a população em

geral e o suicídio está entre as três principais causas de morte nesses pacientes, junto com câncer e doenças cardiovasculares (Pompili *et al*., 2013).

As causas do comportamento suicida são múltiplas e complexas. Embora a presença do TB seja um fator predisponente importante, a existência dessa patologia por si só não é suficiente para explicar completamente o comportamento suicida, sem a interação com outros fatores, como presença de desesperança, de impulsividade e de agressividade, entre outras. Os preditores clínicos do comportamento suicida não são, geralmente, robustos, o que significa que não são reprodutíveis para diferentes amostras de pacientes ou para um determinado paciente individualmente, em parte porque o suicídio e o comportamento suicida são resultados de uma combinação de fatores de risco individuais, além da influência da ocorrência de estressores e das características da doença naquele determinado momento da vida do indivíduo (Goodwin *et al*., 2007). Portanto, o manejo da depressão e a prevenção do suicídio continuam sendo um grande desafio para clínicos e pesquisadores. Estudos prospectivos são fundamentais para compreender e aprimorar as estratégias de avaliação, de detecção e, principalmente, de prevenção do suicídio nesses indivíduos.

1.5 ETIOLOGIAS E ASPECTOS NEUROBIOLÓGICOS

Investigações neurobiológicas de pacientes suicidas adultos relataram decréscimo de metabólitos serotoninérgicos no fluido do SNC. A serotonina, neurotransmissor crucial para humor e cognição, e a norepinefrina, aparecem diminuídas em tais casos, desencadeando estados depressivos, devido à produção insuficiente, reabsorção excessiva na fenda sináptica ou falha do sistema receptor (Coelho; Mello-Santos; Wang, 2011; Kutcher; Chehil, 2007). Indivíduos deprimidos com histórico de suicídio apresentam níveis mais baixos de 5-hidroxi-triptamina (5-HT), quando comparados com deprimidos sem histórico semelhante (Mann; Oquendo; Arango, 1999).

A depressão é uma condição multifatorial, oriunda da interação entre suscetibilidade endógena e estressores ambientais. Segundo o DSM-V, a herdabilidade é de 40%, com traços de personalidade neurótica contribuindo para essa suscetibilidade genética. Pessoas com familiares de primeiro grau com depressão têm duas a quatro vezes mais chances de desenvolver a doença. Em um estudo, a herdabilidade da depressão maior foi de 42% (Edvardsen *et al*., 2009; Juruena *et al*., 2015; Shorter *et al*., 2007). O TB também é

multifatorial e desencadeado pela interação entre fatores ambientais e genéticos. O consumo de álcool, a dependência de drogas, traumas precoces e eventos indesejáveis significativos influenciam seu desencadeamento e evolução (AAS *et al*., 2014; Goodwin *et al*., 2012; Kerner, 2014). A literatura sugere alta herdabilidade do TB, com uma taxa de recorrência variando de 40 a 70% em gêmeos monozigóticos e de 5 a 10% em familiares de primeiro grau (Berçot, 2018; Potash *et al*., 2000; Smoller *et al*., 2003).

1.5.1 A Neuroinflamação no episódio depressivomaior

As alterações em vias biológicas relevantes, como sistemas de neurotransmissores, neurotrofinas, inflamação, sinalização de cálcio, estresse oxidativo e nitrosativo, desregulação do eixo HHA, transporte e bioenergética celular, e cronobiologia, são cruciais para a compreensão do TDM (Sigitova *et al*., 2017).

A ativação de micróglia e a infiltração de células imunes periféricas causam stress e lesão neuronal, gerando danos progressivos no SNC. O controle dessa atividade inflamatória é crucial em diferentes estágios da doença para o desenvolvimento de terapias neuroprotetoras apropriadas.A neurodegeneração nos TDM está associada à geração de espécies reativas de oxigênio e nitrogênio, disfunção mitocondrial e excitotoxicidade glutamatérgica. No entanto, os principais reguladores da desregulação cerebral no TDM ainda não estão bem definidos.

Varias doenças neuropsiquiatricas, incluindo TDM e comportamento suicida, apresentam alterações na regulação epigenética. Medicações e tratamentos de neuromodulação agem sobre esses mecanismos epigenéticos desregulados, como ilustrado pelo ácido valpróico, risperidona, clozapina e imipramina (Wang *et al*., 2018).

A inflamação é uma resposta imune essencial do organismo a estressores. As citocinas são a chave para coordenar essa resposta, mas o equilíbrio é fundamental para evitar danos maiores causados pelo próprio processo inflamatório (Alvaro-González *et al* 2002; Poon *et al*., 2013). Os processos inflamatórios podem causar danos teciduais e alterar a função celular, como visto em doenças autoimunes. Anteriormente, acreditava-se que o SNC estava protegido de processos inflamatórios periféricos, mas estudos recentes mostram que a inflamação pode aumentar a permeabilidade da barreira hematoencefálica e influenciar o SNC (Stolp *et al*., 2005).

Desde os anos 90, os pesquisadores estudam a relação entre inflamação e doenças mentais (Maes, 1995; Smith, 1991). Pacientes com doenças mentais, como depressão, apresentam as principais características de uma resposta inflamatória (Maes, 1999; Miller *et*

al., 2019). A inflamação, especialmente no SNC, parece estar intimamente relacionada a transtornos psiquiátricos (Passos *et al*., 2015; Stein *et al*., 2018).Estudos recentes também sugerem que a resposta do sistema imunológico inato pode ser ativada em situações adversas sociais (Slavich; Cole, 2013). Com base nisso, pesquisadores começaram a investigar o papel da inflamação nas doenças mentais (Soskin *et al*., 2012). As citocinas, moléculas sinalizadoras do sistema imune, são cruciais para a comunicação de astrócitos e células da glia no cérebro. Razões leucocitárias, tradicionalmente usadas como marcadores em doenças inflamatórias, têm sido progressivamente incluídas no estudo da progressão de transtornos mentais, como TDM e esquizofrenia, e também como marcadores de resposta terapêutica (Kapczinski *et al*., 2017; Özdin; Böke, 2019; Yüksel *et al*., 2019).

Dada a crescente evidência de uma ligação entre inflamação e depressão, há um interesse considerável na identificação de novos alvos terapêuticos e estratégias de tratamento. Vários estudos recentes sugerem que a modulação do sistema imunológico pode ser uma abordagem promissora para o tratamento da depressão. Por exemplo, foi descoberto que um conjunto de genes relacionados ao sistema imunológico e à inflamação estava superexpresso em pacientes com depressão grave (Leday *et al*., 2018). Além disso, foi observado que o tratamento com antagonistas de proteínas inflamatórias (TNFα) pode melhorar os sintomas depressivos em pacientes com biomarcadores inflamatórios elevados (Raison *et al*., 2013). Assim, os marcadores inflamatórios podem ter um papel importante na personalização do tratamento antidepressivo no futuro, permitindo a identificação de pacientes que provavelmente responderiam ao tratamento anti-inflamatório (Slavich; Cole, 2013).

1.5.2 Sistema Glutamatérgico na Fisiopatologia no episódio depressivo maior

O glutamato, principal neurotransmissor excitatório no SNC, é crucial para a neuroplasticidade (Pittenger; Sanacora; Krystal, 2007). A neurotransmissão glutamatérgica ocorre via receptores ionotrópicos, como o α-amino-3-hidroxil-5-metil-4-isoxazol-propionato (AMPA) e o NMDA (Deutschenbaur *et al*., 2016; Johansen *et al*., 2011; Pape; Pare, 2010).

No contexto doTDM, a hipótese glutamatérgica sugere que o estresse crônico promove uma elevação de glicocorticóides, que ativam neurônios expressando receptores para estes, resultando em maior liberação de glutamato (Stein-Behrens; Lin; Sapolsky, 1994; Sanacora *et al*., 2012; Venero; Borrell, 1999). O acúmulo extracelular de glutamato e a ativação dos receptores NMDA extrassinápticos podem levar à disfunção na regulação do Fator

Neurotrófico Derivado do Cérebro (BDNF), comprometendo a plasticidade sináptica e mecanismos de sobrevivência celular (Pittenger; Duman, 2008).

Alterações no metabolismo do glutamato, observadas através de estudos com espectroscopia por ressonância magnética, foram associadas a transtornos de humor (Tokita; Yamaji; Hashimoto, 2012). Portanto, a disfunção na transmissão glutamatérgica pode ser um fator essencial na etiologia das doenças psiquiátricas (Deutschenbaur *et al*., 2016; Pittenger; Duman, 2008; Sanacora *et al*., 2012). Consequentemente, a modulação da liberação de glutamato ou antagonismo do receptor NMDA podem ser estratégias promissoras para o tratamento da depressão. Notavelmente, a cetamina, um antagonista do receptor NMDA, mostrou efeitos antidepressivos em estudos pré-clínicos e clínicos (Tokita; Yamaji; Hashimoto, 2012).

1.6 CETAMINA

A Cetamina, inicialmente identificada como CI-581 (Domino, 1980), é uma arilcicloalquilamina, análoga à fenciclidina, com potência decrescida em um décimo. Caracteriza-se pela lipossolubilidade e peso molecular de 238 Daltons (Oliveira *et al*., 2004). Foi administrada pela primeira vez em 1964 na Universidade de Michigan (Denomme, 2018). Atualmente, é comercializada como uma mistura racêmica de dois isômeros opticamente ativos: o S(+) (levógiro) e o R(-) (dextrógiro) (Kohrs; Durieux, 1998; Luft; Mendes, 2005). Cada isômero apresenta propriedades farmacológicas específicas, que afetam a ação do fármaco (Plenninger; Durieux; Himmelseher, 2002). O isômero S(+) tem potência anestésica três vezes maior e analgésica duas a quatro vezes superior ao isômero R(-) (White *et al*., 1985; Jaksch *et al*., 2002). Adicionalmente, o S(+) tem maior seletividade e afinidade pelo receptor NMDA (Hemelrijck; White, 1997; Plenninger; Durieux; Himmelseher, 2002), e costuma produzir menos efeitos adversos que o R(-) (Evers; Crowder, 2001).

A farmacocinética da cetamina apresenta uma alta lipossolubilidade e uma baixa capacidade de ligação às proteínas plasmáticas (até 30%), o que facilita a sua rápida passagem através da barreira hematoencefálica e a sua rápida distribuição pelo corpo (Rommanelii; Smith, 2002; Valadão, 2010). Este fármaco tem altas concentrações no tecido adiposo, no SNC, nos pulmões e no fígado, e concentrações mais baixas no coração, no músculo esquelético e no plasma (Silva *et al*., 2010). A meia-vida da cetamina depende da via de administração, mas é geralmente curta (1 a 3 horas) (Koob, 1992), com efeitos evidentes rapidamente e uma duração aproximada de 30 a 45 minutos (Jansen, 2000; Rommanelii;

Smith, 2002; Valadão, 2010).

A metabolização da cetamina é realizada principalmente pelas enzimas hepáticas CYP3A4, CYP2B6 e CYP2C9, que fazem parte do citocromo P450 (Hijazi; Boulieu, 2002). A cetamina é convertida no metabólito norcetamina, que é transformado em 4OH-cetamina, 5OH-cetamina e 6OH-cetamina pela ação das enzimas CYP2B6 e CYP2C9. O principal metabólito ativo da cetamina é a norcetamina, que tem entre um terço e um quinto da potência do fármaco original e pode produzir efeitos anestésicos e analgésicos prolongados (Morgan; Curran, 2011; Valadão, 2010). Os metabólitos da norcetamina são então hidroxilados em hidroxinorcetina e, em seguida, conjugados com ácido glicurônico para formar derivados de glicuronídeos hidrossolúveis que são principalmente excretados na urina (Hijazi; Boulieu, 2002).

Um estudo realizado por Loo *et al*. (2016), analisou a concentração de cetamina no sangue em pacientes psiquiátricos. Amostras de sangue foram coletadas no início do estudo e em vários pontos depois disso, com os resultados mostrando que as concentrações plasmáticas de cetamina estavam linearmente correlacionadas com a dosagem de cetamina. Os resultados também sugerem que todas as três vias de administração (endovenosa, intramuscular, SC) produziram efeitos antidepressivos comparáveis, com menos efeitos adversos observados quando a cetamina foi administrada por via subcutânea. A resposta antidepressiva, os efeitos adversos e as concentrações de cetamina foram relacionados à dose (<0,5 mg/kg).

1.6.1 Mecanismo de Ação da Cetamina

A cetamina é um fármaco com um mecanismo de ação complexo, sendo que a sua principal ação é como um antagonista do receptor NMDA, um dos três tipos de receptor de glutamato, que é o principal neurotransmissor excitatório no cérebro de mamíferos (Bannerman *et al*., 1995). O antagonismo dos receptores NMDA pela cetamina é responsável pelas suas principais propriedades, como a analgesia, os efeitos amnésicos e psicossensoriais, e a neuroproteção (Potter; Choudhury, 2014). A cetamina também interage com outros sistemas de neurotransmissores, embora com menos intensidade. Ela tem efeitos nos receptores muscarínicos de acetilcolina, nos receptores de ácido gama-aminobutírico (GABA), na liberação de dopamina e nos receptores opioides (Oye; Paulsen; Maurset, 1992; Rabiner, 2007). Na neurotransmissão monoaminérgica, a cetamina pode bloquear a recaptação neuronal e potencializar os efeitos das monoaminas, contribuindo para as suas

propriedades analgésicas secundárias não relacionadas ao receptor do tipo NMDA (Meller, 1996; Quibell *et al*., 2011).

A interação da cetamina com o sistema GABA é particularmente interessante. O GABA é o principal neurotransmissor inibitório no SNC e a cetamina potencializa os efeitos da inibição sináptica mediada pelo GABA. Isso resulta na hiperpolarização da membrana neuronal e no desenvolvimento de um potencial pós-sináptico inibitório, contribuindo para os efeitos sedativos da cetamina (Irifune *et al*., 2000). Além disso, também há relatos de que a cetamina possa agir através da modulação de outros sistemas, como o sistema de serotonina, no qualela pode interferir com a recaptação de serotonina e norepinefrina, contribuindo para os seus efeitos antidepressivos.

Em um estudo (Lasič *et al.,* 2019) apontou para um mecanismo adicional da cetamina em sua ação antidepressiva rápida, destacando o papel dos astrócitos. Foi observado que a cetamina eleva a concentração intracelular de cAMP ([cAMP]i) em astrócitos, diferentemente de seu efeito inibitório sobre a exocitose, o que sugere mecanismos distintos de ação. Além disso, a cetamina promove a redistribuição do colesterol na plasmalema de astrócitos, afetando a densidade e distribuição desses domínios ricos em colesterol. Essa redistribuição é específica para astrócitos e poderia influenciar a plasticidade sináptica e a atividade funcional de redes neuronais, através da modulação do fluxo de colesterol para neurônios e da atividade da adenilil ciclase.

Em sintése, a cetamina é um fármaco com múltiplos mecanismos de ação (figura 2). A sua principal delas é o antagonista do receptor NMDA, mas ela também influencia uma série de outros sistemas de neurotransmissores. Essa diversidade de ações é responsável pelo amplo espectro de efeitos clínicos da cetamina, que inclui a analgesia, efeitos amnésicos, psicossensoriais e a neuroproteção, entre outros (Irifune *et al*., 2000).

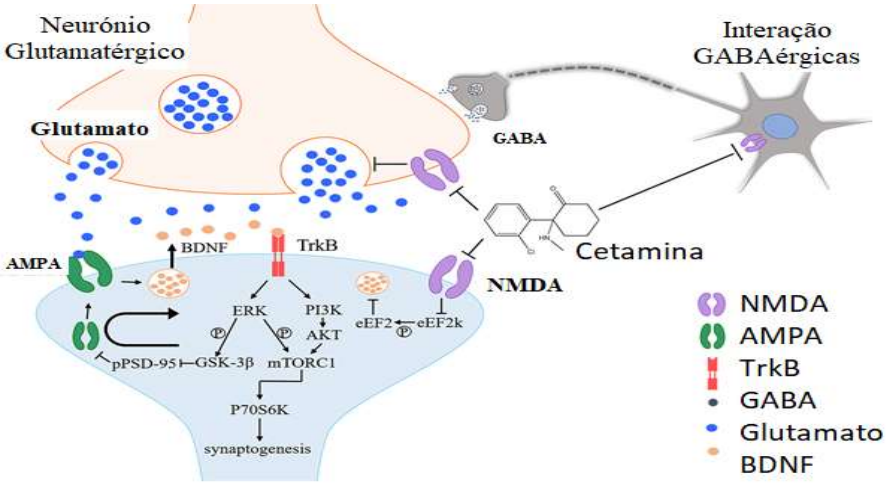

Figura 2 - Mecanismos propostos da cetamina atuando na plasticidade sináptica. As sinapses no sistema glutamatérgico e o ciclo glutamato-glutamina. O glutamato é originário da glutamina que, por ação da enzima glutaminase, é convertida a glutamato. Após ser liberado na fenda sináptica o glutamato pode atuar sobre seus sítios de ligação localizados nos receptores do tipo mGluR, cainato, AMPA e/ou NMDA. Posteriormente, na célula da glia, o glutamato é convertido novamente a glutamina pela ação da enzima glutamina sintetase, caracterizando o ciclo glutamatoglutamina. NMDA = receptor do tipo N-Metil D-Aspartato; AMPA = receptor do tipo α-amino-3-hidroxi-metil-5-4-isoxazolpropiônico; mGluR = receptor metabotrópico.
Fonte: Adaptado de Peng *et al.* (2020).

1.6.2 Cetamina no episódio depressivo maior

Os agentes moduladores de glutamato são eficazes no tratamento de transtornos de humor devido a suas alterações na neurotransmissão glutamatérgica, implicada na patofisiologia do TDM (Machado-Vieira *et al.*, 2015). Estes agentes atuam regulando a atividade neuronal através da modulação dos receptores ionotrópicos de glutamato - NMDA, AMPA, e KA. Pacientes com TDM comumente apresentam níveis elevados de glutamato, levando à superativação dos receptores NMDA e subsequente sobrecarga de íon cálcio no neurônio, potencializando o estresse oxidativo e a liberação de citocinas pró-inflamatórias (Altamura *et al.*, 1993; Hashimoto; Sawa; Iyo, 2007; Leonard; Maes, 2012; Mossner *et al.*, 2007; Stork; Renshaw, 2005;Yanning *et al.*, 2017). A cetamina antagoniza as ações do glutamato e exerce efeitos anti-inflamatórios, modulando os níveis de citocinas pró-inflamatórias no SNC (Cosman; Boyle; Porsteinsson, 2007; Garcia *et al.*, 2008; Yanning, *et al.*, 2017). Além disso, ela também atua nos receptores pré-sinápticos NMDA, localizados em regiões específicas do SNC, como interneurônios gabaérgicos, reduzindo a liberação de

GABA (Bouvier *et al*, 2015; Iadarola *et al*, 2015).

Estudos clínicos, desde 2000, evidenciam a capacidade da cetamina em reduzir rapidamente os sintomas de TDM (Berman *et al*., 2000; Zarate *et al*., 2006). As taxas de resposta à cetamina após 24 horas são comparáveis às relatadas após seis a oito semanas de tratamentos antidepressivos convencionais (Machado-Vieira *et al*., 2015). Estudos recentes também relatam o uso promissor de cetamina em pacientes com TDM e ideação suicida (Ionescu *et al*., 2016; Ionescu *et al*., 2019; Zhan *et al*., 2019; Domany *et al*., 2020), no qual foi observado que a infusão repetida de cetamina resultou em uma diminuição rápida e robusta da ideação suicida, que foi mantida por pelo menos três meses após a infusão final em alguns pacientes. Entretanto, a eficácia dessas doses requer investigação adicional.

2 JUSTIFICATIVA

O episódio depressivo maior, seja parte do TB ou do TDM, é uma psicopatologia que gera um impacto negativo na saúde e na funcionalidade destes indivíduos, principalmente na qualidade das relações interpessoais e na esferasocial. Estima-se que cerca de 16% e 1% da população irá apresentar algum episódio de depressão unipolar ou de TB, respectivamente, uma ou mais vezes durante a vida, conforme o National Comorbidity Survey Replication (Kessler *et al*., 2003), sendo que custos anuais pela perda de produtividade relacionada com a depressão nos EUA ultrapassem US$36 bilhões (Kessler *et al*., 2005), e essa depressão, em especial, pode levar ao suicídio.

Estudos demonstram que a depressão e o uso de substâncias foram responsáveis por dois terços dos suicídios em 2010 e, quando considerado o déficit pelo suicídio, passaram de quinta para terceira principal causa de prejuízo por doenças (Ferrari *et al*., 2014). Em 2019, dados da OMS, apontaram que 703.000 pessoas comentaram suicídio em todo o mundo, estimando-se que há outras 20 pessoas fazendo uma tentativa de suicídio. Entre jovens de 15 a 29 anos, o suicídio é a quarta principal causa de morte. Em 2019, na região das Américas, 97.339 pessoas morreram por suicídio. No Brasil, 0.5 - 1% das mortes foram por suicídio.

É também observado que o TDM de início precoce na idade adulta concentra-se em uma faixa etária de 18 a 30 anos (Zisook *et al*., 2004), sendo a idade média de início dos trantornos de humor de 25 a 32 anos (Kessler *et al*., 2005). Quando comparados ao TDM de início tardio (participantes com idade de início igual ou superior a 30 anos), os participantes com TDM de início precoce apresentam maior prevalência de tentativas de suicídio, maior duração da doença, maior número de episódios e gravidade dos sintomas (Liu *et al*., 2015), ou seja, o TDM é, sem dúvida, um transtorno multidimensional. Neste contexto, a neuroinflamação surge como um elemento de destaque, tendo em vista que seu papel na gênese de diversas doenças psiquiátricas tem sido cada vez mais reconhecido (Al-Atram, 2018; Al-Diwani *et al*., 2017).

Na procura por delimitações mais claras entre essas patologias, tem sido realizada a busca por marcadores periféricos, por meio de métodos não invasivos, como a coleta de sangue para pesquisar diferenças entre parâmetros bioquímicos que possam servir como auxílio no diagnóstico diferencial dessas doenças. Uma vez que o manejo da depressão e a prevenção do suicídio continuam sendo um grande desafio para clínicos e pesquisadores, estudos científicos prospectivos continuam sendo de extrema relevância para impulsionar o conhecimento nesta área, bem como a busca por novas e eficazes terapias que visam

biomarcadores são fundamentais para compreender e aprimorar as estratégias de avaliação, de detecção e, principalmente, de prevenção do suicídio.

Navegar pelos intricados desafios éticos e metodológicos que surgem na condução de pesquisas psiquiátricas efetivas para o tratamento da depressão e comportamento suicida é uma tarefa complexa. Esta realidade amplia o hiato existente na literatura científica, sublinhando a necessidade de investigações meticulosas e profundas na área. A cetamina emergiu de uma série de estudos como uma potencial ferramenta terapêutica para combater a depressão e a ideação suicida, exibindo resultados promissores no tratamento desses transtornos psiquiátricos. No entanto, um dos passos cruciais, para a implementação eficaz desta abordagem é a padronização de um protocolo terapêutico, simultaneamente à investigação de biomarcadores séricos, que têm o potencial de auxiliar no monitoramento da eficácia do tratamento. Esta abordagem multidimensional é necessária para promover a melhoria contínua, a evolução na terapia para a depressão e o comportamento suicida, bem como facilitar a execução de estudos controlados de larga escala.

3 OBJETIVOS

3.1 OBJETIVO GERAL

O objetivo geral desta tese é estudar a neurobiologia da depressão e seu tratamento, e, assim, foi estruturadaem três etapas interconectadas. A primeira etapa envolve a identificação de biomarcadores inflamatórios distintivos entre a Depressão de Início Precoce e Depressão de Início Tardio. A segunda etapa foca no desenvolvimento e na padronização de um protocolo de intervenção com cetamina, especificamente projetado para pacientes com sintomas depressivos e ideação suicida. A terceira e última etapa consiste na condução de um estudo naturalístico, aplicando o protocolo padronizado, com o objetivo de investigar o impacto terapêutico da cetamina na mitigação do risco de suicídio e na redução da sintomatologia depressiva nesses pacientes, através de uma abordagem multidimensional e baseada em evidências.

3.2 OBJETIVOS ESPECÍFICOS

a) Identificar Biomarcadores Distintivos: Realizar uma análise quantitativa para diferenciar biomarcadores inflamatórios específicos e identificar subgrupos de pacientes com TDM, como aqueles com início precoce ou tardio;

b) Desenvolver e Padronizar Protocolo com Cetamina: Formular e estabelecer um protocolo de intervenção terapêutica com cetamina, abordando a via subcutânea (SC) e avaliando critérios como durabilidade, efeito e tolerabilidade, com base em diretrizes clínicas e padrões farmacológicos;

c) Conduzir Estudo Naturalístico: Implementar um estudo naturalístico longitudinal, aplicando metodologias de pesquisa robustas para investigar o impacto terapêutico da cetamina na sintomatologia depressiva e no risco de suicídio.

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PARTE II

Metodologia e Resultados

4 METODOLOGIA E RESULTADOS

A metodologia adotada e os resultados obtidos nesta tese são articulados e apresentados na forma de capítulos individuais. Especificamente, os capítulos de 1 a 5 são estruturados como artigos científicos independentes, cada um fundamentado nos modelos teóricos e metodológicos descritos abaixo:

CAPÍTULO 1

Artigo: Earlier age of onset is associated with a pro-inflammatory state in major depressive disorder

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Earlier age of onset is associated with a pro-inflammatory state in major depressive disorder

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ABSTRACT

Major depressive disorder (MDD) is a common condition that affects the general population over a wide range of ages, regardless of gender and social background. Early-onset of MDD in adulthood, between ages of 18 and 30 years, is associated with worse outcomes and increased years of disability. Stress load and physical health have been associated with age of onset in MDD. We aim to investigate whether early onset MDD might be associated with changes in systemic inflammatory markers. We examined levels of following cytokines: IL-1β, IL-6, IL-10 and TNFα in 234 patients with MDD. Higher serum levels of TNFα and IL-1β are associated with the early onset of the disorder in patients with MDD. IL-6 levels were also higher in the early onset group and IL-10 levels were higher in the late onset group, but with no significant difference. Changes in the anti-inflammatory/pro-inflammatory balance have been described in mood disorders and may be implicated in its severity and pattern of progression. Our findings reinforce that higher serum levels of IL-1β and TNFα may be associated with the earlier onset subgroup of MDD patients. Future research that target inflammatory markers of immune modulation may be, key in the search for novel preventative therapeutics.

1. Introduction

Major depressive disorder (MDD) is a common condition that affects the general population over a wide range of ages, regardless of gender and social background. According to the National Comorbidity Survey Replication, 51% of the Americans will experience a mental health disorder during their lifetime, with some evidence that this proportion may be increasing in younger cohorts and half of these disorders starting at the age of 14 (Kessler et al., 2005). Thus, MDD in young adults warrants attention. In fact, MDD is currently the third cause of years lived with disability in the world (James et al., 2018) and is expected to become the second largest contributor to the overall disease burden by 2030 (Mathers and Loncar, 2006).

Early-onset MDD in adulthood is concentrated in an age group of 18–30 years (Zisook et al., 2004). The average age of mood disorders onset is between 25 and 32 years (Kessler et al., 2005) and almost half of adolescents with subliminal depressive symptoms in a community sample developed major depressive disorder in their first 30 years(Klein et al., 2009). These results were confirmed in samples of young adults using a onset age cut-off point at 30 years (Bukh, 2011). When compared to late-onset MDD (participants with onset age equal or greater than 30 years) the participants with early-onset MDD show higher prevalence of suicide attempts, of psychiatric comorbidities such as personality disorders, higher levels of neuroticism (Van Lang et al., 2007), longer duration of the disease, greater number of episodes and severity of symptoms (Liu et al., 2015).

In a large subgroup of patients, MDD is associated with or triggered by stress. Chronic stress, particularly early life stress, has long been

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associated with depressive symptoms. This association may be mediated in part by a dysfunction of the hypothalamic-pituitary-adrenal axis (HPA) (McEwen, 2000) which alters monoaminergic systems (Lichtblau et al., 2013). Deregulation of the HPA axis has been shown to be a vulnerability factor for MDD (Modell et al., 1998). This provided basis to the subsequent studies of depression and general inflammatory status. Since the 1990s, initial reports of neural-immune interactions, the action of pro and anti-inflammatory cytokines in brain cells have pointed to an important response associated with psychiatric symptoms (Brambilla, 1998; Dowlati et al., 2010; Primo and Alves, 2020; West and Maes, 1999). Several studies reported that patients with depression hiper-activated inflammatory pathways, with increased levels of pro-inflammatory cytokines (interleukin (IL) -1β (Thomas et al., 2005; Uint et al., 2019), IL-6 (Dentino et al., 1999; Ting et al., 2020), IL-8 (Kuzior et al., 2020; Zou et al., 2019) and tumor necrosis factor (TNFα) (Brymer et al., 2019; Moorman et al., 2007) and acute phase proteins, besides an increased expression of chemokines and adhesion molecules (Simon et al., 2008). Also, studies with monocytes from depressed patients have already demonstrated abnormalities in the production of cytokines (Lanquillon et al., 2000). Nevertheless, the findings about inflammatory changes in MDD are not universal and may be highly associated with subsets of depressive patients, but not all.

Therefore, the role of inflammation on the long-term neuropathology of depressive symptoms is even less clear. There are morphological and biochemical changes that occur in the brain of patients with MDD, which are associated with number of episodes, starting at the beginning of the disease and getting worse with its progression (Kunz et al., 2011). In addition, evidence points to an association of chronic depressive symptoms with dementia later in life (Byers and Yaffe, 2012; Cantón-Habas et al., 2020).

In summary, despite these important findings about the long-term course of depressive illness, and the evidence of inflammatory changes during depressive episodes, it is not clear yet if cytokines may be associated with MDD subtypes, such as earlier onset. Previous studies have suggested that inflammation may be restricted to subgroups of patients with MDD, such as melancholics (Haroon et al., 2012; Spanemberg et al., 2014). Our hypothesis is that patients who had an early onset of the disease (<30 years) will show different levels of pro-inflammatory interleukins compared with those with late-onset MDD. Thus, the aim of this study was to investigate if the early onset of MDD may be associated with greater serum levels of cytokines.

2. Methods

2.1. Participants

This study includes outpatients with a clinical diagnosis of MDD confirmed by the Mini International Neuropsychiatric Interview (MINI) who began treatment between 2005 and 2015 at the Mood Disorders Program of Hospital de Clínicas de Porto Alegre (PROTHUM-HCPA), in the city of Porto Alegre, southern Brazil.

Our inclusion criteria were patients: (1) aged 18 to 65 years; (2) diagnosed with MDD according to MINI; (3) score equal to or greater than 8 points on the HAM-D scale; (4) that were on regular care in outpatient clinic (PROTHUM, HCPA).

Our exclusion criteria were patients: (1) diagnosed with bipolar disorder; with schizophrenia; with schizoaffective disorder or with a primary diagnosis of substance use disorder; (2) with current unstable/unremitted or untreated systemic inflammatory disease; (3) currently on corticosteroids, immunosuppressants or immunomodulators.

The study was approved by the Research Ethics Committee of the Hospital de Clínicas de Porto Alegre (protocol no. 2016-0540). The authors state that all procedures that contribute to this work are in accordance with the ethical standards of the relevant national and institutional committees on experimentation in humans and with the Declaration of Helsinki in 1975, which was revised in 2008. All patients provided their written consent prior to inclusion in the study.

2.2. Demographic, behavioral and clinical assessments

A diagnosis of unipolar depression was carried out according to the Diagnostic and Statistical Manual of Mental Disorders, 4° Edition (DSM-IV) criteria and evaluated by the Mini International Neuropsychiatric Interview, Brazilian version (MINI-Plus) 5.0 Plus. The 17-item Hamilton Depression Rating Scale (HDRS-17) was used to assess the severity of major depression. General demographic and behavioral data were acquired using a standard questionnaire.

The early age of onset group included those with an onset of depressive symptoms before age 30 (Klein et al., 2009; Liu et al., 2015; Parker et al., 2003; Price et al., 1987; Zisook et al., 2004). This cut-off point was obtained from previous studies (Kessler et al., 2005; Klein et al., 2009) that have showed the average age of onset of mood disorders is between 25 and 32 years, with almost 50% of adolescents with depressive symptoms developing major depressive disorder by the age of 30 years. These results were confirmed in samples of young adults using an age onset cutoff at 30 years (Bukh, 2011). Information related to age at onset of depressive symptoms was obtained from the standard protocol and confirmed with the best information available (if necessary, medical record review, check with family and/or assistant physician).

The Cumulative Disease Assessment Scale (CIRS) was used to quantify the total impact of medical disorders. This scale consists of 14 disease categories relevant to different organ systems. Each category is evaluated from 0 (no problem) to 4 (extremely severe / necessary immediate treatment / severe deficiency in function). For this study, the total score was obtained by adding the scores of the first 13 categories (excluding psychiatric disorders). A clinical interview and a review of medical records were conducted to investigate the presence of diseases that could exclude the patient from the study.

Childhood trauma was assessed using the validated English version of the Childhood Trauma Questionnaire (CTQ), which has 28 items, which investigates five subscales (Grassi-Oliveira et al., 2006). Patients were grouped as "Emotional Abuse, Physical Abuse, Sexual Abuse, Emotional neglect, Physical neglect and Any abuse/neglect". The criteria, originally proposed by the authors (Bernstein, 1998; Bernstein et al., 1997), show that each subscale starts with "When I was growing up", and respondents indicate on a 5-point scale the frequency of the particular incident. Each 5-item subscale ranges from 5 (no history of abuse) to 25 (very extreme history of abuse).

To assess the severity of depressive symptoms, we used the HDRS-17 and the Beck Depression Inventory (BDI). The scale HDRS-17 was used (Hamilton, 1960), translated and adapted to Portuguese, with a structured interview guide (Moreno and Moreno, 1998). The scale consists of 17 items whose total score ranges from 0 to 52. Although the author has not proposed a standard cutoff point, in practice, scores above 24 are considered to identify a severe depressive episode; between 18 and 24, moderate depressive episode; between 7 and 17, mild depressive episode and, below 7, no depressive episode or remission (Blacker, 2000). The scale BDI (Beck et al., 1961) consists of a 21-item, range 0-3, self-report measure that assesses the presence and severity of depressive symptoms, identifying and quantifying mild, moderate and severe depression in both inpatients and outpatients, being the most widely used instrument for self-assessment of depressive symptoms in clinical research (Richter et al., 1998). For samples of patients with affective disorder, we used the recommended cutoff points as follows: <10, no depression or minimal depressive symptoms; from 10 to 18, mild to moderate depression; from 19 to 29, moderate to severe depression; and from 30 to 63 severe depression.

2.3. Blood collection and processing

Blood samples were collected from each patient and allowed to clot in blood collection tubes with no additive. Subsequently, whole blood

was centrifuged for 10 minutes at 1000 xg and serum was removed, aliquoted and stored at -80 °C until assayed.

2.4. Cytokine assay

Serum cytokine (IL-6, IL-1β, IL-10 and TNFα) were measured by multiplex immunoassay using the commercial kit ProcartaPlex™ Multiplex Immunoassay - Human Custom HS ProcartaPlex 4-plex (PPXS-04-MXEPTJ4) (Invitrogen, Austria). In brief, magnetic beads were added into all wells, the plate was washed, and each diluted standard and samples (without dilution) were pipetted into the appropriate wells and the plate was sealed and incubated on a plate shaker overnight at 4 °C. After that, the plate was washed followed by the addition of detection antibodies into each well and - after 30 min incubation with agitation at room temperature (RT) - streptavidin conjugated to the fluorescent protein phycoerythrin was added and the plate was incubated on the plate shaker at RT for 30 min. Thereafter, the plate was washed to remove the unbound streptavidin-phycoerythrin, reading buffer was pipetted into all wells and the beads were resuspended on a plate shaker for 5 min at RT. The beads (minimum of 50 beads per cytokine) were analyzed in the Luminex® 200 TM instrument, which monitored the spectral properties of the beads while simultaneously measuring the amount of fluorescence associated with phycoerythrin. Raw data (median fluorescent intensity, MFI) was analyzed using a 5-parameter logistic method to determine the concentrations of the analytes (TNFα, IL-6, IL-1β and IL-10) in each sample (Luminex Xponent software 3.1).

2.5. Statistical analysis

Participants with onset age equal or greater than 30 were classified as late onset. All cytokines' results were normalized for parametric analysis using the R package best Normalize (version 1.6.1). First, a Multivariate Analysis of Variance (MANOVA) was performed for an overall test of the degree to which cytokines levels showed differential multivariate profiles (more than one dependent variables) as a function of group. The alpha for this test was 0.05. To identify which cytokines was associated with the groups, we performed ANOVA using Bonferroni p-values correction. Cytokines significantly different between the groups were evaluated by linear regression controlling by age, education, ethnicity (Caucasian or not), autoimmune diseases and anticonvulsants, these variables were included in the model because they were significantly different between early and late onset. The depressive symptoms variable (total HDRS-17) was placed in the model because depressive symptoms are associated with interleukin levels (Zou et al., 2019) and the variable sex was analyzed considering the difference in the levels of interleukins between men and women (Engler et al., 2016).

Missing values and outliers were handled using the R package mice (version 3.11) with "pmm" method and R package rstatix (version 0.6). Shapiro-Wilk test was used to evaluate normal distribution. The t-student and Mann-Whitney tests were used to detect differences between groups in demographical and clinical variables. Participants with missing values (not detected) in at least one cytokine were excluded to perform MANOVA. Jasp and R (version 4.0.2) software were used to conduct the statistical analysis.

3. Results

Most of the sample (77.78%) were composed by female sex with similar proportion in both groups. Early onset MDD participants were younger and more frequently went to college or graduate school (Table 1).

The clinical characteristics of participants with early and late onset MDD are shown in Table 2. As expected, patients showed significant differences in the onset age of the disease ($p < 0.001$). The two groups were similar in terms of depression severity, impact of comorbid medical diseases and childhood trauma Fig. 1.

Table 1
Sociodemographic variables in patients with major depression.

Variable		Early onset (99)	Late onset (135)	p
Sex (female)		82,83% (82)	74,08% (100)	0.112
Age (yr) mean (S.D)		45.75 (12.65)	54.21 (8.25)	<0.001
Education	Elementary	31.96% (31)	47.41% (64)	0.017
	High School	44.33% (43)	40.74% (55)	
	College/ Graduate	23.71% (23)	11.85% (16)	
Ethnicity (Caucasian)		84.61% (77)	87.72% (100)	0.520

Fig. 2 shows the results for the levels of cytokines. For the analysis of cytokines, eight (TNFα=1, IL-1β=6 and IL-6=1) participants were excluded, because they had levels below the detection threshold in at least one cytokine. MANOVA indicated the cytokine's profile is different between early and late onset ($F=5.97$, $p < 0.001$), specifically in IL-1β ($p < 0.001$) and TNFα ($p < 0.001$) levels, as showed by ANOVA, in this analysis, eight participants were excluded for presenting levels below the detection threshold of the method in at least one cytokine (Tables 3 and 4).

Differences in IL-1β and TNFα levels remained relevant even when controlling for age, sex, educational ethnicity, Hamilton's total and autoimmune disease, age onset was significant, specifically, late onset was reducing TNFα (beta=-0.36, $p = 0.008$) and IL-1β concentrations (beta=-0.51, $p < 0.001$). Autoimmune diseases were significantly associated with increased levels of TNFα (beta = 0.61, $p = 0.019$) and IL-1β (beta = 0.76, $p = 0.003$) (Table 5).

4. Discussion

The present study showed that higher serum levels of TNFα and IL-1β (pro-inflammatory) are associated with the early onset of MDD. Regardless of the comorbidity with autoimmune diseases, a significant difference remained. Interestingly, IL-6 levels were also higher in the early onset group and IL-10 (anti-inflammatory) levels were higher in the late onset group, but with no significant difference possibly due to lack of power to detect medium or small effect sizes. Changes in the anti-inflammatory/pro-inflammatory balance have been largely implicated in the symptoms and progression of mood disorders.

These results support the hypothesis that an imbalance between pro-inflammatory and anti-inflammatory cytokines may be associated with subsets of depressive symptoms of earlier onset. We believe these findings provide a hint that early-onset MDD may be a particular subtype in which the pro-inflammatory state plays a greater role than in late-onset MDD. The prodrome conditions associated with this proinflammatory state remain to be investigated.

In a study (Bukh, 2011) which assessed the differences between patients with early and late onset MDD, there was a lower prevalence of stressful life events prior to the early onset of MDD compared to patients with late onset age. The authors hypothesized that early onset of MDD in adulthood may be associated with a higher level of neuroticism and a higher prevalence of comorbidities. Our results corroborate this finding, since serum cytokine changes occurred in early onset of MDD. Thus, the results point to etiological divergences not only between extreme age groups, such as childhood and geriatric depressions, but also between early adult onset and late adult depression.

Therefore, although the etiological factors that contribute to the

Table 2
Clinical characteristics of patients at early and late onset of major depression.

Variable	Early onset (99)	Late onset (135)	*p*
Onset age of illness. mean (S.D)	18.91 (6.13)	43.42 (8.79)	<0.001*
CIRS Mean (S.D)	4.18 (2.71)	4.02 (2.63)	0.735
HDRS 17 Mean (S.D)	21.21 (5.26)	21.42 (5.79)	0.781
BDI Mean (S.D)	36.11 (9.14)	36.57 (9.64)	0.718
Emotional Abuse	27.17% (25)	26.77% (34)	0.947
Physical Abuse	16.30% (15)	21.87% (28)	0.304
Sexual Abuse	10.99% (10)	9.45% (12)	0.710
Emotional neglect	14.13% (13)	13.39% (17)	0.874
Physical neglect	15.38% (14)	19.53% (25)	0.429
CTQ - Any abuse/ neglect	43.96% (40)	46.46% (59)	0.715
Smoking	24.49% (24)	27.07% (36)	0.659
Autoimmune disease	7.29% (7)	5.34% (7)	0.547
Medication Tricyclics	42.71% (41)	41.79% (56)	0.890
SSRI	68.75% (66)	77.61% (104)	0.131
BZDs	33.68% (32)	29.32% (39)	0.483
Lithium	14.58% (14)	14.18% (19)	0.931
Anticonvulsants	7.37% (7)	18.66% (25)	0.015*

onset of psychiatric symptoms are still poorly understood, early onset generally indicates greater general severity and may predispose the patients to particular features of the disorder which are associated with unfavorable outcomes (Bellivier et al., 2003; Tsai et al., 1999). Early onset of MDD is associated with greater severity of symptoms, higher prevalence of medical and psychiatric comorbidities, greater risk of depressive episodes and suicide attempts (McGorry et al., 2011; Zisook et al., 2007).

The pleomorphic cytokines, IL-1β and TNFα can vary from person to person due to pathological conditions (Sarkar et al., 2018). Serum concentrations of IL-1β and TNFα are still controversial in MDD. It is suggested that one of the reasons for these results to be controversial is because the authors evaluated patients as a single group, without separating them into early and late onset. Several studies have reported that altered levels of some pro-inflammatory and anti-inflammatory cytokines are responsible for the pathogenesis of depression (Cassano et al., 2017; Lopresti et al., 2014).

However, it has been proposed that many factors may influence the serum level of cytokines (eg, suicide attempts, lifestyle).

Patients with MDD are also at increased risk for the development of metabolic and cardiovascular diseases, being, on average, 1.58 times more likely to have metabolic syndrome (MS) compared to the general population (Vancampfort et al., 2015). MS is an umbrella term for clinical and biochemical changes, which include: central obesity, dyslipidemia, hyperglycemia, and hypertension (IDF, 2006). Excess central adiposity, assessed by waist circumference, is an important clinical marker in MS, because adipose tissue is recognized as an endocrine tissue that secretes hormones involved in several biological responses, including inflammation (Scherer, 2006).

It is known that smoking and high body mass index (BMI) are common in MDD (Dierker et al., 2002; Stunkard et al., 2003) and can also significantly affect the levels of peripheral inflammatory markers (Brooks et al., 2010; Yanbaeva et al., 2007). Our results also suggest that patients with early onset of MDD may have greater comorbidities than patients with late onset. To the date, there are few available studies of immunological markers in depression that have systematically considered the effect of clinical confounders, such as smoking or high BMI, on immunological changes. Thus, it is still unclear to what degree the association between depression and peripheral inflammatory markers is secondary to smoking or high BMI (Osimo et al., 2019).

Studies also correlate inflammation with suicide (Halaris et al., 2013). People who attempt suicide have also been reported to have higher levels of IL-6 in their cerebrospinal fluid and higher levels of IL-6 and TNFα in their plasma compared to healthy controls (Janelidze et al., 2011).

When considering the link between IL-1β and depression, the results are mixed. Authors reported high levels of IL-1β in MDD (Mota et al., 2013) and a meta-analysis found elevated IL-1β in patients affected with MDD. These finding occurred only when the included studies were classified as having high quality methodologies (Ellul et al., 2016). However, other meta-analysis did not find a general association between IL-1β and MDD (Dowlati et al., 2010; Haapakoski et al., 2015; Köhler et al., 2017), although it has been suggested that this could be due to measurement problems, since IL-1β concentrations in blood are very low (Haapakoski et al., 2015).

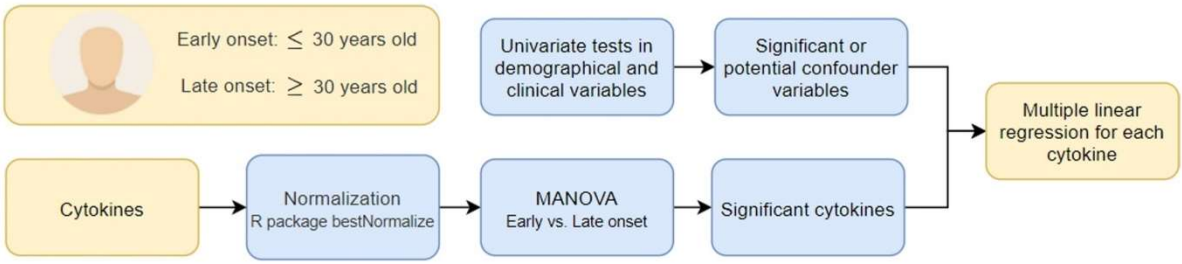

Fig. 1. Statistical analysis workflow.

Table 5
Results of linear regression.

	TNFα			IL-1β		
Characteristic	**Beta**	**95% CI**	**p-value**	**Beta**	**95% CI**	**p-value**
Age	-0.10	-0.23, 0.03	0.14	-0.07	-0.20, 0.07	0.3
Onset						
Early						
Late	-0.36	-0.62, -0.09	**0.008**	-0.51	-0.77, -0.24	**<0.001**
Sex						
Female						
Male	-0.16	-0.45, 0.13	0.3	0.13	-0.17, 0.43	0.4
Caucasian						
No						
Yes	0.34	-0.02, 0.69	0.061	0.07	-0.29, 0.44	0.7
Education						
Primary						
Secondary	-0.03	-0.30, 0.24	0.8	-0.07	-0.34, 0.21	0.6
Tertiary	0.13	-0.23, 0.48	0.5	0.22	-0.13, 0.58	0.2
Hamilton total	-0.01	-0.13, 0.11	0.8	0.03	-0.10, 0.15	0.7
Autoimmune disease						
No						
Yes	0.61	0.10, 1.1	**0.019**	0.76	0.25, 1.3	**0.003**
Anticonvulsivant						
No						
Yes	0.05	-0.30, 0.39	0.8	0.02	-0.34, 0.37	>0.9

Robert Dantzer et al., 2000). However, due to the presence of the blood-brain barrier, changes in cytokine levels in peripheral blood may be understood as a systemic inflammatory state. Fifth, the use of nonsteroidal anti-inflammatory drugs in patients without systemic inflammatory disease was not controlled as it was an occasional occurrence. Zou et al. (2019) in their study controlled for the use of nonsteroidal anti-inflammatory agents (eg, acetylsalicylic acid, ibuprofen, or indomethacin) and also observed that patients exhibit abnormal immune regulation and immune system activation. However, not all serum cytokines showed increased levels or (necessarily) participated in immune activation in patients with depression, but immune activation was related to disease severity.

MDD is, with no doubt, a multidimensional disorder. As far as we know, this is the first study that examined the association of serum levels of IL-1β and TNFα with early and late onset of MDD in adults. Therefore, elevated serum levels of IL-1β and TNFα may be associated with the development of early onset of MDD. However, the results presented here can be generalized only in hospitalized for outpatient care.

In conclusion, elevated serum levels of IL-1β and TNFα may be associated with the development of early onset of MDD and future research, in particular for the development of novel therapeutics that target IL-1β and/or TNFα. These will be very important for designing preventive strategies and for the identification of new drug targets for a subgroup of early-onset depressed patients with immune dysregulation.

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Additional information

The authors declare that they have no conflict of interest.

CRediT authorship contribution statement

Ana Paula Anzolin: Conceptualization, Writing – original draft, Methodology. **Jacson Gabriel Feiten:** Methodology, Formal analysis, Data curation. **Giovana Bristot:** Writing – review & editing, Methodology. **Gabriela Maria Pereira Possebon:** Methodology. **Marcelo Pio de Almeida Fleck:** Conceptualization, Writing – review & editing, Resources. **Marco Antonio Caldieraro:** Conceptualization, Writing – review & editing, Resources. **Marcia Kauer-Sant'Anna:** Conceptualization, Supervision, Project administration, Writing – review & editing.

Declaration of Competing Interest

All of the researchers declare not to have any conflict of interest. In the last 5 years, Dr. Kauer-Sant'Anna has received grant or research support from CAPES, CNPq, CNPq-Produtividade FIPE-HCPA and INCT-TM-CNPq (2008/09009-2); has been a speaker for Daiichi-Sankyo.

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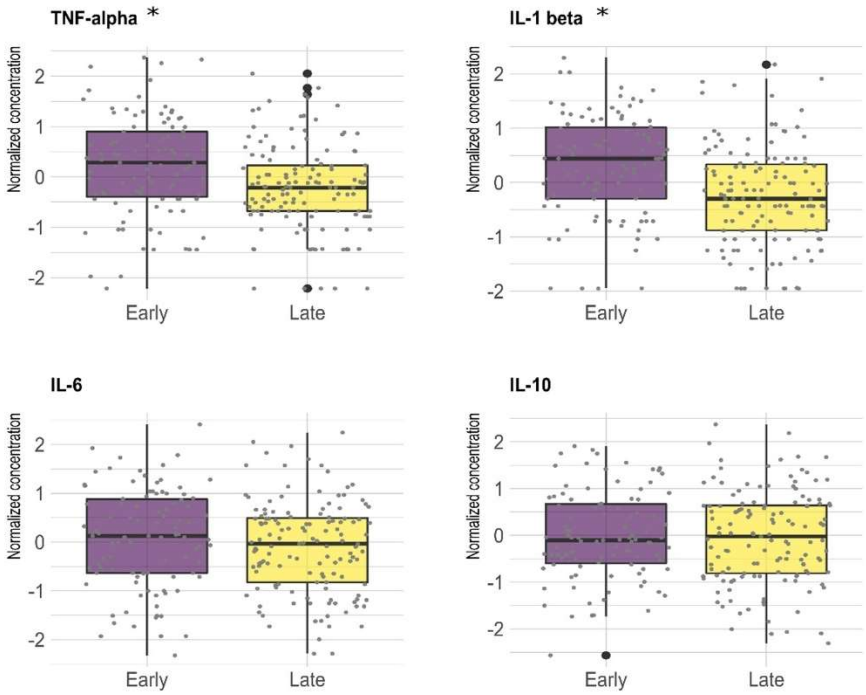

Fig. 2. Cytokines levels for early and late onset groups.

Table 3
Results of the multivariate analysis (MANOVA) results of cytokines as a function of age onset group, with patients who had autoimmune diseases.

Interleukins (226)	F value	NumDF	DenDF	p-value	Bonferronip-value
TNFα	12.6	1	224	0.0004	0.0019*
IL-1β	22.1	1	224	< 0.0001	< 0.0001*
IL-6	1.42	1	224	0.234	0.9360
IL-10	0.003	1	224	0.956	1

* p values were adjusted using Bonferroni procedure.

Table 4
Results of the multivariate analysis (MANOVA) results of cytokines as a function of age onset group, no patients who had autoimmune diseases.

Interleukins (226)	F value	NumDF	DenDF	p-value	Bonferroni p-value
TNFα	10.7	1	208	0.001	0.004*
IL-1β	20.6	1	208	< 0.0001	< 0.0001*
IL-6	1.12	1	208	0.291	1.00
IL-10	0.004	1	208	0.949	1.00

* p values were adjusted using Bonferroni procedure.

An admissible interpretation of our finding is that in early onset MDD, the stress system through autonomic nervous system may be implicated as an underlying mechanism in MDD. It has been observed that IL-1β can induce over activation of the HPA axis (Maes et al., 1991) and TNFα can increase the concentrations of adrenocorticotropic hormone and cortisol, which can also lead to hyperactivity of the HPA axis (O'Brien et al., 2004). Hyperactivation of HPA, on the other hand, can disrupt the normal functions of the glucocorticoid receptor (GR) (Cavanagh and Mathias, 2008), which plays an important role in the response to exogenous glucocorticoids. Taken together, the above results suggest the presence of hyperactivity of the HPA axis. Overall, HPA axis hyperactivity has been the most reproducible pathological marker in patients with clinical depression (Pariante and Lightman, 2008; Stetler and Miller, 2011; Zhang et al., 2016). In addition, TNFa can affect the levels of the neurotransmitter serotonin (5-HT) (Han and Yu, 2014; Ng et al., 2018) and can increase dopamine metabolism, which may contribute to depressive symptoms (Van Heesch et al., 2013).

The lack of association between early and late onset of MDD and IL-10 and IL-6, can be explained by previous studies that found no significant difference in these biomarkers both in patients with MDD and in control groups (Al-Hakeim et al., 2015; Hiles et al., 2012; Sadia Anjum et al., 2020; Schmidt et al., 2014). Also lack of power to detect medium or small effect sizes power.

In our results, most patients were on antidepressants. With the use of such drug class, a reduction in peripheral cytokine levels may occur. However, a study of Zou et al. (2019) showed evidence of changes in cytokine levels in antidepressant-naive patients with MDD. These patients also had an abnormality in immune regulation and activation of the immune system. The authors suggest that depression may be a process of intensifying inflammation, however not all serum cytokines had increased levels or participated in immune activation in patients with MDD. The cytokines IL-1β, IL-10 and TNFα showed a significant difference, while IL-6 and TGF-β1 had no difference. Corroborating this study, our findings also show the difference in cytokines IL-1β and TNFα and no difference in IL-6 in patients with early and late onset MDD, even with the use of antidepressants.

Our study, however, has some limitations. The first one is the absence of a control group. The second, the disease age onset was assessed retrospectively, and its measurements may be influenced by the participant's recall or reporting bias. Third, although we have studied several cytokines in this investigation, our results do not reflect changes in the entire immune network response. Fourth, there is some evidence to suggest that serum levels of cytokines reflect, at least in part, levels of cytokines in the brain. Increased levels of cytokines in the blood of patients who have not undergone surgery are accompanied by increased levels of cytokines in the cerebrospinal fluid (Bromander et al., 2012;

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CAPÍTULO 2

Artigo: Ketamine study: Protocol for naturalistic prospective multicenter study on subcutaneous ketamine infusion in depressed patients with active suicidal ideation

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Ketamine study: Protocol for naturalistic prospective multicenter study on subcutaneous ketamine infusion in depressed patients with active suicidal ideation

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Background: Psychiatric disorders are associated with more than 90% of reported suicide attempts worldwide, but few treatments have demonstrated a direct effect in reducing suicide risk. Ketamine, originally an anesthetic, has been shown anti-suicide effects in clinical trials designed to treat depression. However, changes at the biochemical level were assessed only in protocols of ketamine with very limited sample sizes, particularly when the subcutaneous route was considered. In addition, the inflammatory changes associated with ketamine effects and their correlation with response to treatment, dose-effect, and suicide risk warrant further investigation. Therefore, we aimed to assess whether ketamine results in better control of suicidal ideation and/or behavior in patients with depressive episodes and whether ketamine affects psychopathology and inflammatory biomarkers.

Materials and methods: We report here the design of a naturalistic prospective multicenter study protocol of ketamine in depressive episodes carried out at *Hospital de Clínicas de Porto Alegre* (HCPA) and *Hospital Moinhos de Vento* (HMV). The study was planned to recruit adult patients with Major depressive disorder (MDD) or Bipolar disorder (BD) types 1 or 2, who are currently in a depressive episode and show symptoms of suicidal ideation and/or behavior according to the Columbia-Suicide Severity Rating Scale (C-SSRS) and have been prescribed ketamine by their assistant psychiatrist. Patients receive

ketamine subcutaneously (SC) twice a week for 1 month, but the frequency can be changed or the dose decreased according to the assistant physician's decision. After the last ketamine session, patients are followed-up *via* telephone once a month for up to 6 months. The data will be analyzed using repeated measures statistics to evaluate the reduction in suicide risk as a primary outcome, as per C-SSRS.

Discussion: We discuss the need for studies with longer follow-ups designed to measure a direct impact on suicide risk and that additional information about the safety and tolerability of ketamine in particular subset of patients such as those with depression and ideation suicide. In line, the mechanism behind the immunomodulatory effects of ketamine is still poorly understood.

Trial registration: https://clinicaltrials.gov/, identifier NCT05249309.

KEYWORDS

suicide, bipolar depression, unipolar depression, depression, ketamine

Introduction

Depression is a chronic, recurrent, and highly prevalent condition associated with functional disability and compromised physical health (1). Its etioloy is multifactorial and combines endogenous susceptibility with exposure to environmental stressors (1, 2). The association between depression and suicidal behavior has been widely described in literature; for example, according to the WHO (3), psychiatric illnesses are associated with more than 90% of suicide ideation cases and are responsible for 90% of deaths by suicide reported cases worldwide (3, 4). Furthermore, studies across different populations have confirmed the relationship between depression and suicide (5–7): population-based investigations in the United States (5, 8), Canada (6), and China (7) indicate that depression is the main nosological entity associated with suicidal ideation, suicidal plans, and suicide attempts. Despite these facts, treatments for depression that also impact suicidal behavior are scarce; thus, drugs with anti-suicide effects are highly desirable and one of the main research needs in psychiatry (4).

There is an increasing interest in the benefits of ketamine, its racemic compound, and its enantiomers [i.e., S-ketamine (esketamine) (9) and R-ketamine (arketamine) (10) for the treatment of psychiatric disorders. Indeed, intranasal esketamine for treating depression was recently approved by the Food and Drug Administration (FDA) and European Regulatory Authorities (11, 12) for treating Major depressive disorder (MDD). Ketamine acts on glutamate, the principal excitatory neurotransmitter, as a non-competitive antagonist on the *N*-methyl-*D*-aspartate (NMDA) receptor; the effectiveness of glutamate modulating agents in the treatment of mood disorders regulates of glutamatergic neurotransmission, contributing to the pathophysiology of depression, as well as to the mechanisms of antidepressants (13).

Glutamate acts pre- and post-synaptically through the activation of several receptors. Ionotropic glutamate receptors-NMDA, alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and kainate (KA)–are channels that allow the influx of ions into the cell, regulating polarization of the neuronal surface, which activates intracellular signaling cascades. It is believed that NMDA and AMPA are directly involved in the antidepressant actions of ketamine (14). The pharmacokinetic characteristics of ketamine allow its administration by various routes, including intravenous (IV) (15, 16), subcutaneously (SC) (17, 18), intranasal (11, 12), oral (19, 20), sublingual (21), and intramuscular (22). The SC route of administration has comparable efficacy to conventional IV infusion but fewer side effects (23, 24). In a recent systematic review (18) that included 12 studies (two randomized clinical trials, five case reports and five retrospective studies), the authors observed that racemic ketamine and its enantiomer esketamine, *via* SC, seems to be a promising treatment in depression, given its efficacy and tolerability. The literature has already verified that doses of 0.5 mg/kg are unsuitable for patients with chronic depression (25). Repeated and staggered doses of ketamine (0.5 mg/kg for the first three infusions to 0.75 mg/kg for the last infusions) reinforced the antidepressant and antisuicidal properties of ketamine in a sample of patients with severe depression (25–27).

Some studies have associated proinflammatory cytokines with the severity of depressive symptoms (28, 29). Innate immune cells present in the central nervous system (CNS), such as microglia, participate in the process of neuroinflammation; when this process is activated, the production of cytokines that affect synaptic plasticity in regions important for mood regulation increases (19). Based on pharmacological properties and animal studies (30), it is hypothesized that depressed patients with higher levels of inflammation is more responsive to ketamine treatment (31). Furthermore, in an animal model of treatment-refractory depression with chronic administration of adrenocorticotropic hormone (ACTH), animals that responded to ketamine exhibited higher baseline plasma concentrations of C-reactive protein (CRP) and tumoral necrosis factor-α (TNF-α) (32, 33). Blood biomarkers [TNF-α, Interleukin (IL)-6] predicted a favorable antidepressant response to Ketamine administration in a small sample of depressed patients. Other studies report that increased body mass index

(BMI) and plasma concentrations of adipokine (both associated with inflammation) correlated with the ketamine response, in other words, lower baseline adiponectin levels correlated with superior antidepressant response to ketamine (percent change from baseline) at 230 min post-infusion [Montgomery-Åsberg Depression Rating Scale (MADRS): $r = 0.25$, $p = 0.03$; Hamilton Depression Rating Scale (HAM-D): $r = 0.22$, $p = 0.051$] and at day 1 (MADRS: $r = 0.28$, $p = 0.01$; HAM-D: $r = 0.34$, $p = 0.002$) (13, 34).

Patients with depression are also at increased risk for developing metabolic and cardiovascular diseases, being, on average, 1.58 times more likely to have metabolic syndrome (MS) compared to the general population (35). Among the hormones secreted by adipose tissue, leptin seems to be involved with depressive disorders (36–38). In addition, some works have investigated the involvement of central and peripheral leptin as a potential biomarker for suicide risk (39).

New biomarkers are also essential to predict the outcome of treatment in the future with the application of conventional antidepressants and anti-inflammatory drugs. For example, alterations in the expression of sirtuin 3 (SIRT3) are associated with the pathophysiology of depressive disorders (40). Similarly, serum levels of soluble urokinase plasminogen activator receptor (suPAR) are positively correlated with inflammatory proteins previous reported in mood disorders, such as tumor necrosis factor-alpha [TNF-α_ and ultra-sensitive C-reactive protein (us-CRP) (41)]. Moreover, studies have observed that high levels of suPAR were associated with a higher probability of depression diagnosis and recent suicide attempts (42, 43).

Therefore, given the potential challenges of conducting a definitive randomized control trial (RCT) of ketamine as a rapid-onset antidepressant in suicidal ideation, a feasibility study is needed to inform tolerability, acceptability, safety, effect, as well as the better understanding of the biochemical changes of ketamine in the treatment of suicide risk in patients with depression. In addition, these data could serve as the basis for the larger RCT using an individualized dose ketamine approach.

Aims

This article aims to describe the protocol of a multicenter prospective naturalistic study, which allows an analysis of the response to ketamine *via* SC in relation to the treatment of suicidal ideation and behavior. We hypothesize that ketamine, through its mechanisms of action on NMDA and neuroplasticity, would reduce suicidal ideation or/and behavior in patients with a depressive episode, according to the Columbia Suicide Severity Rating Scale (C-SSRS) and other rating scales.

Objectives

Our main objective is to evaluate whether ketamine can reduce the frequency and intensity of suicidal ideation or behavior and improve depressive symptoms in patients with depressive episodes. Our secondary aims are the investigation of the impact of ketamine on other psychoathology symptoms, clinical factors, inflammatory biomarkers, and metabolic factors.

Materials and methods

Study design and setting

This is an observational naturalistic, prospective multicenter study performed in two reference centers in ketamine treatment in Porto Alegre, Brazil. The participants were recruited in our reference due to their depression and active suicide ideation or behavior. The ongoing ketamine study started data collection on July 2021 with a target of 45 participants. Data include clinical and psychiatric assessment, blood sampling, and diet on site with a follow-up assessment at 6 months performed by phone call. In addition, an exploratory analysis assesses the risk of suicide throughout ketamine treatment based on subgroups of interest.

Sample size calculation

The sample size of 45 participants over 2 years is projected to be an appropriate number to inform study feasibility; the sample size calculation was performed using the WINPEPI program, version 11.65 to detect a difference of 1 point on the C-SSRS scale, considering results from previous studies (44) with a power of 80% at a significance level of 0.05.

Population and eligibility criteria

The target population are adult patients diagnosed with Major depressive disorder (MDD) currently in depressive episodes, were diagnosed using the Diagnostic and Statistical Manual of Mental Disorders–fifth edition, DSM-5 (45), and confirmed using the Mini International Neuropsychiatric Interview (MINI; updated Version 7 for DSM-5) (46).

All patients are treated with subcutaneous (SC) ketamine and continue to use psychiatric medications prescribed by their attending physician. Information on medications (single or in combination) as well as dosage were collected using a structured questionnaire (**Appendix 1**). Patients did not receive psychotherapy and/or physiotherapy.

The inclusion criteria are (1) adult patients ($\geq$ 18 years); (2) that meet the DSM-5 diagnostic criteria for MDD, BD-1, or BD-2 currently in a depressive episode; (3) with a total score on the Montgomery-Åsberg Depression Rating Scale (MADRS) $\geq$ 12 and score on items 1 (apparent sadness) and 2 (expressed sadness) $\geq$ 2 during the triage period (baseline); (4) and a total Young Mania Rating Scale (YMRS) score $\leq$ 11 during baseline; (5) having current symptoms of suicidal ideation or suicidal behavior, according to the Columbia Suicide Severity Rating Scale (C-SSRS) score $\geq$ 1; (6) indication/prescription of their assistant physician for the use of SC ketamine; (7) use effective contraceptive methods for heterosexual women of childbearing age; (8) patients with BD-1 is taking lithium, valproic acid, or an atypical antipsychotic at therapeutic doses for at least 4 weeks before the initial assessment; (9) patients with BD – 2 is taking lithium, valproic acid, lamotrigine, or an atypical antipsychotic at therapeutic doses for at least 4 weeks before the initial assessment; (10) can provide consent and comply with study procedures.

The exclusion criteria are (1) Patients with an unstable, defined, or suspected systemic medical condition; (2) Women who are pregnant, breastfeeding or planning to become pregnant within the next year; (3) Patients who do not tolerate the use of ketamine or with previous side effects associated with medications; (4) Inability to comply with informed consent or treatment protocol needs; (5) Patients with psychotic symptoms (according to DSM-5 criteria); (6) Patients with a current diagnosis of any substance use disorder according to the DSM-5 Criteria, except for smoking; (7) Patients with immune, inflammatory, cancer or infections.

Withdrawal Criteria: (1)Patients not using the medication or being considered non-adherent by the responsible clinician; (2) Patients who stop taking contraceptives or become pregnant; (3) In case of modifying doses or adding/deleting medication, patients be kept in the study, but the changes be counted as a primary endpoint; (4) Serious adverse reactions; (5) Withdrawal of consent by the patient; (6) Patients with manic or psychotic episodes as clinically assessed and according to DSM-5 criteria.

Comparator

The recruitment of the control group occurs in the form of an invitation to blood donors at the HCPA Blood Bank and blood collection performed in the routine collection performed during the blood donation. This group included only in biochemical analyzes to compare the levels of interleukin 6 (IL-6), IL-10, IL-1β, TNFα, SIRT3, suPAR, us-CRP, and leptin of depressed patients with levels of healthy subjects.

The control group consists of 45 healthy volunteers (age $\geq$ 18). The inclusion criteria for the healthy controls are: (1) Not having a history of psychiatric or neurological diseases; (2) Do not present unstable clinical illnesses or autoimmune diseases; (3) Not being pregnant or breastfeeding.

Interventions

The study procedure is illustrated in **Figures 1**, **2**. The application of ketamine for the study follows the routine related to the care in force applied to patients for treatment with ketamine at HCPA and HMV.

The psychiatrist reassesses the patient to exclude current criteria that contraindicate treatment with ketamine. In addition, it is verified whether the patient has ingested solids for at least 6 h and at least 2 h before the procedure. Finally, the patients are comfortably accommodated sitting in a reclining chair or lying on a stretcher, and vital signs are checked (blood pressure measurement, digital oximetry, and heart rate).

The medication is administered at an initial dose of 0.5 mg/kg. The nursing staff with undiluted ketamine prepare the syringe for SC administration; the psychiatrist administers the SC injection, preferably in the abdominal wall. The tolerability of the patient treated for the first time with ketamine is evaluated by dividing the dose administered at least in the first two infusions and whenever the dose is increased. In this case, the medication is injected using half of the planned dose and the other half 30 min after or when there is remission of adverse events. Blood pressure and pulse oximetry are checked during this period.

The initial SC infusion is 0.5 mg/kg ketamine; if there is no adequate response with the dose of 0.5 mg/kg, a second infusion is performed at least 2 days after the first, using 0.75 mg/kg and the subsequent 1 mg/kg. If the patient responds adequately to those doses (0.5 or 0.75 mg/kg), it is repeated throughout the course of treatment; these eight sessions, twice a week. Then, after a consolidation phase of 8 more sessions, once a week. The general and clinical data of the patients is obtained in person, along with the care procedure. After the end of the ketamine sessions, the psychiatric scales are applied *via* telephone once a month until the 6th month. In the first and last session of Ketamine (beginning and end of treatment), peripheral blood is collected from patients (15 mL) by a technician trained in blood collection.

Blood sampling

Blood samples are collected from each patient and allowed to clot in blood collection tubes with no additive. Subsequently, whole blood is centrifuged for 10 min at 1,000 *xg* and serum is removed, aliquoted and stored at −80°C until assayed. Blood samples are collected from each patient in an anticoagulant tube. Subsequently, the blood is centrifuged for 10 min at 1,000 mg. and the plasma is removed, aliquoted and stored at −80°C until the time of the assay.

Measurements

Primary outcome measures

C-SSRS

To measure the risk of suicide and the improvement in suicidal ideation with ketamine, we used the Brazilian version of the C-SSRS (translated and validated to Brazilina Portuguese). The C-SSRS has different versions that assess symptoms in different periods, depending on the characteristics of the study. In the present study, we use the baseline/screening version at the beginning of the first session, which assesses the worst period of suicidal ideation during life and in the last month. For later measurements (before each ketamine application), we use the modified version for use in serial assessment, which tracks symptoms since the last assessment.

The C-SSRS is applied by the researcher through a semi-structured interview and is divided into four subscales: (a) severity of suicidal ideation (5-point ordinal scale); (b) intensity of ideation (5-point ordinal scale); (c) suicidal behavior [nominal scale with binary response (yes/no)]; (d) lethality of effective attempts. The researchers performed the necessary training to apply the C-SSRS scale.

Secondary outcome measures

These questionnaires below are applied before all ketamine sessions and, after that, *via* telephone once a month until the 6th month. In addition, the Childhood Trauma Assessment Questionnaire (CTQ) is applied before the first ketamine session.

Childhood Trauma Assessment Questionnaire (CTQ)

The CTQ (47) is a self-assessment instrument for exposure to abuse situations up to fifteen years of age. It consists of 28 items, classifiable on a 5-point Likert scale, originating from the 70-item

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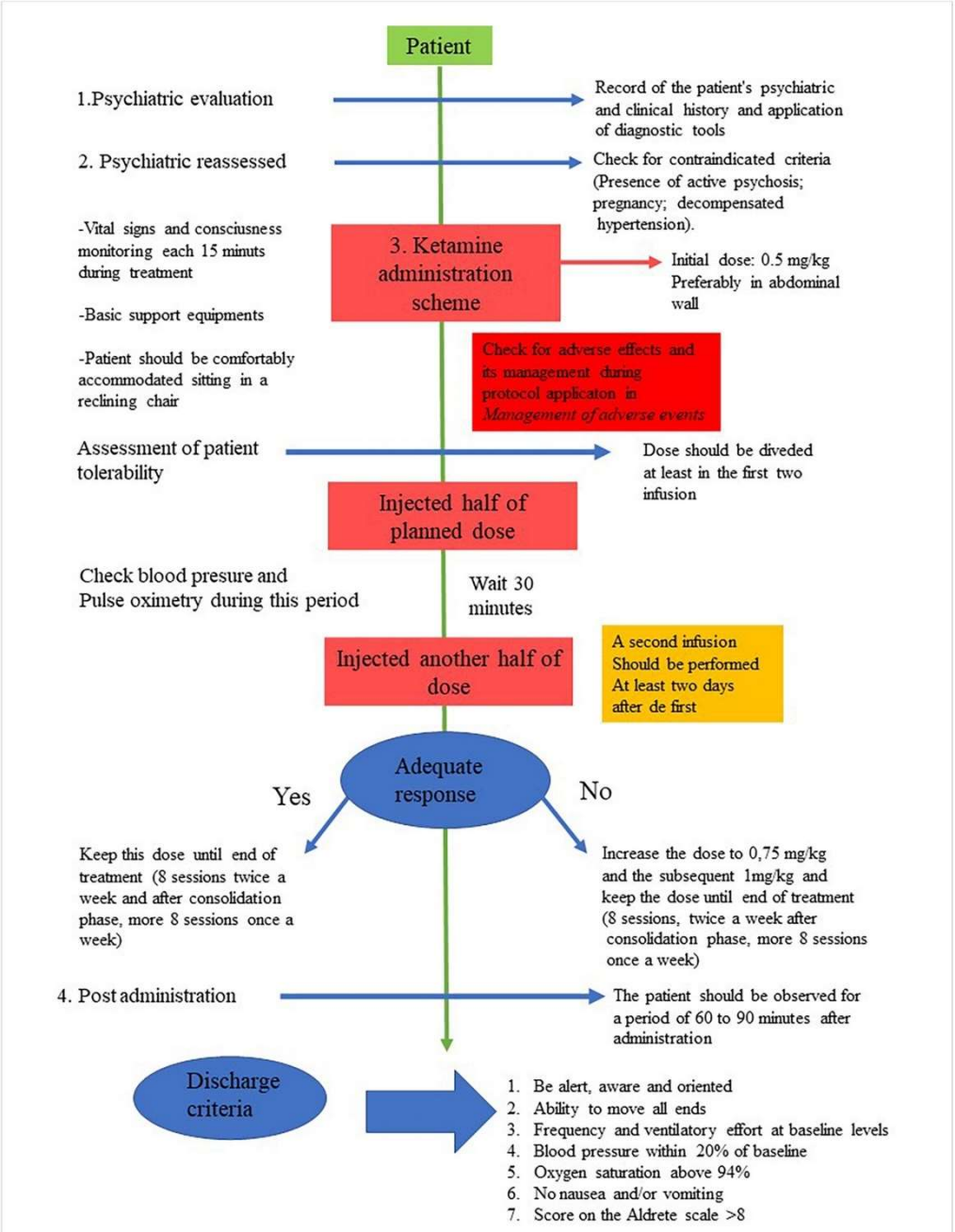

FIGURE 2
Graph scheme of ketamine protocol.

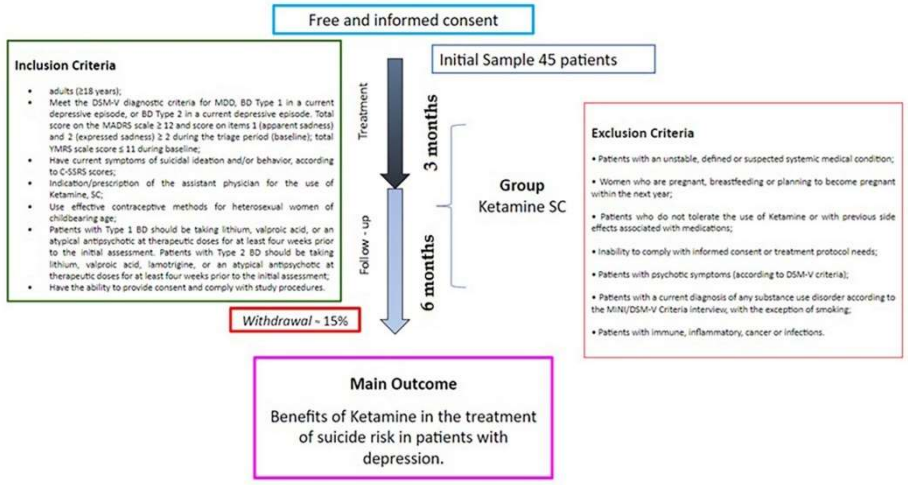

FIGURE 1
Study protocol. The protocol may have its care attention time changed/decreased, due to the amounts of ketamine sessions (determined by the attending physician), after the end of these sessions the patient is monitored once a month to 6 months, *via* telephone.

long version developed by Bernstein et al. (47). Items that describe childhood experiences are classified according to how often they occurred: 1–never, 2–a few times, 3–sometimes, 4–often or 5–always, being formulated with experiences of abuse or adequate care during childhood.

Young Mania Rating Scale (YMRS)

The YMRS (48), translated and adapted to Brazilian Portuguese (44), is used to evaluate the appearance of manic symptoms as an adverse effect of the use of ketamine. The YMRS is a scale the researcher applies through direct observation and unstructured interviews. This scale contains eleven items, and the score ranges from 0 to 62. A score less than or equal to 12 indicates no manic episode.

Montgomery-Åsberg Depression Rating Scale (MADRS)

In its final version, the MADRS Scale (49) consists of ten items that do not include somatic or psychomotor symptoms. This characteristic makes it a more suitable scale to assess patients with general medical comorbidities, as it reduces the risk that symptoms resulting from somatic illness are counted as depressive symptoms. The evaluator can score defined scale grades (0, 2, 4, 6) or intermediate categories (1, 3, 5).

Hamilton Depression Rating Scale (HAM-D)

To measure ketamine effectiveness on the severity of depressive symptoms, we also use the seventeen-item version of the HAM-D (49) translated and adapted to Brazilian Portuguese, with a structured interview guide. The HAM-D is a scale whose total score ranges from 0 to 52. Although the author has not proposed a standard cutoff point, in practice, scores above 24 are considered to identify a severe depressive episode; between 18 and 24, moderate depressive episode; between 7 and 17, mild depressive episode and below 7, no depressive episode or remission (50).

Brief psychiatric rating scale (BPRS)

This scale assesses the presence and level of severity of psychotic symptoms, emotional states, and psychomotricity disorders, among other symptoms (51). The BPRS score adopted is based on the criteria suggested by Elkis et al. (51), with scores ranging from 0 to 6 for each item. Thus, 0 (zero) means absence or non-observation of the symptom and six corresponds to the most severe level. Intermediate values correspond, in turn, to intermediate levels of severity. For the evaluation of mental alterations compatible with disorders of a psychotic nature, the total BPRS score is considered.

Functional assessment short test (FAST)

We use the translated and adapted version for Brazil (52). It is a hetero-applied instrument for the objective and multidimensional assessment of functionality related to the last fifteen days. It consists of 24 items, divided into six specific subscales. Autonomy refers to the subject's ability to perform actions alone or to make their own decisions. Occupational functioning refers to the subject's ability to maintain a regular job, to have a stable performance and to work in an area compatible with their qualification and position at work. Cognitive functioning concerns the subject's ability to concentrate, make simple mental calculations, solve routine problems, learn new information and remember this learned information. Financial skills involve the subject's ability to manage their finances in a balanced way; the item "interpersonal relationships" refers to the quality of relationships with friends and family, the ability to participate in social activities and sexual relationships, and the ability to defend personal ideas and opinions. Leisure activities relate to performance in physical activities (sports, exercise) and

having activities. The score is determined by the sum of the items, which range from 0 (indicating no limitation) to 3 (indicating severe limitation) (53).

Anthropometric measurements

a) Body weight

Body weight is evaluated before the first application of ketamine, in the fourth, eighth and twelfth weeks after the first application of ketamine. Body weight is measured with individuals barefoot, wearing as little clothing as possible and positioned in the center of the platform during the reading. An electronic scale with a maximum capacity of 150 kg and a precision of 0.1 kg is used.

b) Stature

Height is evaluated before the first application of ketamine. To perform the measurement, an anthropometric ruler fixed to the wall is used. Height is considered as the distance from the sole of the bare feet to the top of the head, compressing the hair, with the patient in a vertical position, on the flat surface, looking fixed on the horizon (54).

c) Body mass index (BMI)

Body mass index (BMI) is calculated from weight (kg) and height (m) data using the following formula:

$$BMI = Weight\ (Kg) \div Height\ (m)^2$$

For the classification of BMI, the cutoff points established by the WHO is used, where: BMI < 18.5 Kg/m^2 is classified as low weight; BMI 18.5–24.9 Kg/m^2 is classified as adequate weight; BMI 25–29.9 Kg/m^2 is classified as overweight; BMI 30–34.9 Kg/m^2 is classified as class I obesity; BMI 35–39.9 Kg/m^2 is classified as class II obesity; BMI > 40 Kg/m^2 is classified as class III obesity.

d) Waist circumference (WC)

Waist circumference is evaluated before the first application of ketamine, in the fourth, eighth and twelfth weeks after the first application of ketamine. WC is measured with the aid of an inelastic measuring tape 1.5 m long and accurate to 0.1 cm. The measurement is performed with the patient standing in an upright position, abdomen relaxed, arms extended along the body and feet separated at 25–30 cm. The midpoint between the iliac crest and the lower edge of the last rib, in an orthostatic position, without clothes on the chest and at the end of expiration (54). The reference value used is the one proposed by the IDF (55).

e) Blood pressure

Blood pressure is measured before the first application of ketamine, in the fourth, eighth and twelfth weeks after the first application of ketamine. The measurement is performed according to the HCPA and HMV nursing protocol.

f) Inflammatory profile of the diet

The Dietary Inflammatory Index (DII) is calculated according to previous studies (56, 57) and from the average of food recalls of the last 24 h (R24h) to the interview with intervals between them according to the ketamine applications. The pro-inflammatory nutritional parameters included in the DII score is total calories (kcal); carbohydrates (g); fat (g); protein (g); cholesterol (mg); total saturated fatty acids (g); iron (mg); and vitamin B12 (μg). The anti-inflammatory dietary parameters included in the DII score is: alcohol (g); caffeine (g); fiber (g); total monounsaturated and polyunsaturated fatty acids (g); n-3 and n-6 polyunsaturated fatty acids (g); niacin (mg); riboflavin (mg); thiamine (mg); vitamins A (retinol equivalents), B6 (mg), C (mg), D (μg), E (mg); β-carotene (μg); magnesium (mg); selenium (μg); zinc (mg); and folate (μg). Scores are centered at 0, with positive scores indicating a pro-inflammatory diet and negative scores indicating an anti-inflammatory diet. Continuous DII scores is standardized (mean = 0, standard deviation = 1) for better interpretation of results.

Outcome measures

Primary and secondary outcomes and endpoints that correspond to the secondary objectives are listed according to the various assessment time points in Table 1. As a primary outcome, this work is expected to prove the benefits of ketamine in the treatment for suicidal ideation in patients with MDD or BD, as well as the durability of the antidepressant effect, and the transdiagnostic comparison of the effect of ketamine. The C-SSRS scale score over 6 months in relation to the initial score is used to assess this primary outcome.

As a secondary outcome, we are verifying the occurrence of changes before, during and after treatment on psychiatric scales (e.g., BPRS, MADRS, HAMD, YMRS, FAST) and serum concentration of IL-6, IL-10, IL-1β, TNFα, suPAR, us-CRP, and leptin as well as gene expression and immunocontent of SIRT3. In addition, the presence of MS is evaluated as a potential moderator of treatment response together with the predictors mentioned above.

The standard tools BPRS, MADRS, and HAM-D are used for comparison with other ketamine literature available in psychiatry (23, 24, 58, 59). Clinical response is defined as MADRS score reduction of $\geq$ 50% from baseline and remission as MADRS score ≤ 9 (22), and relapse is defined as MADRS ≥ 16 after an initial remission. The time points for measurements of the scales used were chosen to verify the initial and maximum response time (during treatment) and the duration of response (up to 6 months).

Assessment integrity

Under the guidance of PBA, LNC and MKS (staff psychiatrists), the APA researcher participated in training to perform psychiatric assessments. APA then provides on-site initiation and training for the rest of the research team members (nutritionist, undergraduate students, and other researchers).

Eligible participants undergoing ketamine treatment are monitored regularly in each treatment application. Upon completion of treatment, participants go through the follow-up phase, in which they are monitored monthly by telephone (months 1–6) (Table 1). Each participant receives a unique identification number. All study data is recorded on the study case report forms and entered by study researchers into REDCap, a sophisticated platform for collecting and managing research data protected by Secure Sockets Layer encryption. All source

Neurobiological studies with adult suicide patients have found reduced levels of serotonin metabolites in central nervous system (CNS) fluid. Deficiency of this and other neurotransmitters (such as norepinephrine) has been observed in cases of suicide since the deficiency of these neurotransmitters in critical places in the brain results in depressive states. Such a deficiency can occur due to insufficient production, excessive neurotransmitter reuptake in the synaptic cleft or failure of the receptor system (64).

Serotonin and norepinephrine are the most studied neurotransmitters when it comes to suicide. Studies have also described the association of a decrease in the level of 5-HT in the brain of the deceased who had a diagnosis of depression. In the case of those who died by suicide, there was a decrease in 5-hydroxyindoleacetic acid (5-HIAA), the main metabolite of 5-HT. Depressed individuals who committed suicide or serious attempts had reduced levels of 5-HT, when compared to patients with depression, but who did not commit suicide or serious attempts (65).

Sirtuin 3 (SIRT3) is the main NAD + deacetylase dependent mitochondria that acts as a regulator of mitochondrial protein function, being essential for maintaining mitochondrial integrity. Abe et al. (66) analyzed whether there were alterations in sirtuin messenger RNA (mRNA) expression in peripheral white blood cells of BD patients and also examined whether altered sirtuin mRNA expression is state or characteristic dependent in BD patients who were in a remissive state. As a result, they observed that mRNA sirtuin levels in BD patients significantly decreased in those who were in a depressed state, compared to healthy controls. Therefore, altered sirtuin expression is state-dependent and is associated with the pathogenesis or pathophysiology of bipolar depression. A study correlated SIRT3 with depression, using semiquantitative Western blotting methods, associating the pathogenesis of depression with the expression of SIRT3 (67).

The urokinase plasminogen activating receptor (uPAR) is part of the plasminogen activation system. It is also involved in cell adhesion and migration and is important for the recruitment of immune cells (68). The soluble form of the receptor, suPAR, results from cleavage and release of membrane-bound uPAR into the blood and reflects activation of the immune system. In most cases, serum levels of suPAR positively correlate with inflammatory proteins such as TNFα and C-reactive protein (CRP) (41). It was also observed that high levels of suPAR were associated with a higher probability of diagnosis of depression (42). Ventorp et al. (43) evaluated plasma levels of suPAR as a biomarker of low-grade inflammation in patients with DDM and in patients who had recently attempted suicide. It was observed that both depressed patients and those who attempted suicide increased plasma suPAR, which may in the future be a prognostic in relation to the outcome of treatment with the application of conventional antidepressants in conjunction with anti-inflammatory drugs.

Patients with MDD are at increased risk for the development of metabolic and cardiovascular diseases, being, on average, 1.58 times more likely to have MS compared to the general population (35). On a global scale, it is estimated that 31% of patients diagnosed with BD have MS (35). MS is an umbrella term for clinical and biochemical changes, which include central obesity (waist circumference or body mass index), dyslipidemia (elevated triacylglycerols and reduced high-density lipoprotein-HDL), hyperglycemia, and hypertension (55). The presence of metabolic syndrome components in BD, mainly excess adiposity, is associated with reduced neurocognition, in addition to an association with executive function deficits and global cognitive deterioration (69). Excess central adiposity, assessed by waist circumference, is an important clinical marker in MS, because adipose tissue is recognized as an endocrine tissue that secretes hormones involved in several biological responses, including inflammation (70).

Among the hormones secreted by adipose tissue, leptin appears to be involved with depressive disorders, including MDD and depression in BD (36–39, 71–73). Leptin was described in 1995 as a hormone responsible for the feeling of satiety, which acts mainly on the CNS and inhibits the action of orexigenic neurons and stimulates the action of anorectic neurons, in addition to increasing basal energy expenditure and being secreted in proportion to the adipose tissue stock (74).

In addition to the metabolic aspects involved in mood disorders and suicide, nutritional aspects and diet quality have recently come to be considered in mental disorders (75), mainly as process-stimulating inflammatory agents. In a meta-analysis of prospective cohort studies, individuals grouped in extracts with better quality dietary patterns had a lower odds ratio for incidence of depression or depressive symptoms compared to individuals in extracts with lower quality dietary patterns (76). In the same study, those individuals who were in the lowest quintiles of the Dietary Inflammatory Index (DII) also had a lower odds ratio of incidence of depression and depressive symptoms (76), suggesting that nutritional factors may influence the development of mood disorders *via* stimulation of the inflammatory process. The dietary inflammatory index (IBD) is a global measure of the inflammatory potential of the foods consumed, considering macronutrients and micronutrients and their association with inflammatory markers such as IL-1b, IL-4, IL-6, IL-10, TNF –α, and CRP (57). In the case of MDD and suicidal ideation, the positive index of IBD, indicating a pro-inflammatory eating pattern, was associated with suicidal ideation and MDD in a cross-sectional study with a representative sample of the American adult population (56). In this sense, a systematic review of cross-sectional studies showed a positive association between a dietary pattern consisting of a high intake of red meat and derivatives and a low intake of fruits and vegetables with the concentration of CRP, IL-6 and IL-18 (77), all inflammatory markers involved in mood disorders. However, the clinical potential of IBD as a reference for the inflammatory profile of the diet still needs to be better characterized in patients with MDD and using ketamine.

The main limitation of this study is its inability to report the definitive efficacy of ketamine, since a multicenter, randomized, controlled clinical trial is required for assess these outcome. Secondly, severely depressed or psychotic patients who cannot consent will be excluded. Thirdly, patients remained on antidepressant medications. Therefore, we cannot rule out the possibility that the improvements in suicidal ideation and depressive symptoms are due to the intensifying effects of ketamine and not just ketamine which would require a RCT. However, this problem can be partially solved based on the well-known antidepressant effect of rapid rise and fall of ketamine compared to gradual changes of antidepressants that take weeks to months. Fourth: have not evaluate criteria for Treatment-Resistant Depression (TRD) and, given the naturalistic design, we cannot

documents and the master list linking participant identification information and identification numbers are stored in a locked cabinet at HCPA. All information is accessible only to those directly involved in the study. There is no advance sharing of data beyond the group of investigators. Study records are maintained for 5 years after study completion in secure archival facilities per the National Council for Health and Medical Research and Good Clinical Practice guidelines.

Data analysis

We will use the Shapiro-Wilk test to assess the normality of the variables. Clinical and demographic data with normal distribution will be assessed with parametric tests (e.g., *t*-test for independent samples) and those with non-asymmetric data will be analyzed with non-parametric tests (e.g., Mann-Whitney test). Categorical variables will be compared through the chi-square test or Fisher's exact test, as appropriate.

For the analysis of the C-SSRS suicidal ideation severity scale, as it is an ordinal scale, we plan to use the Wilcoxon test to detect differences between the scores for each evaluation point throughout the study. In addition, the generalized estimation equations will be used to evaluate the durability of the antidepressant effect and tolerability of SC ketamine.

The association between the variables is evaluated by the Pearson correlation test or Spearman, as per the distribution pattern. The margin of error used is 5%.

Trial duration

July 2021–December 2023.

Ethics and dissemination

This study is supervised by the Research Ethics Committee of *the Hospital de Clínicas de Porto Alegre* (CEP-HCPA) and Research Ethics Committee of the *Hospital Moinhos de Vento* (CEP-HMV). The same is a collegiate instance, of a consultative, deliberative, and educational nature, whose objective is to assess the ethical and methodological aspects (through the issuance of an opinion) and to monitor research projects involving human beings, carried out or proposed by the institution. The instance is registered with OHRP/USA (Office for Human Research Protections): IORG0000588, CEP Registration (IRB – Institutional Review Board) with OHRP/USA (Office for Human Research Protections): IRB 00000921 and Federal wide Assurance (FWA), certificate of commitment that the Institution undertakes to follow the requirements established by the HHS (U.S. Department of Health and Human Services) Protection of Human Subjects: FWA00002409.

This study was approved by Research Ethics Committee the HCPA (CAAE: 33589320300005327) on the 18 June 2020 and Research Ethics Committee the HMV (CAAE: 33589320.3.2001.5330). This trial has been registered in U.S. National Library of Medicine–Clinical Trial Registry (Reference Number: NCT05249309), with recruitment commenced on the May 2021.

The results of this study will be submitted for publication in peer-reviewed journals and presented at relevant conferences.

Discussion

To the best of our knowledge, this study is the first naturalistic investigation designed to verify the therapeutic effects of SC ketamine in reducing suicide risk in patients with mood disorders. Furthermore, it is the first study to assess the impact of ketamine on serum levels of SIRT3, IL-6, IL-10, TNFα, leptin, us-CRP, suPAR, metabolic parameters, and dietary inflammatory index.

This study includes a period of ketamine administration (treatment) and a 6-months follow-up to determine not only the acute effects of ketamine but also its impacts in the long term. This duration was chosen to obtain adequate data on effect and durability in the short, medium, and long term, maintaining the feasibility of the study. Therefore, this study may provide relevant information for a future definitive study exploring the safety, tolerability, and effects of ketamine for suicidal ideation and/or behavior.

A study carried out in Denmark, observed that recent psychiatric hospitalization was the factor most strongly associated with suicide (60). This finding reinforces the idea that severe mental disorders is one of the leading indicators of suicide risk. Later, in 2010, mental disorders and substance use disorders were found to be responsible for two-thirds of suicides. Considering the additional burden of mental and substance use disorders as a risk factor for suicide, increased mental and substance use disorders have risen from the fifth most common disease category in the global burden to the third most common disease category (61).

The risk of suicide increases more than twenty-fold in individuals with DDM and is even greater in subjects with comorbidity with other psychiatric disorders or medical conditions (62). Psychological autopsy data show that approximately half of the individuals who died by suicide were suffering from depression. Lee et al. (7) observed that, compared to anxiety disorders, the diagnosis of MDD was associated with an odds ratio about ten times higher.

The causes of suicidal behavior are multiple and complex. Although the presence of MDD is an important predisposing factor, the existence of this pathology alone is not enough to fully explain suicidal behavior, without the interaction with other factors, such as the presence of hopelessness, impulsiveness, and aggression, among others. Furthermore, clinical predictors of suicidal behavior are generally not robust, meaning they are not reproducible for different patient samples, since suicidal behavior results from a combination of individual risk factors (63).

A systematic review recently verified that ketamine and esketamine are promising treatments for MDD, given their efficacy and tolerability (18). The authors analyzed 12 articles (two randomized controlled trials, five case reports and five retrospective studies). SC ketamine was administered to unipolar and bipolar patients in single or multiple doses, weekly or twice a week; the dose ranged from 0.1 to 0.5 mg/kg. In all studies, SC Ketamine showed a rapid and robust antidepressant effect, with remission rates of 50 to 100% after single or multiple doses, with transient side effects.

TABLE 1 Assessment schedule.

Assessments	Eligibility	1	2	3	4	5	6	7	8	Follow up (1 month)	Follow up (2 months)	Follow up (3 months)	Follow up (4 months)	Follow up (5 months)	Follow up (6 months)
Informed consent	X (Re-affirm)	X													
General data information		X													
Clinical information		X													
Nutritional measures		X							X						
Inflammatory profile of the diet		X		X		X		X							
CTQ		X													
Bloods (IL-6, IL-10, IL-1β, TNFα, SIRT3, suPAR, us-CRP and leptin)		X							X						
C-SSRS		X	X	X	X	X	X	X	X	X	X	X	X	X	X
MADRS	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
BPRS		X	X	X	X	X	X	X	X	X	X	X	X	X	X
YMRS	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
HAMD		X	X	X	X	X	X	X	X	X	X	X	X	X	X
FAST		X	X	X	X	X	X	X	X	X	X	X	X	X	X

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exclude that part of the sample could be TRD. Lastly, the lack of blinding and placebo group to analyze the study primary outcomes (clinical outcomes), as the control group will be used only to analyze the biochemical markers.

The use of standard psychiatry research instruments (e.g., MADRS, YMRS, FAST, HAM-D, and BPRS) allows direct comparison of this study with other psychiatric studies. In particular, using YMRS and BPRS allow for a better characterization of the side effect of ketamine confusion on various psychotomimetic and dissociative symptoms as well as manic symptoms. The C-SSRS is widely used in research that evaluates other drugs for suicidal ideation and has high impact power (43–45). The Centers for Disease Control and Prevention (CDC) and the FDA also recommend using the C-SSRS as a reference predictive measure in the analysis of suicide-related issues.

This protocol provides important information for a future definitive study exploring the safety, tolerability, and effects of ketamine for suicidal ideation and/or behavior in addition to investigating the specific molecular mechanism behind the immunomodulatory effects of ketamine.

Ethics statement

This study was approved by Research Ethics Committee the HCPA (CAAE: 33589320300005327) and Research Ethics Committee the HMV (CAAE: 33589320.3.2001.5330). Participants consent by signing the consent form.

Author contributions

MK-S, PB-D-A, and AA prepared the body of the protocol. AA, MK-S, PB-D-A, KC, and JVP prepared all information regarding sample size and statistical analysis. MK-S, JVP, PB-D-A, and LC contributed to the preparation of the entire body of the protocol. AA, JG, VC, and KC prepared study flow and assessment description, table, diagram, references, and formatted the manuscript. All authors read and approved the final manuscript.

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Conflict of interest

In the last 5 years, MK-S has received grant or research support from CAPES, CNPq, CNPq-Produtividade FIPE-HCPA and INCT-TM-CNPq; has been a speaker for Daiichi-Sankyo.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Appendix

Appendix 1: Patient assessment form.

General patient data sheet
Name, number, and initials:
Have you been diagnosed with depression by a psychiatrist?
Have you had depressive symptoms for the past 2 weeks most of the time?
Are you taking any psychotropic medication (antidepressants, antipsychotics, tranquilizers or mood stabilizers)? If so, which drugs and what doses?
-
-
–
Have you been taking the same medications at the same doses for more than 4 weeks in a row daily?
Have you started any psychotherapy (psychological follow-up) recently? If yes, how long ago?
Do you have any known medical illnesses? If yes, what diseases?

CAPÍTULO 3

Artigo: Repeated doses of subcutaneous esketamine in patients with treatment-resistant depression: Case series in a general hospital in Southern Brazil

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Repeated doses of subcutaneous esketamine in patients with treatment-resistant depression: Case series in a general hospital in Southern Brazil

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ABSTRACT

The use of esketamine appears to be a promising alternative, with rapid improvement in depressive symptoms and suicidal ideation. This study aimed to describe the benefits of subcutaneous (SC) esketamine in patients with treatment-resistant depression. The patients in this study were between 22 and 60 years of age, were diagnosed with treatment-resistant depression, and received SC esketamine. This study highlights that repeated esketamine doses may be a promising treatment option for patients with treatment-resistant depression. Eight of the nine patients met th e criteria for response to depressive symptoms at the end of the treatment period, as evaluated using the HAMD-17 scale. In addition, a reduction in suicidal ideation was observed in patients with depression according to item 9 of the HAM-D17 and item 3 of the PHQ-9. Adverse reactions were mild and transient, with no persistent complications. Thus, SC esketamine may be a safe and beneficial alternative for the treatment of resistant depression and suicidal ideation and/or behavior. The use of the SC route is shown to be possible, mainly because of its ease of use and cost reduction in both equipment and human resources.

1. Introduction

Depression is a chronic, recurrent, and highly prevalent condition associated with functional disability and compromised physical health (Juruena et al., 2015) affecting approximately 320 million people worldwide (Friedrich, 2017). Its etiology is multifactorial and combines endogenous susceptibility with exposure to environmental stressors (Juruena et al., 2015). According to a study conducted by (Blackburn, 2019), one-third of patients with Major Depressive Disorder (MDD) did not respond to current antidepressants, leading to an unmet medical need for innovative treatments that are effective in this population.

The association between depression and suicidal behaviors has been widely described in the literature. According to the World Health Organization (WHO) (WHO, 2002), psychiatric illnesses are associated with more than 90% of suicidal ideation cases and are responsible for 90% of suicide-reported deaths worldwide (Brådvik, 2018). Studies across different populations have confirmed the relationship between depression and suicide (Lee et al., 2007). Despite these facts, treatments for depression that also impact suicidal behavior are scarce; thus, drugs with anti-suicide effects are highly desirable and are one of the main research needs in psychiatry (Brådvik, 2018).

In this context, there is an increasing interest in understanding the benefits of ketamine, its racemic compound, and its enantiomers [that is, S-ketamine (esketamine) (Singh et al., 2016) and R-ketamine (arketamine)] (Leal et al., 2021) for the treatment of psychiatric disorders. Intranasal esketamine for treating depression was recently approved by the Food and Drug Administration (FDA) in the United States of America and European Regulatory Authorities (Daly et al., 2019; Popova et al., 2019) for treatment-resistant depression. Ketamine acts

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on glutamate, the principal excitatory neurotransmitter, as a noncompetitive antagonist of the N-methyl-D-aspartate (NMDA) receptor. The effectiveness of glutamate-modulating agents in the treatment of mood disorders involves the regulation of glutamatergic neurotransmission, contributing to the pathophysiology of depression as well as to the mechanisms of antidepressants (Machado-Vieira et al., 2017). Glutamate acts pre- and post-synaptically to activate various receptors. Ionotropic glutamate receptors, such as NMDA, alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), and kainate (KA), are channels that allow the influx of ions into the cell, regulate neuronal surface polarization, and activate intracellular signaling cascades. According to Machado-Vieira et al. N-methyl-D-aspartate (NMDA) and AMPA are directly involved in the antidepressant effects of ketamine (Machado-Vieira et al., 2015).

The pharmacokinetic characteristics of ketamine can be administered intravenously (IV) (Phillips et al., 2020), subcutaneously (SC) (Cavenaghi et al., 2021), intranasally (Daly et al., 2019), orally (Can et al., 2021), sublingually (Lara et al., 2013), and intramuscularly (Cusin et al., 2012). The SC route of administration has comparable efficacy to conventional IV infusion, but has fewer side effects (Loo et al., 2016). In a recent systematic review that included 12 studies (two randomized clinical trials, five case reports, and five retrospective studies), the authors observed that racemic ketamine and its enantiomer, esketamine, administered via SC seem to be a promising treatment for depression, given its efficacy and tolerability (Cavenaghi et al., 2021). Previous studies have verified that repeated and staggered doses of ketamine reinforce its antidepressant and anti-suicidal properties in patients with severe depression (Cusin et al., 2017; Ionescu et al., 2019, 2016).

Studies on repeated doses of IV ketamine have shown promising results, supporting its acute antidepressant effects (Ghasemi et al., 2014; Ionescu et al., 2019; López-Díaz et al., 2017; Murrough et al., 2013). Ketamine SC is also effective and safe in patients with depression (Cavenaghi et al., 2021). Therefore, the main purpose of this study was to describe the results of a clinical experience by applying a protocol of repeated doses of SC esketamine in a tertiary hospital in the city of Porto Alegre, Southern Brazil.

2. Methods

2.1. Participants

This study included patients clinically diagnosed with treatment-resistant depression, who were prescribed SC esketamine and met the inclusion and exclusion criteria. This study was conducted between 2019 and 2022 at the Hospital Moinhos de Vento (HMV-POA) in Porto Alegre, southern Brazil.

The inclusion criteria for the study were patients with: 1) age ≥21 years; 2) and a diagnosis of treatment-resistant depressive (TRD) episode (confirmed diagnosis of depression and lack of response to at least two antidepressants from different classes used in adequate doses and for a minimum period of 6 weeks).

The exclusion criteria were as follows: 1) presence of active psychosis; 2) history of stroke; 3) pregnancy or lactation; 4) uncompensated systemic arterial hypertension; 5) chronic obstructive bronchopulmonary disease; 6) congestive heart failure; 7) coronary artery disease; 8) renal failure; 9) hypersensitivity to esketamine and its components; and 10) porphyria.

This study was approved by the Research Ethics Committee of Hospital Moinhos de Vento in Porto Alegre (CAAE: 21,994,919.4.0000.5330). All study procedures were performed in accordance with the ethical standards of the relevant national and institutional committees on human experimentation and the 1975 Declaration of Helsinki, which was revised in 2008. All patients were informed about the nature and procedures of the study and provided informed consent before enrollment.

2.2. Demographic, behavioral and clinical assessments

A depressive episode was diagnosed according to the criteria of the Diagnostic and Statistical Manual of Mental Disorders, 5th Edition (DSM-5) (American Psychiatric Association and American Psychiatric Association, 2013) and evaluated using the Mini International Neuropsychiatric Interview, Brazilian version (MINI) 4.0 Plus (Amorim, 2000). General demographic and behavioral data were acquired using a standard questionnaire. The instruments used to monitor the patients in this study were the 17-item Hamilton Rating Scale for Depression (HAMD-17) (Hamilton, 1960, p. 196) and the Patient Health Questionnaire (PHQ-9) (Kroenke et al., 2001).

To assess the severity of depressive symptoms, the HAMD-17 was used (Hamilton, 1960, p. 196) translated and adapted into Portuguese, with a structured interview script (Ramos de Carvalho et al., 1993). The scale consists of 17 items with total scores ranging from 0 to 52. Although the author (Hamilton, 1960, p. 196) has not proposed a standard cut-off point, in practice, scores above 24 are considered to identify a severe depressive episode; 18–24, a moderate depressive episode; 7–17, a mild depressive episode; and below 7, no depressive episode or remission.

The PHQ-9 (Kroenke et al., 2001) was used to assess depressive symptoms and suicidal ideation. It is used in the general population, consisting of nine questions that assess the presence of each of the symptoms of a major depressive episode, and has been translated and validated for the Brazilian population. The score is based on the frequency of each symptom in the previous two weeks and recorded on a Likert scale from 0 to 3, corresponding to the answers "never," "several days," "more than half of the days" and "almost every day," respectively. It also included a tenth question assessing the interference of these symptoms with the performance of the patient's usual activities.

To assess remission and response to treatment, cutoff points already used in the literature (Faries et al., 2000; Lin and Lin, 2019) were considered: response was defined as ≥ 50% improvement in the HAMD-17 score from baseline and remission as a HAMD-17 total score of ≥ 7 at the endpoint. Suicidal ideation/behavior was assessed using item 3 of HAMD-17 and item 9 of PHQ-9. Adverse effects were assessed by self-reporting and observation during esketamine administration.

2.3. Procedure

The patients received SC esketamine at an initial dose of 0.5 mg/kg. If an adequate clinical response (decrease in HAMD-17 and PHQ-9) was not obtained, a second infusion was administered at least two days after the first, using 10% more than the first dose. When the patient responded clinically to a dose with a dissociative effect, this dose was repeated throughout the treatment in eight sessions performed 2–3 times a week. In some cases, based on the clinical evaluation by the attending physician and/or the recurrence of depressive symptoms and patient acceptance, a reinforcement/maintenance phase was carried out with repetition of the SC esketamine sessions initially scheduled every fortnight.

Patient safety was ensured by monitoring vital signs, side effects, cognitive/psychiatric status, and physical symptoms for approximately 1 h after the injection. The main effect was the dissociative effect of SC esketamine, with altered sensory perception of light and sounds, which lasted for 20–40 min, followed by post-treatment drowsiness and nausea. The patients were then sent home with their companions.

3. Results

This study was developed based on nine cases of patients, aged 22–60 years, with treatment-resistant depression who used SC esketamine, with mean increasing doses between 0.5–1.0 mg/kg (Table 1). As described previously, repeated doses of SC esketamine were associated with a reduction in depressive symptoms (mean improvement of 56%

Table 1
Sociodemographic and clinical variables in patients.

Variable ($n = 9$)	
Sex	female (5)
(n)	male (4)
Age (yr)	43,33 (13,09)
mean (S.D)	
Ethnicity	caucasian (8)
(n)	malaysian (1)
Marital status	married (3)
(n)	separated/divorced (3)
	single (3)
Children	no (5)
(n)	yes (4)
Education	completed high school (2)
(n)	incomplete superior (2)
	complete superior (5)
Years of education (yr)	14,22 (2,22)
mean (S.D)	
Worked in the last 30 days	no (5)
(n)	yes (4)
Use of illicit drugs	no (7)
(n)	yes (2)
Smoker	no (9)
(n)	
Comorbidities	hypertension (3)
(n)	rheumatological (1)
	diabetes (1)
	cardiological (0)
	respiratory (1)
	chronic kidney failure (0)
	metabolic syndrome (1)
	hepatitis B/C(0)
	cirrhosis (0)
	tuberculosis (0)
	human immunodeficiency virus (0)
	cancer (0)
Psychiatric	bipolar disorder (1)
Comorbidities	panic disorder (1)
(n)	agoraphobia (0)
	social phobia (0)
	obsessive-compulsive disorder (2)
	post-traumatic stress disorder (2)
	alcohol abuse (1)
	alcohol addiction (2)
	abuse of other substances (1)
	dependence on other substances (0)
	psychotic syndrome (0)
	anorexia nervosa (0)
	bulimia nervosa (1)
	generalized anxiety disorder (1)
Medication	antidepressants (9)
(n)	mood stabilizers (3)
	atypical antipsychotics (4)
	typical antipsychotics (2)
	benzodiazepines (4)
	other non-psychotropic medications (5)

HAMD-17 and 50% PHQ-9), stabilization of the depressive state, and a decreased risk of suicide (mean improvement of 83% item 3 HAMD17 and 79.6% PHQ-9) (Tables 2 and 3). It was also observed that the treatment was safe and well tolerated by all patients. No serious adverse effects were observed.

3.1. Cases

Each case included in the study being reported in this paper is presented below.

Case 1

Male, 38 years old, 134 kg with a history of depression starting at the age of 17 years, hypertensive and obese [body mass index (BMI) 46 kg/m^2]. The patient had previously used venlafaxine, duloxetine, bupropion, risperidone, and aripiprazole without adequate response. He is currently using desvenlafaxine 150 mg QD, lithium 750 mg QD, and clozapine 25 mg QD, as prescribed by his clinical physician. The patient had a history of electroconvulsive therapy (ECT) sessions and an improvement in depressive symptoms, with cessation due to accentuated cognitive deficits. Currently, he has persistent depressive symptoms (isolation, sadness, and suicidal ideation), with a score of 18 on the HAMD-17 scale. Based on the MINI evaluation, the patient was diagnosed with TRD without melancholic features or suicide risk. Therefore, the protocol for the use of SC esketamine was initiated with increasing doses between 0.5 and 1.0 mg/kg. The patient underwent nine sessions (the first eight were conducted every seven days, and the ninth session conducted after 15 days). Applications were interrupted because there was no sustained improvement or lack of financial support from the patient. Initially, a relative improvement was observed after the sixth application in depressive symptoms [a decrease of 3 points (17%) and 1 point (0.5%) in relation to the baseline HAMD-17 and PHQ-9 scores, respectively]. However, the improvement was not sustained after the end of weekly SC esketamine, which has been referred for ECT. During the sessions, the patient reported well-being, but presented mild transient adverse effects of dizziness, paresthesia, drowsiness, distorted vision, and disorganized thoughts.

Case 2

Woman, 47 years old, 77 kg, and BMI of 28.63 kg/m^2; according to evaluation, the MINI diagnosed her with TRD, with melancholic characteristics and suicidal ideation (score 19 points on the HAMD-17 scale). The patient also had hypothyroidism, vitiligo, and severe migraines. She had previously used lithium, escitalopram, vortioxetine, modafinil, amisulpride, sodium divalproex, lamotrigine, and duloxetine, with guidance, followed by a pharmacogenetic panel, without an adequate response. In the past, she had improved with ECT sessions, which ceased because of a pronounced cognitive deficit. Currently, nortriptyline 50 mg QD, venlafaxine 225 mg QD, topiramate 50 mg QD, aripiprazole 5 mg QD, lurasidone 120 mg QD, and zolpidem 10 mg QD are prescribed by assistant physicians. The protocol for the use of SC esketamine was started, with increasing doses between 0.5 and 1.0 mg/kg, and at the end of 8 sessions, there was an improvement of 53% and 57% in relation to the baseline score of the HAMD-17 and PHQ-9, respectively. However, 30 days after the last application, the patient had to return and required another five sessions of SC esketamine for maintenance. The last five sessions were carried out monthly for 5 months, and the patient remained asymptomatic despite the melancholic characteristics. During these sessions, patients reported relaxation and dissociation. However, she experienced mild transient adverse effects, such as sweating, paresthesia, nausea and vomiting, dry mouth, feeling of having no memory, and relaxation.

Case 3

Woman, 28 years old, 88 kg, and BMI of 31.89 kg/m^2; according to evaluation, the MINI diagnosed her with Obsessive-Compulsive Disorder and TRD with melancholic features and suicidal risk (score 39 points on the HAMD-17 scale). The patient was hospitalized after a suicide attempt and had a history of three previous attempts. History of childhood trauma (sexual abuse) and psychiatric hospitalization at 27 years of age The protocol for using SC esketamine was started on the patient, with increasing doses of 0.5–1.0 mg/kg, for 8 sessions. At the end of these sessions, the patient's condition improved (59% and 65% with respect to the baseline HAMD-17 and PHQ-9 scores, respectively). During the follow-up visits, two months after discharge, she showed sustained improvement in suicidal ideation, depression, and feeling able to work. The patient continued to use fluoxetine (60 mg QD), valproic acid (1000 mg QD), and chlorpromazine (100 mg QD). During these sessions, patients reported relaxation and dissociation. Moreover, she presented

Table 2
Depressive symptoms before and after the repeated doses SC esketamine.

Patient No./Sex/ Age, y	Race	HAM-D17			PQH9		
		Baseline	After final treatment	Improvement, No. (%)	Baseline	After final treatment	Improvement, No. (%)
1/M/38	White	18	15	17%	20	19	0,5%
2/F/47	White	19	9	53%	21	9	57%
3/F/28	White	39	16	59%	26	9	65%
4/M/60	White	41	11	73%	20	11	45%
5/F/22	Brown	22	10	55%	27	8	70%
6/M/42	White	29	8	73%	25	8	68%
7/F/53	White	19	9	55%	20	10	50%
8/M/57	White	27	8	70%	22	11	50%
9/M/34	White	23	11	52%	21	11	48%

Table 3
Suicidal ideation before and after the repeated doses SC esketamine.

Patient No./Sex/ Age, y	Race	ITEM 3 HAMD-17			ITEM 9 PQH9		
		Baseline	After final treatment	Improvement, No. (%)	Baseline	After final treatment	Improvement, No. (%)
1/M/38	White	2	2	0%	1	1	0%
2/F/47	White	3	0	100%	2	0	100%
3/F/28	White	4	1	75%	2	1	50%
4/M/60	White	4	1	75%	3	1	67%
5/F/22	Brown	3	0	100%	3	0	100%
6/M/42	White	1	0	100%	3	0	100%
7/F/53	White	2	0	100%	2	0	100%
8/M/57	White	2	0	100%	2	0	100%
9/M/34	White	2	0	100%	1	0	100%

with mild transient adverse effects such as restless legs, double vision, dry mouth, and a feeling of erasing her thoughts.

Case 4

Male, 60 years old, 83 kg, and BMI of 27.41 kg/m^2, former smoker, comorbid with moderate alcohol use disorder. According to the MINI assessment, the patient was diagnosed with TRD and exhibited melancholic features with suicidal risk (HAMD-17 score of 41). The patient was admitted to the hospital because of three previous attempts at suicide. He had already been treated for a depressive episode with two antidepressants at the maximum dosage (paroxetine 80 mg and bupropion 450 mg) for a period longer than six weeks without a therapeutic response. The patient is currently receiving venlafaxine 225 mg QD and quetiapine 100 mg QD. The protocol for the use of SC esketamine was initiated, with increasing doses of 0.5–1.0 mg/kg, during 8 sessions, at the end of which there was an improvement of 73% and 45% in relation to the baseline score of the HAMD-17 and PHQ-9, respectively. During these sessions, the patient reported experiencing tranquility and dissociation. He also experienced mild transient adverse effects, including dry mouth, fear, sensation of blood in the mouth, and the sensation of death.

Case 5

Woman, 22 years old, 64 kg, and BMI of 24.39 kg/m^2, puerperal patient who was hospitalized for premature delivery (34 weeks). The patient was diagnosed with TRD, exhibiting melancholic features and a high suicide risk (HAMD-17 score, 22). The protocol for the use of SC esketamine began seven days postpartum, with increasing doses of 0.5–0.6 mg/kg, during 8 sessions. At the end of the protocol, there was an improvement of 55% and 70% in relation to baseline HAMD-17 and PHQ-9 scores, respectively. During the sessions, she presented with mild transitory adverse effects of dry mouth, headache, dizziness, anxiety, anger, desire to cry, and a feeling of being drunk.

Case 6

Male, 42 years old, 72.4 kg, and BMI of 22.35 kg/m^2; according to the MINI assessment, the patient was diagnosed with bipolar disorder, in a depressive period (HAMD-17 scale score 29 points). Substance abuse, such as lysergic acid diethylamide (LSD) and solvents. Patient with a history of use of agomelatine 50 mg, paroxetine 40 mg, venlafaxine 225 mg, risperidone (manic phase), and lithium carbonate 1200 mg/day (not tolerated and discontinued due to diarrhea). Currently, the patient has had a depressive episode for 5 months, with characteristics of hypersomnia, excessive fatigue, lack of energy, and suicidal ideation. The patient was administered brexpiprazole 4 mg QD, bupropion 150 mg QD, escitalopram 20 mg QD, methylphenidate 80 mg QD, and oxcarbazepine 1200 mg QD. The protocol for the use of SC esketamine was initiated, with increasing doses of 0.5–0.9 mg/kg, during 8 sessions; at the end of the protocol, there was an improvement of 73% and 68% in relation to the baseline score of the HAMD-17 and PHQ-9, respectively. During the sessions, he experienced transient adverse effects, such as dry mouth, difficulty speaking, and increased perception of light, color, and sound.

Case 7

Woman, 53 years old, 72 kg, and BMI of 26.45 kg/m^2, underwent bariatric surgery three years ago, with hyperparathyroidism; according to the MINI evaluation, diagnosed with TRD and use of alcohol (0.5–1 g/day on the weekends). History of use of fluoxetine 60 mg, escitalopram 60 mg, and melatonin. The patient exhibited irritability and delusional ideas of extreme poverty, insomnia, anxiety, and hopelessness with a score of 19 on the HAMD-17 scale. The patient concomitantly received vortioxetine 20 mg QD, zolpidem 10 mg QD, alprazolam, and clonazepam 0.25 mg QD. The protocol for the use of SC esketamine was started, with increasing doses of 0.5–0.8 mg/kg, during 8 sessions and 5 more maintenance sessions every 15 days. At the end of the protocol, there was an improvement of 55% and 50% in relation to baseline HAM-D17 and PHQ-9 scores, respectively. During the sessions, she presented with mild transient adverse effects such as dry mouth, crying, nausea, and a floating sensation.

Case 8

Male, 57 years old, 106 kg, and BMI of 34.22 kg/m^2, diagnosed with TRD and obsessive-compulsive disorder, as assessed by the MINI. His-

tory of use of venlafaxine, citalopram, fluoxetine, and imipramine. The patient had a depressive episode with symptoms of insomnia and somatization with chronic pain, irritation, anxiety, pessimism, and hopelessness, with a score of 27 on the HAMD-17. The patient was administered quetiapine 200 mg daily, and clonazepam and paroxetine 75 mg daily. The protocol for the use of SC esketamine began with increasing doses of 0.5–0.9 mg/kg, during 8 sessions and 4 more maintenance sessions every 15 days. At the end of the protocol, there was an improvement of 70% and 50% in relation to baseline HAM-D17 and PHQ-9 scores, respectively. During the sessions, the patient experienced a mild transient adverse effect of dry mouth.

Case 9

Male, 34 years old, 90 kg, and BMI of 24.41 kg/m^2; according to the MINI evaluation, he was diagnosed with TRD. The patients with a history of using lithium 900 mg and desvenlafaxine (150 mg) reported having used several other drugs but without good adherence due to side effects. The patient exhibited destructive self-criticism, negativism, self-sabotage, hypersomnia, and eventual suicidal ideation, with an score of 23 points on the HAMD-17 scale. She concomitantly used lisdexamfetamine 70 mg QD, vortioxetine 10 mg QD, zolpidem 10 mg QD, and clonazepam 20 mg QD. The protocol for the use of SC esketamine was initiated, with increasing doses of 0.5–1.0 mg/kg, during 8 sessions. At the end of the protocol, there was an improvement of 52% and 48% in the baseline HAM-D17 and PHQ-9 scores, respectively. During the sessions, he experienced mild transient adverse effects such as dry mouth, a floating sensation, and disinhibition (talking more than usual).

4. Discussion

In this study, it was observed that repeated doses of SC esketamine may be a promising treatment for depressive symptoms and suicide risk, given that eight patients met the response criteria (≥ 50% improvement in the HAMD-17 score from baseline). A reduction in suicidal ideation was also observed in patients with depression according to item 3 of the HAM-D17 scale and item 9 of the PHQ-9 scale (Table 3). The adverse reactions were mild and transient.

Ketamine metabolism is influenced by the expression of CYP enzymes in various biological tissues (intestine, liver, and brain), the route of drug administration, and the sex and age of rodents and patients (Nguyen et al., 2022). Ketamine is a fat-soluble drug with a bioavailability of almost 100% and can easily cross the blood-brain barrier. When ketamine is administered, it is distributed directly into the systemic circulation and reaches the brain (target organ). The highest levels of ketamine are metabolized by CYP enzymes in the brain into norketamine and then into hydroxynorketamine, that is, it has increased bioavailability because it avoids first-pass hepatic metabolism; thus, ketamine metabolites are not formed (Loo et al., 2016; Nguyen et al., 2022). The concentration of ketamine in the blood of psychiatric patients was evaluated based on blood samples obtained at baseline and at 5, 15, 30, 120, and 240 min after IV dosing, and 15, 30, 120, and 240 min after IM/SC injection. Plasma concentrations recorded after IV showed a peak between 350 and 400 ng/mL (0.5 mg/kg dose), with a peak below 200 ng/mL in the SC route. Plasma concentrations were linearly correlated with ketamine dosage (IV, $r = 0.88$, $p<0.001$; IM, $r = 0.92$, $p<0.001$; SC, $r = 0.86$, $p<0.001$) and Clinician-Administered Dissociative States Scale (CADSS) scores at 40 min ($r = 0.44$, $p = 0.001$). All three routes of administration resulted in comparable antidepressant effects, with fewer adverse effects observed with the SC route. Antidepressant response, adverse effects, and ketamine concentrations were dose-related (<0.5 mg/kg) (Loo et al., 2016). This suggests that the application of ketamine SC is a practical, feasible, and effective treatment approach.

In 2022, a systematic review was published (Smith-Apeldoorn et al., 2022) on esketamine administered via a non-intranasal route to assess its antidepressant effects and safety. The authors noted that SC, IV, and possibly oral administration might be effective in reducing depressive symptoms in most patients with MDD, bipolar depression, and treatment-resistant depression. A clinical response was also observed after repeated administration of esketamine throughout the treatment. Regarding tolerability, esketamine was well tolerated by most patients; however, open data indicated marked psychotomimetic symptoms in exceptional cases.

In the present study, increasing doses of SC esketamine were administered during treatment, and decreases in suicidal ideation and depressive symptoms were observed. The literature has already indicated that doses of 0.5 mg/kg may not be sufficient in patients with severe and chronic depression (Ionescu et al., 2019). Repeated and escalating doses of SC ketamine (0.5 mg/kg for the first three infusions to 0.75 mg/kg for the last infusions) reinforced the antidepressant and anti-suicidal properties of ketamine in a sample of patients with severe depression (Ionescu et al., 2019). This study also corroborates the idea (Ionescu et al., 2019) that patients with extremely severe disease and/or TRD can tolerate doses above 0.5 mg/kg.

The literature on SC ketamine is limited. A recent systematic review (Cavenaghi et al., 2021) found 12 studies, the vast majority of which were case reports or retrospective studies. For depression (unipolar and bipolar), data from a retrospective analysis (Lucchese et al., 2021), of 70 patients showed that 50% of the patients were responders (50% improvement in the final MADRS score), with doses of 0.5–1.0 mg/kg. In the responder group, the mean final MADRS score was 9.9, and 25.7% of patients achieved complete remission (MADRS score ≤10). The authors also observed that greater resistance to treatment was the main factor related to poor response to ketamine. identified BMI, history of suicide attempts, and family disorders due to alcohol use (Rong et al., 2018). In a recently published study conducted by Tham et al. (2022), a study of 10 patients with treatment-resistant moderate and severe depression; the authors noted that at the end of treatment, response (≥50% reduction in score from baseline to endpoint) was achieved in 8/10 cases on the Montgomery-Åsberg Depression Scale (MADRS) and 6/10 on the Rapid Inventory of Depressive Symptomatology, whereas Self-Report (QIDS-SR 16) and remission was achieved in 8/10 (based on MADRS ≤10) and 5/10 (based on QIDS-SR 16 ≤ 6). The initial dose was 0.25 mg/kg for at least two sessions. If there was an inadequate response, the dose was increased to 0.5 mg/kg for at least two sessions, and then, if necessary, increased to a maximum dose of 0.75 mg/kg. Up to six sessions were conducted, varying according to the response and clinical tolerability of the patients.

George et al. conducted a double-blind randomized controlled trial with a 6-month follow-up in 14 elderly patients (≥60 years old) with TRD (George et al., 2017). The authors observed that SC doses of ketamine (0.1–0.5 mg/kg) administered in separate sessions (≥1 week apart) with an active control (midazolam), led to the remission of depressive symptoms in 50% of patients. Doses ≥ 0.2 mg/kg esketamine was significantly more effective than midazolam, and repeated esketamine sessions resulted in a greater likelihood of remission or a longer time to relapse. These results, with SC esketamine, are comparable to response rates seen in studies using multiple-dose ketamine IV protocols (Domany et al., 2020; Ionescu et al., 2019, 2016). For comparison, in the IV route, remission rates of 23% and response rates of 59% were observed (Phillips et al., 2020), 29% and 71% response (Zarate et al., 2006), 50% response (Berman et al., 2000), and 64% response (Murrough et al., 2013).

In 2022, Surjan et al. evaluated 70 patients with resistant MDD or bipolar depression who received SC esketamine once a week for 6 weeks (Surjan et al., 2022). The authors observed significant decreases in suicidal ideation with a rapid onset of action. A significant difference in suicide scores was observed 24 h after the first administration ($p < 0.001$), and a further reduction was observed with repeated administration, with good tolerability and safety. The same research group also observed that dissociative symptoms did not correlate with antidepressant responses. They conducted another retrospective analysis of 394 esketamine ad-

ministrations at doses between 0.5–1.0 mg/kg. It was found that 30 min after administration, there was no difference between the mean scores of the CADSS and no clinical correlation between response×dissociative symptoms, time×demographic and clinical characteristics, or interaction between time×combined medication (Del Sant et al., 2022).

Our patients continued to take other medications concomitantly with esketamine as described in Table 1. Owing to the high complexity of the patients, all patients used other concomitant psychiatric drugs. A recent systematic review (Veraart et al., 2021), which included 24 studies provided an overview of the pharmacodynamic interactions between ketamine and mood stabilizers, benzodiazepines, monoamine oxidase inhibitors, antipsychotics, and psychostimulants. The authors noted no significant interaction between ketamine and the monoamine oxidase inhibitor, tranylcypromine. However, they also noted an interaction between ketamine×haloperidol and ketamine×risperidone, which showed an attenuating effect of risperidone on ketamine-induced brain perfusion changes. Clozapine significantly attenuated the positive symptoms induced by ketamine in patients with schizophrenia but not in healthy participants. Benzodiazepines have been repeatedly shown to shorten the duration of the antidepressant effects of ketamine. Therefore, when using benzodiazepines and lamotrigine, a reduction in the outcome of ketamine treatment should be considered. There is also limited evidence regarding the interactions between ketamine and clozapine, haloperidol, or risperidone (Veraart et al., 2021). BZDs are commonly prescribed as psychotropics and are often added to antidepressants during the initial treatment of depression. They act as GABA-A receptor agonists, allosterically increasing the inhibitory tone of GABA interneurons and may thus interfere with the therapeutic effect of ketamine in light of ketamine's blockade of NMDA receptors in an identical population of GABAergic interneurons (Abdallah et al., 2015). Diazepam selectively blocks the influence of ketamine on limbic system metabolism and inhibits ketamine-induced hyperlocomotion in rodents (Irifune et al., 1998). Furthermore, over five decades of experience in anesthesiology has documented that BZDs restrict the psychotomimetic side effects of ketamine during general anesthesia (Cartwright and Pingel, 1984). These clinical experiences, along with the opposing effects of ketamine and BZD on GABAergic interneurons, suggest that BZD may negatively interfere with the antidepressant effect of ketamine and treatment outcomes. This assumption was supported by two case series suggesting a modulatory influence of BZDs on the antidepressant effects of ketamine (Albott et al., 2017).

This study included patients with extremely severe depression and/or TRD, and a simple and low-cost clinical protocol may be a promising, viable, and accessible alternative for patients with severe and resistant depression. We used two validated scales (HAM-D17 and PHQ-9) to compare and validate the symptoms observed in these patients.

The limitations of this study include the inability to report on the efficacy of SC esketamine, as a multicenter, randomized, controlled clinical trial would be required; patients' continued to use of other medications (antidepressants, antipsychotics, mood stabilizers, etc.), which might have caused interference between multiple medications and esketamine (negative pharmacodynamic interactions); and dissociative symptoms not assessed using an appropriate scale, such as the CADSS (Bremner et al., 1998).

In conclusion, the study results show the benefits of SC esketamine for depressive symptoms and suicide risk in patients already on psychotropic treatment and with suicidal ideation and/or behavior. Thus, the SC route might be further encouraged in the treatment of resistant depression, as it allows medication to be administered conveniently, safely, and with less waiting time for patients. Medications can be administered on an outpatient basis, allowing patients to receive treatment more easily and with a lower risk of adverse effects. Additionally, owing to less complex equipment and personnel, the SC route is an alternative, especially in underdeveloped countries where the cost of intranasal ketamine is high. Randomized controlled clinical trials are needed to understand the mechanism of action of SC esketamine, particularly with regard to biochemical biomarkers, and to evaluate the efficacy and safety of this therapy on a larger scale. Our group is conducting a larger study to assess the serum levels of inflammatory biomarkers and their correlation with clinical effects, as well as follow-up (6 months), to better understand the duration of action of SC esketamine (NCT05249309).

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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CAPÍTULO 4

Artigo: Allergic reaction induced by subcutaneous administration of ketamine: a case report

Periódico: International Clinical Psychopharmacology

Fator de impacto:2.6 (2023), **Qualis CAPES**A3

Allergic reaction induced by subcutaneous administration of ketamine: a case report

Victor Hugo Schaly Cordova[a,b,c], Ana Paula Anzolin[d], Marcia Kauer Sant'Ana[c,d], Acioly Lacerda[e] and Paulo Silva Belmonte-de-Abreu[d]

Ketamine can be used for depression and suicidal ideation due to its effectiveness and low complication rates; moreover, allergic reactions are rare. Immediately after subcutaneous (SC) ketamine administration, a 22-year-old man rapidly developed hives on the trunk and face without oxygen desaturation. Symptoms disappeared after treatment with prednisolone. This case presents an allergic reaction to ketamine compatible with mast cell activation and release of preformed mediators, without being able to prove whether the event was mediated by immunoglobulin E. This is the only case reported to date of an allergic reaction to SC ketamine for psychiatric treatment. *Int Clin Psychopharmacol* XXX: 000–000

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Keywords: COVID-19, glucose-6-phosphate dehydrogenase deficiency, major depressive disorder, suicide attempt, urticaria

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Ketamine has been recently approved in different countries to treat resistant depression and control suicidal ideation. Protocols for ketamine use propose provision of drugs for hypertension, nausea and anxiety, without describing measures for eventual allergic reactions. Though there are eventual descriptions of skin reactions in ketamine use for pediatric anesthesia (Bylund *et al.*, 2017; Boynes *et al.*, 2007), a systematic review of SC ketamine/esketamine use in major depressive disorder (MDD) failed to report any episode of dermatologic reactions such as skin rash (urticaria) (Cavenaghi *et al.*, 2021). Therefore, it is relevant to report this kind of adverse effect and its acute treatment, for better identification and management.

We report the case of a 22-year-old man treated at the outpatient service of a major teaching hospital (Hospital de Clínicas de Porto Alegre) with a history of MDD with suicidal ideation with ketamine indication after failure to 8 weeks respond to escitalopram at 20 mg/day, with prior diagnosis glucose-6-phosphate dehydrogenase (G6PD) deficiency that contraindicated further antidepressant use and with history of allergy to amoxicillin, egg white, and Neosaldina (caffeine + dipyrone + isometheptene). Patient was under continued use of enalapril 40 mg/day for 9 years and stopped escitalopram after starting SC ketamine.

Fourteen months before (November 2020), the patient contracted COVID-19 with symptoms of coughing, throat secretion and worsening of previous depressive symptoms. After 7 months, he developed pericarditis treated with colchicine 0.5 mg/day for 90 days. He received three COVID-19 vaccines, in May, September and December 2021 (first and second doses of Oxford/Astra Zeneca and third dose of Bio NTech/Pfizer). Patient experienced tremors, fever, headache and tachycardia after the first dose but no adverse reaction after the second and third doses.

Patient presented at the hospital with suicidal ideation (ideation level 5 and 12 points of intensity of ideation, as measured by Columbia-Suicide Severity Rating Scale), without suicide attempt, no psychiatric hospitalization and without history of electroconvulsive therapy. The patient weight was 71.90 kg (BMI, 18.9).

The procedure was performed with the patient comfortably resting on the stretcher with basic life support equipment, with 4 h of fasting (Lacerda *et al.*, 2019). Patient received a half-calculated dose of SC esketamine (0.24 mg/kg) into the abdominal wall. A skin rash (urticaria) was observed on the patient's chest and forehead 10 min after application (Fig. 1). Pulse raised from 65 to 100 BPM and blood pressure from 125/63 to 148/98 mmHg, keeping normal sinus rhythm and pulse oximetry (SpO2) of 99%. Therefore, patient received a 10-mg oral prednisolone single dose, and 15 min after urticaria began to resolve, vital signs remained stable. The patient reported diffuse and slight analgesia, with

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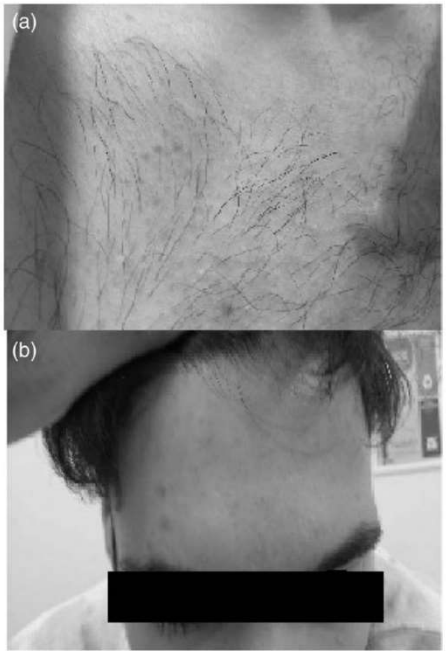
Fig. 1



Urticarial reaction in (a) chest and (b) forehead.

no dissociative effects. The patient followed the protocol of seven additional applications of ketamine (the second with 0.43 mg/kg, the third with 0.56 mg/kg, the fourth of 0.83 mg/kg, the fifth of 0.66 mg/kg and sixth–eighth 0.69 mg/kg), preliminarily assuming a 10-mg single dose of prednisolone before each application. All the other seven administrations of ketamine were divided into three applications (10 min apart) until reaching the total dose. The patient no longer had any allergic reaction.

Allergic reactions related to ketamine administration appear to be extremely rare. There is a report of extensive macular rash in a female patient who received ketamine for wart removal, developing generalized rash and laryngospasm requiring endovenous (EV) epinephrine. The remaining reports include an allergic reaction with severe urticarial rash and wheezing similar to our case report, after intramuscular ketamine and midazolam coadministration before a dental procedure, with urticarial rash, respiratory distress and subsequent 90% O_2 saturation hypoxia, and a type I hypersensitivity reaction to EV infusion of ketamine and midazolam, with pruritic urticarial eruption and perioral edema (Bylund *et al.*, 2017). Further reports concern a veterinary use.

Urticaria following SC ketamine administration is more suggestive of mast cell activation and mediator release (histamine), instead of immunoglobulin E (IgE) release. Along this line, it is more probable that the reported allergic reaction to ketamine in this patient was more likely to result from direct stimulation of mast cells, instead of consequence of IgE mechanism. It must also be questioned about the long-term effect of COVID-19 in this patient, since cutaneous reactions are common in COVID-19 too, with reports of rash with petechiae, chilblain-like eruptions on fingers and toes, and others (Wollina *et al.*, 2020). However, in this patient, urticaria appeared only on the first ketamine application, around 10 min after the shot, but not during COVID-19 infection. The choice of prednisolone instead of an antihistamine was decided at the moment considering potential risk of the G6PD gene mutation carried by the patient and its potential risk of inducing hemolysis after several drugs administration.

Given all above-mentioned considerations, we can argue that this case is consistent with an SC ketamine induced adverse drug reaction. The missing point is about the mechanism, whether by direct mast cells stimulation followed by histamine release or by IgE-mediated anaphylactic reaction, and requires additional studies with the use of biochemical markers. Although absolutely rare, clinicians should be aware that allergic skin reactions may be a consequence of SC ketamine administration. Therefore, pharmacological treatments for allergic reaction should be available when administering this drug, even in psychiatric conditions.

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Author contributions

Conceptualization: V.H.S.C. and A.P.S.A. Methodology: A.P.S.A. Investigation: V.H.S.C. Data Curation: V.H.S.C. and A.P.S.A. Writing-original draft: V.H.S.C. Writing-review and editing: A.P.S.A., A.L., and P.S.B.-A. Supervision: M.K.S. and P.S.B.-A.

Data availability statement: data of this paper can be accessed by contacting the authors. For ethical reasons, patient's identity is confidential.

Conflicts of interest

There are no conflicts of interest.

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CAPÍTULO 5

Artigo: Efficacy of ketamine for depressive symptoms and suicidal ideation: 6-month naturalistic study

STATUS: a ser submetido

PARTE III

Discussão e Conclusões

5 DISCUSSÃO

A etiologia dos transtornos psiquiátricos é complexa e intrinsecamente multifatorial, refletindo uma interação entre fatores genéticos, comportamentais e ambientais. Nesse contexto, abordagens multidimensionais, emergem como cruciais para decifrar as sutilezas e heterogeneidades associadas aos transtornos mentais e suas correlatas disfunções psicobiológicas. Diante da complexidade dos transtornos psiquiátricos e da urgente necessidade de terapias mais eficazes, a busca por biomarcadores séricos, detectáveis perifericamente, ascendeu a um patamar de primordial importância. Tais biomarcadores podem transformar o panorama do diagnóstico, monitoramento e intervenção em condições psiquiátricas, proporcionando uma visão mais elucidativa da fisiopatologia subjacente.

Nesse contexto, o artigo que constitui o capítulo I desta tese demonstrou que níveis séricos mais elevados de TNFα e IL-1β (pró-inflamatórios) estão associados ao início precoce do TDM,mesmo desconsiderando a comorbidade com doenças autoimunes, a diferença significativa permaneceu. Curiosamente, os níveis de IL-6 também foram mais altos no grupo de início precoce e os níveis de IL-10 (anti-inflamatório) foram mais altos no grupo de início tardio, mas sem diferença significativa, possivelmente devido à falta de poder para detectar tamanhos de efeito médio ou pequeno. Estes resultados apoiam a hipótese de que um desequilíbrio entre citocinas pró-inflamatórias e anti-inflamatórias pode estar associado a subconjuntos de sintomas depressivos de início mais precoce. Desta forma, observa-se que uma indicação de que o TDM de início precoce pode ser um subtipo particular, no qual o estado pró-inflamatório desempenha um papel maior do que no TDM de início tardio, contudo as condições prodrômicas associadas a este estado pró-inflamatório precisam ser investigadas.

Nossos resultados também corroboram com a literatura existente indicando que mudanças nas citocinas séricas ocorreram no início precoce do TDM e apontam para divergências etiológicas não apenas entre grupos etários extremos, como depressões infantis e geriátricas, mas também entre início adulto precoce e depressão adulta tardia. Bukh *et al*., (2011) avaliou as diferenças entre pacientes com TDM de início precoce e tardio, e observou que houve uma menor prevalência de eventos estressantes da vida antes do início precoce do TDM em comparação com pacientes com idade de início tardio. Os autores também tinham como hipótese que o início precoce do TDM na idade adulta pode estar associado a um maior nível de neuroticismo e uma maior prevalência de comorbidades. Em nosso estudo, a maioria dos pacientes utilizava antidepressivos. Sabe-se quecom o uso dessa classe de medicamentos,

poderá ocorrer uma redução nos níveis periféricos de citocinas (Liu *et al*., 2019). No entanto, um estudo de Zou *et al*. (2019) mostrou evidências de mudanças nos níveis de citocinas em pacientes com TDM que não usavam antidepressivos. Esses pacientes também apresentaram anormalidade na regulação imunológica e ativação do sistema imunológico. Os autores sugerem que a depressão pode ser um processo de intensificação da inflamação, no entanto, nem todas as citocinas séricas apresentaram níveis aumentados ou participaram da ativação imunológica em pacientes com TDM. Neste estudo, as citocinas IL-1β, IL-10 e TNFα mostraram uma diferença significativa, enquanto IL-6 e TGF-β1 não apresentaram diferença. Corroborando este estudo, nossos achados também mostram a diferença nas citocinas IL-1β e TNFα e nenhuma diferença em IL-6 em pacientes com início precoce e tardio de TDM, mesmo com o uso de antidepressivos. Outros estudos também correlacionam a inflamação com suicídio (Halaris *et al*., 2013). Pessoas que tentam suicídio apresentam níveis mais altos de IL-6 no líquido cefalorraquidiano e níveis mais altos de IL-6 e TNFα no plasma em comparação com controles saudáveis (Janelidze *et al*., 2011).

Mais recentemente, a cetamina emergiu como uma molécula de interesse devido aos seus efeitos antidepressivos e anti-suicídas (Cavenaghi *et al*., 2021). No entanto, a literatura atual ainda carece de estudos longitudinais robustos que avaliem o impacto direto da cetamina no risco de suicídio em longo prazo. Além disso, é crucial entender a segurança e a tolerabilidade dessa substancia, especialmente em subpopulações específicas, como a de pacientes com episódio depressivo maior acompanhados de ideação suicida.

Uma das questões ainda não totalmente elucidada é o mecanismo imunomodulador da cetamina e como ele se relaciona com seus efeitos terapêuticos. Neste sentido, o capítulo II desta tese foi delineado com o propósito de investigar a relação entre a administração de cetamina SC e a modulação de biomarcadores inflamatórios em pacientes com episódio depressivo maior. Apresentou-se um protocolo de estudo multicêntrico prospectivo naturalístico da cetamina em episódios depressivos realizado no HCPA e no HMV. O estudo foi planejado para recrutar pacientes adultos com TDM ou TB, que estavam atualmente em um episódio depressivo maior e apresentavam sintomas de ideação e/ou comportamento suicida de acordo com a Escala C-SSRS. Os pacientes receberam cetamina SC duas vezes por semana durante 1 mês, mas a frequência poderia ser alterada ou a dose reduzida e/ou aumentada conforme decisão do médico assistente. Após a última sessão de cetamina, os pacientes foram acompanhados por telefone uma vez por mês por até 6 meses.

No Capítulo III, são apresentados os resultados preliminares e a validação da metodologia proposta, fundamentados na implementação do protocolo delineado no Capítulo

II. Esta série de casos, compreendendo nove pacientes, evidencia uma resposta terapêutica à administração SC de cetamina. A via de administração SC emergiu como uma abordagem terapêutica viável e eficaz, com destaque para a potencial necessidade de titulação da dose, particularmente em pacientes com quadros depressivos de alta refratariedade ou gravidade. Estes achados estão em consonância com estudos recentes, como os conduzidos por Lucchese *et al*. (2021) e Tham *et al*. (2022), que também corroboram a eficácia antidepressiva da cetamina via SC. Adicionalmente, conforme elucidado por Ionescu *et al*. (2019), doses superiores de cetamina podem não somente ser bem toleradas, mas também demonstrar maioreficácia em determinados subgrupos de pacientes.

Contudo, é importante salientar as limitações intrínsecas do presente estudo. A ausência de um ensaio clínico randomizado e controlado restringe a capacidade de generalização dos resultados obtidos. Ademais, a concomitância de múltiplas medicações pode introduzir variáveis confundidoras, exigindo uma análise meticulosa e criteriosa para discernir os efeitos terapêuticos isolados da cetamina. Uma revisão sistemática recente (Veraart *et al*., 2021) destacou interações farmacodinâmicas entre a cetamina e estabilizadores de humor, benzodiazepínicos, inibidores da monoamina oxidase, antipsicóticos e psicoestimulantes. Foi observado que os benzodiazepínicos podem reduzir a duração dos efeitos antidepressivos da cetamina. Além disso, experiências clínicas sugerem que os benzodiazepínicos podem interferir negativamente no efeito antidepressivo da cetamina. Esta suposição foi corroborada por estudos de caso que indicam uma influência moduladora dos benzodiazepínicos sobre os efeitos antidepressivos da cetamina (Albott *et al*., 2017).

Os protocolos atuais para a administração de cetamina propõem intervenções farmacológicas para efeitos adversos como hipertensão, náusea e ansiedade, contudo, não abordam adequadamente medidas preventivas ou terapêuticas para reações alérgicas. Embora existam relatos isolados de reações cutâneas associadas ao uso de cetamina em contextos de anestesia pediátrica (Boynes *et al*., 2007; Bylund *et al*., 2017), uma revisão sistemática conduzida por Cavenaghi *et al*. (2021), não identificou episódios de reações dermatológicas adversas em pacientes com TDM tratados com cetamina SC.

No capítulo IV, é apresentado um caso clínico de um paciente masculino, 22 anos, diagnosticado com TDM e ideação suicida, que foi submetido ao protocolo terapêutico descrito no Capítulo II. Este paciente manifestou uma reação de urticária após a administração SC de cetamina. A intervenção imediata com prednisolona oral mitigou a reação alérgica, que não se repetiu em administrações subsequentes da cetamina. A etiologia desta reação pode estar associada à ativação direta de mastócitos com subsequente liberação de mediadores

inflamatórios, como a histamina, em detrimento de uma resposta mediada por IgE. É imperativo considerar o histórico médico do paciente, incluindo a infecção prévia por COVID-19, dada a associação documentada com reações cutâneas (Wollina *et al*., 2020). A decisão de tratar com prednisolona foi influenciada pelo potencial risco de hemólise associado à mutação genética G6PD identificada no paciente. Com base nas evidências e observações clínicas apresentadas, sugere-se que este caso ilustra uma reação adversa induzida pela cetamina SC. A natureza exata do mecanismo subjacente, seja ele mediado por células mastócitas ou uma reação anafilática de tipo IgE, permanece incerta e justifica investigações adicionais com marcadores bioquímicos específicos. Apesar da raridade de tais reações, é essencial que os profissionais de saúde estejam preparados para identificar e gerenciar potenciais reações adversas associadas à cetamina SC, garantindo a disponibilidade de intervenções farmacológicas apropriadas, mesmo em contextos psiquiátricos.

Após a análise detalhada do protocolo de tratamento, mecanismos de ação, efeitos adversos e potenciais interações medicamentosas da cetamina, conforme discutido nos capítulos anteriores, o capítulo V se concentra nos resultados finais do protocolo de estudo apresentado no Capítulo II. A eficácia da cetamina, especialmente na administração SC foi avaliada em relação aos sintomas depressivos e suicidas.

Os resultados apresentados no capítulo V, vem em consonância com as evidências anteriores (Cavenaghi *et al*., 2021; George *et al*., 2017; Smith-Apeldoorn *et al*., 2022; Tham *et al*., 2022) e ofereceu insights inovadores sobre o uso terapêutico da cetamina SC em episódio depressivo maior. Observou-se uma melhoria significativa nos sintomas depressivos após oito a dez administrações SC de cetamina, com destaque para a redução da ideação suicida. Os resultados mantiveram sua relevância clínica e estatística no acompanhamento de 6 meses, reforçando a eficácia sustentada da cetamina SC no manejo da depressão.

A farmacocinética e o metabolismo da cetamina são influenciados por múltiplos fatores, como destacado por Nguyen *et al*. (2022). A cetamina exibe alta lipossolubilidade e biodisponibilidade, o que permite uma distribuição rápida na circulação sistêmica e uma passagem eficiente pela barreira hematoencefálica. Os estudos de Loo *et al*. (2016) e Jollant *et al*. (2023) também foram consistentes com nossos achados, destacando a tolerabilidade e os efeitos colaterais transitórios da cetamina. Desta forma a análise de nossos resultados, em combinação com a literatura existente, sugere que a cetamina SC pode ser uma opção de tratamento promissora para a depressão, especialmente em casos resistentes ao tratamento. A estratégia de dosagem utilizada neste estudo reforçou os efeitos antidepressivos e anti-suicidas da cetamina, apoiando os achados de Ionescu *et al*. (2019).

Com base nas análises e discussões apresentadas ao longo desta tese, enfatizamos a importância da cetamina como uma alternativa terapêutica promissora no tratamento da depressão e da ideação suicida. No entanto, é evidente que condições complexas, como episódio depressivo maior e a ideação suicida, não podem ser totalmente abordadas apenas por uma intervenção farmacológica, dada a complexidade multifatorial que envolve genética, ambiente e fatores sociais. Como observado, a eficácia da cetamina, embora promissora, pode variar entre os indivíduos e pode ser influenciada por diversos fatores. Além disso, a compreensão profunda das alterações biológicas da depressão, bem como dos mecanismos de ação da cetamina, como discutido nos capítulos anteriores, podem abrir novos caminhos para a identificação de alvos terapêuticos e biomarcadores. A via de administração SC da cetamina, que foi o foco principal desta tese, demonstrou ser uma alternativa viável e eficaz, mas a busca contínua por otimizar e entender completamente seu potencial é essencial para avançar no campo da psicofarmacologia.

6 CONCLUSÕES

Esta tese sugere a possibilidade de identificar subgrupos de pacientes através do perfil de biomarcadores inflamatórios, em que o perfil mais inflamatório tem início mais precoce. Além disso, estudamos um protocolo padronizado de cetamina em um estudo naturalístico que teve eficácia nos desfechos de sintomas depressivos e risco de suicídio. Estes achados associados contribuem para intervenções cada vez mais específicas e personalizadas, e como perspectiva, no futuro contribuir para melhor predição de resposta e tratamentos de precisão. Inicialmente, os dados revelaram uma associação significativa entre níveis séricos elevados de TNFα e IL-1β e o início precoce de TDM. Estas descobertas sustentam a hipótese de que alterações nos níveis de IL-1β e TNFα podem identificar um subgrupo específico de pacientes com TDM, marcado por um início mais precoce da doença.

Dada a complexidade do tratamento da depressão e a resistência de muitos pacientes às terapias convencionais, a busca por intervenções rápidas e eficazes é crucial. A cetamina, neste contexto, apresentou-se como uma alternativa terapêutica promissora. Em uma análise preliminar de pacientes submetidos ao protocolo de tratamento com cetamina SC, observou-se uma melhoria notável nos sintomas depressivos e uma redução significativa na ideação suicida.No entanto, é fundamental considerar os potenciais efeitos adversos associados ao uso da cetamina. Observou-se que este agente pode desencadear reações alérgicas, como urticária. A intervenção imediata com prednisolona provou ser eficaz na mitigação desses sintomas. A natureza dessa reação alérgica aponta para uma ativação de mastócitos e a subsequente liberação de mediadores pré-formados. Ainda assim, a causa exata dessa reação, particularmente se é mediada por imunoglobulina E, permanece incerta e requer investigações adicionais.

No estudo naturalístico e prospectivo, o potencial terapêutico da cetamina foi significativamente associado a melhora clínica. Os dados obtidos indicam uma relação dose-resposta definida, caracterizada por um incremento progressivo nas concentrações administradas e uma consequente melhoria nos indicadores de depressão, ideação suicida e funcionamento global. A cetamina administrada via SC destaca-se como uma abordagem terapêutica inovadora para a depressão refratária, oferecendo vantagens como administração eficiente, segurança e redução no tempo de espera para os pacientes. A possibilidade de administração ambulatorial facilita o acesso ao tratamento e minimiza o risco de reações adversas. Adicionalmente, a simplicidade dos equipamentos e a menor necessidade de pessoal especializado tornam a via SC particularmente atrativa, sobretudo em contextos de países em

desenvolvimento, onde os custos associados à cetamina intranasal são altos. No entanto, é essencial enfatizar a necessidade de estudos mais rigorosos e extensos. Ensaios clínicos controlados e randomizados, bem como investigações longitudinais, são cruciais para a validação e ampliação das descobertas atuais, assegurando a eficácia e segurança da cetamina via SC como uma intervenção eficaz no tratamento da depressão.

Esta tese, portanto, abre caminhos para a identificação de subgrupos específicos de pacientes com base em perfis biomarcadores inflamatórios, destacando-se aqueles com um início mais precoce da doença. Paralelamente, a pesquisa realizada com um protocolo de cetamina padronizado em um contexto naturalístico demonstrou resultados promissores no alívio dos sintomas depressivos e na diminuição do risco de suicídio. Esses resultados, em conjunto, são fundamentais para o desenvolvimento de abordagens terapêuticas mais direcionadas e personalizadas. Olhando para o futuro, essas descobertas têm o potencial de aprimorar a precisão na predição de respostas a tratamentos, pavimentando o caminho para terapias mais eficazes e adaptadas às necessidades individuais dos pacientes.

7 PERSPECTIVAS

À luz dos resultados obtidos nesta tese sobre a eficácia da cetamina via SC no tratamento da depressão, é importante considerar a necessidade de pesquisas subsequentes para validação. A continuidade do estudo iniciado nesta tese poderá gerar um pós-doutorado, com foco na etapa de avaliar a neurobiologia como preditor de resposta a cetamina e ainda ampliar a compreensão das ações moleculares deste tratamento com ação anti-suicida. A complexidade da depressão e a heterogeneidade da resposta terapêutica entre os pacientes sugerem a necessidade de uma abordagem mais personalizada:

a) *Ampliação da Amostra:* A inclusão de um maior número de participantes permitiria uma análise mais robusta e representativa. Esta ampliação tem o potencial de revelar nuances e especificidades na resposta terapêutica à cetamina, possibilitando a identificação de subgrupos de pacientes com perfis de resposta distintos;
b) *Farmacogenética:* Assim como observado com outros agentes terapêuticos, como o lítio, a resposta à cetamina pode ser influenciada por fatores genéticos. Investigar a farmacogenética associada à cetamina poderia elucidar marcadores genéticos que predizem uma resposta terapêutica favorável, otimizando a seleção de pacientes e aumentando as taxas de sucesso no tratamento;
c) *Alvos Neurobiológicos:*análise de biomarcadores como IL-6, IL-10, IL-1β, TNFα, PCRus e GDF11, e a avaliação sistemática do risco de suicídio e sintomatologia depressiva.A compreensão profunda dos mecanismos neurobiológicos subjacentes à ação da cetamina é crucial. Identificar e caracterizar os alvos moleculares e celulares específicos da cetamina no SNC pode fornecer *insights* sobre como prolongar e potencializar seus efeitos terapêuticos. Adicionalmente, é fundamental entender como a cetamina modula a neurotransmissão, particularmente no que diz respeito aos sistemas glutamatérgico e GABAérgico, para desvendar os mecanismos subjacentes à sua rápida ação antidepressiva. A análise das interações sinápticas, da plasticidade neural e da modulação de circuitos cerebrais específicos em resposta à cetamina pode não só elucidar sua ação, mas também pavimentar o caminho para o desenvolvimento de novas abordagens terapêuticas e o refinamento das estratégias atuais;
d) *Manutenção dos Efeitos Agudos:* Uma das questões centrais no uso terapêutico da cetamina é a durabilidade de seus efeitos, buscando estratégias que permitam manter os benefícios agudos da cetamina a longo prazo, garantindo uma melhoria

sustentada na qualidade de vida dos pacientes;

e) *Comparação com Outras Modalidades de Tratamento:*Comparar a eficácia da cetamina com outras intervenções, como a terapia eletroconvulsiva (ECT), especialmente em pacientes com depressão resistente ao tratamento. Para estabelecer a cetamina como uma alternativa viável ou complementar a outras modalidades de tratamento.

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ANEXOS

ANEXO A -Carta de Aprovação do Comitê de Ética em Pesquisa - Projeto 2020- 0334

HOSPITAL DE CLÍNICAS DE PORTO ALEGRE

Grupo de Pesquisa e Pós Graduação

Carta de Aprovação

Projeto

2020/0334

Pesquisadores:

MARCIA KAUER SANT ANNA

JÉFERSON FERRAZ GOULARTE	PAULO SILVA BELMONTE DE ABREU	KEILA MARIA MENDES CERESER
JAIRO VINICIUS MEREGE DE MELLO CRUZ PINTO	IVES CAVALCANTE PASSOS	ANA PAULA SORDI ANZOLIN

Número de Participantes: 45

Título: ESTUDO NATURALÍSTICO DE CETAMINA NO TRATAMENTO DA DEPRESSÃO: avaliação do risco de suicídio e medidas séricas de SIRT3, suPAR, PCRus, Interleucina 6, hemograma completo, leptina, perfil lipídico e glicemia

Este projeto foi APROVADO em seus aspectos éticos, metodológicos, logísticos e financeiros para ser realizado no Hospital de Clínicas de Porto Alegre.
Esta aprovação está baseada nos pareceres dos respectivos Comitês de Ética e do Serviço de Gestão em Pesquisa.

- Os pesquisadores vinculados ao projeto não participaram de qualquer etapa do processo de avalição de seus projetos.

- O pesquisador deverá apresentar relatórios semestrais de acompanhamento e relatório final ao Grupo de Pesquisa e Pós-Graduação (GPPG).

09/10/2020

Assinado digitalmente por:
PATRICIA ASHTON PROLLA
Grupo de Pesquisa e Pós-graduação
11/10/2020 17:19:56

Impresso do sistema AGHUse-Pesquisa por ANDERSON ZANARDO MACHADO em 09/10/2020 17:21:46

ANEXO B -Parecer Consubstanciado do Comitê de Ética em Pesquisa – Hospital Moinhos De Vento

HOSPITAL MOINHOS DE VENTO - HMV

PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: ESTUDO NATURALÍSTICO DE CETAMINA NO TRATAMENTO DA DEPRESSÃO: avaliação do risco de suicídio e medidas séricas de SIRT3, suPAR, PCRus, Interleucina 6, hemograma completo, leptina, perfil lipídico e glicemia

Pesquisador: LUCIANE NASCIMENTO CRUZ

Área Temática:

Versão: 2

CAAE: 33589320.3.2001.5330

Instituição Proponente: Hospital Moinhos de Vento - HMV

Patrocinador Principal: CONSELHO NACIONAL DE DESENVOLVIMENTO CIENTIFICO E TECNOLOGICO-CNPQ
Hospital de Clínicas de Porto Alegre

DADOS DO PARECER

Número do Parecer: 5.537.529

Apresentação do Projeto:

INTRODUÇÃO

A depressão é uma condição crônica, recorrente e de alta prevalência na população mundial, que está frequentemente associada à incapacitação funcional e ao comprometimento da saúde física dos indivíduos afetados. Entre suas possíveis consequências está o suicídio. De acordo com a OMS, as doenças psiquiátricas estão associadas a mais de 90% dos casos de suicídio, e são responsáveis por 30% dos casos de suicídio relatados em todo o mundo, cujos tratamentos são difíceis e representam uma das lacunas da pesquisa psiquiátrica. Todavia, tem-se observado que a cetamina, utilizada como anestésico, apresenta eficácia na depressão. Considerando a alta taxa de suicídio entre pacientes com depressão e suas possíveis consequências sociais, o presente projeto tem como objetivo examinar o efeito da cetamina na diminuição do risco de suicídio em pacientes com depressão e sua eficácia como agente antidepressivo. Será conduzido um estudo naturalístico no Hospital das Clínicas de Porto Alegre (HCPA).

HIPÓTESE

Espera-se com esse trabalho a comprovação dos benefícios da Cetamina no tratamento para o risco de suicídio em pacientes com depressão unipolar ou bipolar. Bem como a definição da

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de eventos adversos e eventuais emendas ou modificações no protocolo.

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_1959580.pdf	09/07/2022 12:04:53		Aceito
Projeto Detalhado / Brochura Investigador	projeto_hosp_moinhos.docx	09/07/2022 12:03:49	ANA PAULA ANZOLIN	Aceito
Outros	carta_adendo090722.docx	09/07/2022 12:01:12	ANA PAULA ANZOLIN	Aceito
Cronograma	CRONOGRAMA.docx	07/07/2022 23:00:41	ANA PAULA ANZOLIN	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	tcle_HMV.docx	07/07/2022 22:59:42	ANA PAULA ANZOLIN	Aceito
Folha de Rosto	folha_rosto.pdf	22/06/2022 13:50:04	ANA PAULA ANZOLIN	Aceito
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Guilherme Alcides Flôres Soares Rollin
(Coordenador(a))

ANEXO C - artigos científicos publicados em autoria ou coautoria durante o período do doutorado, os quais não estão relacionados diretamente à tese.

Para fins práticos, consta apenas a primeira página de cada uma destas publicações.

Molecular Psychiatry www.nature.com/mp

REVIEW ARTICLE

Inflammatory and oxidative stress markers in post-traumatic stress disorder: a systematic review and meta-analysis

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Post-traumatic stress disorder (PTSD) has been associated with persistent, low-degree inflammation, which could explain the increased prevalence of autoimmune conditions and accelerated aging among patients. The aim of the present study is to assess which inflammatory and oxidative stress markers are associated with PTSD. We carried out a meta-analytic and meta-regression analysis based on a systematic review of studies comparing inflammatory and oxidative stress markers between patients with PTSD and controls. We undertook meta-analyses whenever values of inflammatory and oxidative stress markers were available in two or more studies. Overall, 28,008 abstracts were identified, and 54 studies were included, with a total of 8394 participants. The Newcastle-Ottawa Quality Assessment Scale was used to evaluate the quality of the studies. Concentrations of C-reactive protein (SMD = 0.64; 95% CI: 0.21 to 1.06; $p = 0.0031$; $k = 12$), interleukin 6 (SMD = 0.94; 95% CI: 0.36 to 1.52; $p = 0.0014$; $k = 32$), and tumor necrosis factor-α (SMD = 0.89; 95% CI: 0.23 to 1.55; $p = 0.0080$; $k = 24$) were significantly increased in patients with PTSD in comparison with healthy controls. Interleukin 1β levels almost reached the threshold for significance (SMD = 1.20; 95% CI: −0.04 to 2.44; $p = 0.0569$; $k = 15$). No oxidative stress marker was associated with PTSD. These findings may explain why PTSD is associated with accelerated aging and illnesses in which immune activation has a key role, such as cardiovascular diseases and diabetes. In addition, they pointed to the potential role of inflammatory markers as therapeutic targets.

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INTRODUCTION

Post-traumatic stress disorder (PTSD) is a chronic and severe psychiatric condition, which may develop in approximately one-third of individuals who were exposed to or experienced a significant traumatic event [1, 2]. PTSD presents a lifetime prevalence of about 3.9% (0.3–8.8%) [3]. Family history of psychiatric disorders, chronic medical conditions, the intensity of the traumatic event, being a woman, and cumulative traumatic experiences are risk factors associated with its development [2]. In addition, PTSD has been associated with increased suicide risk [4], premature death [5], coronary heart disease, and elevated economic burden [6]. PTSD presents a complex pathophysiology, and immune activation has been associated with the disorder [1, 7]. Most of the evidence comes from empirical data on blood biomarkers, which have described increased levels of specific proinflammatory cytokines and acute-phase proteins in patients with PTSD compared with healthy controls [7, 8]. PTSD has also been associated with an increased risk of developing autoimmune conditions in longitudinal studies [9, 10]. In addition, PTSD has been linked to accelerated aging, reduced cortical thickness, and neurodegeneration [11–13].

A previous meta-analysis and meta-regression published in 2015 showed increased levels of interleukin 6 (IL-6), interleukin 1β (IL-1β), tumor necrosis factor-α (TNF-α), and interferon-γ (IFN-γ) in patients with PTSD [8]. In addition, its findings suggested the use of IL-1β as biomarker of illness duration, and IL-6 as a biomarker of PTSD severity. Some limitations, however, prevent more robust conclusions. Specifically, few studies were included in the meta-analyses and meta-regression analysis of some inflammatory markers, and the study did not include oxidative stress markers. In addition, type of trauma and the blood fraction assessed (serum vs plasma) were not explored as potential moderators of the effect sizes. Another systematic review and meta-analysis was published in 2020, with the aim of providing an updated account of the presence of immune biomarkers in PTSD patients [14]. In their analysis, patients with PTSD presented significantly higher levels of C-reactive protein (CRP), TNF-α, IL-6, IL-1β, interleukin 2 (IL-2), white blood cells, and IFN-γ in comparison with healthy controls.

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Extended applications the subcutaneous esketamine for major depression with suicidal ideation in autism traits—Case report

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1. Introduction

The noncompetitive N-methyl-D-aspartate receptor (NMDA) antagonist esketamine is primarily used to induce and maintain sedation and analgesia during anesthesia beyond its classical use. Recently, there is an increasing interest in the benefits of ketamine, its racemic compound, and its enantiomers [i.e., S-ketamine (esketamine) and R-ketamine (arketamine) for the treatment of psychiatric disorders (Kang et al., 2021).

The Autism Spectrum Disorders (ASD) and major depressive disorder (MDD) are associated with altered NMDA function (Won et al., 2012). Putative mechanisms involving early NMDA hyperfunction leading to late NMDA hypofunction are supported by experimental induction of autistic-like social behaviors in mice, with additional correction of NMDA dysfunction having a lasting preventing effect of autistic-like social behaviors at later stages. However, the evidence of response to ketamine in those patients with ASD and depressive episodes remains limiting (Choi et al., 2022; Olivola et al., 2022; Ozgen and Brink, 2021). The high prevalence of comorbidity with MDD is a major issue in the field of ASD (Ozgen and Brink, 2021).

Recent findings suggests that Ketamine is a possible treatment for ASD (Won et al., 2012), and attributes the effect on Methyl-CpG binding protein 2 (MeCP2) protein (Choi et al., 2022). The gene encoding MeCP2 is one of a few exceptional genes of established causal effect in ASD and the core symptoms of autism in MeCP2 knockdown rats with social impairment recovered dramatically following a single treatment with Ketamine. A recent paper demonstrated positive effects of intranasal esketamine use in young girls with ASD and high IQ (Olivola et al., 2022).

The pharmacokinetic characteristics of ketamine allow its administration by various routes (Bregin et al., 2019; C. et al., 2012; Cavenaghi et al., 2021; Daly et al., 2019; L. et al., 2019; Popova et al., 2019). The subcutaneous (SC) route of administration has comparable efficacy to conventional IV infusion but fewer side effects (Loo et al., 2016). In a recent systematic review (Cavenaghi et al., 2021) observed that racemic ketamine and its enantiomer esketamine, via SC, seems to be a promising treatment in depression, given its efficacy and tolerability.

To our acknowledgment, no controlled clinical trials studies of SC ketamine/esketamine in patients with comorbid ASD and MDD have been reported. Therefore, at least as far as the authors are concerned, this is the first report using the SC route for administering esketamine in hospital facilities for day care (hospital in southern Brazil) in a patient with suspicion borderline traits of autism, MDD and suicidal ideation showing benefits.

2. Case report

We report the case of a single 58-year-old man, and proper functioning (Functional Assessment Staging Tool (FAST) the baseline score 26), treated at the Outpatient Clinic of a major Tertiary Hospital with a history of MDD (treatment-resistant), suicidal ideation and with suspicion borderline traits of autism (25 points in Autism-Spectrum Quotient Test (AQ)) as well as to clinical judgment of a senior psychiatrist. We used the cutoff of 26 because in clinical patients the literature describes good screening properties of AQ in adults referred for an ASD assessment with a cutoff of 26 (Bemmouna, 2022; Bezemer et al., 2021; Robinson and Wheelwright, 2005).

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Inflammatory markers in outpatients with schizophrenia diagnosis in regular use of clozapine: a cross-sectional study

Victor Hugo Schaly Cordova[1,2]*, Amelia Dias Teixeira[1], Ana Paula Anzolin[3,4], Roberta Moschetta[5] and Paulo Silva Belmonte-de-Abreu[1,6]

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It is known that inflammation worsen the course of schizophrenia and induce high clozapine serum levels. However, no study evaluated this change in function of clozapine daily dose in schizophrenia. We assessed the correlation between inflammation and severity symptoms in patients with schizophrenia that take and do not take clozapine. We also assessed the correlation between clozapine daily dose and inflammatory markers to patients who take this drug. Patients were recruited from Schizophrenia Ambulatory and Psychosocial Care Center of Clinical Hospital of Porto Alegre and from an association of relatives of patients with schizophrenia. Exam results, and other important clinical exam were assessed in patients record or patients were asked to show their exam in the case of outpatients. We included 104 patients, 90 clozapine users and 14 non-clozapine users. We calculate the systemic inflammatory markers [neutrophil-lymphocyte ratio (NLR), systemic immune inflammation index (SII), and the psychopathology severity by the Brief Psychiatric Rating Scaled anchored (BPRS-a)]. These variables were compared between clozapine users and non-clozapine users. It was used mean/median test according to data distributing, with study factor (SII, MLR, and PLR), the clinical outcome: severity of symptomatology (BPRS score), and clozapine daily dose as adjustment factor. Clozapine users exhibited a significantly higher neutrophil count (mean $\pm$ SD: 5.03 $\pm$ 2.07) compared to non-clozapine users (mean $\pm$ SD: 3.48 $\pm$ 1.27; p = 0.031). After controlling for comorbidity, other parameters also showed significant differences. These findings are consistent with previous studies that have demonstrated an inflammatory response following the administration of clozapine.

KEYWORDS

inflammatory response, neutrophil-linphocyte ratio, inflammatory markers, immunoinflammatory systemic index, psychopharmacology, second generation antipsychotic

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Effectiveness of topical ozonated oil in severe osteoarthritis: A randomised, triple-blinded, placebo-controlled study

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ABSTRACT

Background: Osteoarthritis is highly prevalent and a common locomotory disorder in the elderly. The treatments aim improves the quality of life. We aimed to evaluate the effectiveness of topical ozonated oil in relieving pain in patients with osteoarthritis.
Design: A placebo-controlled, triple-blind, randomised controlled trial including osteoarthritis patients older than 50 years.
Interventions: Eighty patients were randomly divided into two groups: treatment (ozonated oil) and placebo (non-ozonated oil). The oils were used twice a day for 60 days. Evaluations were performed using WOMAC (Western Ontario and McMaster Universities) and VAS (visual analogue scale) and laboratory analysis.
Results: Pain relief was observed in all groups except in the placebo group where patients are diagnosed with severe osteoarthritis (degree 4) (p-value treatment and placebo group: 0.021 and 0.345, respectively).
Conclusions: For the first time, the pain relief in patients with severe osteoarthritis was demonstrated by the use of topical ozonated oil.

1. Introduction

Aging is a current reality worldwide, and the increase in life expectancy has also triggered the increase of chronic diseases [1]. Currently, non-communicable diseases, such as heart disease, arthritis, and dementia, which mainly affect adults and the elderly, are pressing health concerns of the general population. Aging impairs sensory, motor, and cognitive functions and thus lowers the quality of life [2]. Approximately 50% of elderly people over 85 years face limitations in their daily activities. Among the common pathologies of the locomotory system, osteoarthritis is the second most prevalent [1,3].

Osteoarthritis is a chronic and degenerative joint disorder that affects millions of people [3]. The injury usually begins in the articular cartilage. Genetic factors and mechanical overload are initiators of the injury, which progresses to inflammation that perpetuates joint degradation [4]. The knees are the most joints affected by osteoarthritis [5] because this region has several muscles, tendons and numerous bursas. However, the spine and fingers amongst others may also be affected.

Despite the molecular understanding of osteoarthritis, little is known about the pathogenesis of pain. The chronic arthritic pain does not reflect the magnitude of tissue injury and the treatments are not completely effective [6]. The treatments are palliative, and the goal is to improve the patient's function and quality of life and provide pain relief [7]. Treatment can be pharmacological, non-pharmacological, surgical and integrative (ozone therapy, acupuncture).

Ozone therapy uses ozone obtained from medicinal oxygen, where an oxygen-ozone mixture is obtained at known concentrations. This therapy has shown good results for herniated discs [8], osteoarthritis [9] and wounds [10] and can be administered by different routes: rectal, vaginal, topical with ozonated oil or ozonated water, intra-articular, intramuscular or subcutaneous [11].

Intra-articular ozone therapy can be used as an adjunctive treatment for osteoarthritis [12] presenting some benefits [13–16] after approximately 1 month of treatment, relieving pain with anti-inflammatory effects.

Ozone therapy modulates Nrf2 [17] producing three effects. First, it

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CROP PRODUCTION

Mycorrhization of strawberry plantlets potentiates the synthesis of phytochemicals during *ex vitro* acclimatization

José Luís Trevizan Chiomento[1,3*], Fabiola Stockmans De Nardi[2], Débora Filippi[1], Thomas dos Santos Trentin[3], Ana Paula Anzolin[4], Charise Dallazem Bertol[4], Alexandre Augusto Nienow[1,3] and Eunice Oliveira Calvete[1]

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ABSTRACT. *Ex vitro* strawberry plantlets from micropropagation and coinoculated with arbuscular mycorrhizal fungi (AMF) and biochar can provide beneficial health effects. In the present study, we evaluated the effects of different proportions of biochar in the presence and absence of AMF on the production of secondary metabolites in the leaves and roots of strawberry plantlets during *ex vitro* acclimatization. Additionally, the enzymatic activity of the substrate enriched with AMF and biochar was analyzed. The experiment consisted of the control (absence of the mycorrhizal community) and four biochar proportions (0, 3, 6, and 9% of the volume of the container) coinoculated with AMF. Plantlets produced on substrates enriched with AMF showed higher levels of polyphenols, flavonoids, phenolic acids, and tannins in the tissues analyzed than control plantlets. The combination of AMF and 9% biochar increased the content of total flavonoids in the leaves of strawberry plantlets and increased the activity of phosphatase. The substrate with up to 6% biochar and mycorrhizae showed increased β-glucosidase activity. In conclusion, mycorrhizae are excellent tools to improve the phytochemical quality of strawberry plantlets acclimatized *ex vitro*. The association between host plants, mycorrhizal symbionts, and bioactivators of these fungi potentiates properties beneficial to health, which can be exploited efficiently in sustainable agriculture.

Keywords: *Fragaria* x *ananassa* Duch.; arbuscular mycorrhiza; biochar; biomolecules; enzymatic activity.

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Introduction

Strawberry (*Fragaria* x *ananassa* Duch., Rosaceae) is a fruit that is appreciated for its excellent flavor, fragrance, and chemical qualities, and has important biological properties, such as antioxidant potential (Chaves, Calvete, & Reginatto, 2017), anti-inflammatory action (Duarte et al., 2018), and antihypertensive and anticancer activities (Giampieri et al., 2015), which are all related to the presence of secondary metabolites, mainly anthocyanins.

Micropropagation is one of the methods of *in vitro* cultivation and is considered a fast form of multiplication that generates plants that are genetically homogeneous and produce disease-free material (Cavallaro, Tringali, & Patanè, 2011). However, micropropagation is a complex procedure that requires the development of appropriate techniques in guaranteeing the success of the process, justifying the high investment in micropropagation. One of the stages of *in vitro* plant production that generates high mortality is acclimatization (Kapoor, Sharma, & Bhatnagar, 2008). After the transfer of plant materials from *in vitro* to *ex vitro* conditions, micropropagated plantlets are susceptible to various stresses owing to environmental changes; thus, the plantlets need to change from heterotrophic to autotrophic feeding. Plantlets grown *in vitro* have poorly developed cuticles, non-functional stomata, and a weak root system (Palei, Das, & Rout, 2015), which can reduce their survival or result in the formation of weak plantlets. This problem can be overcome through special conditions during *ex vitro* cultivation. The coinoculation of the growth substrate with arbuscular mycorrhizal fungi (AMF) and biochar can guarantee success at this stage. This is justified

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ORIGINAL ARTICLE

Influence of processing methods on the content of polyphenols and anthocyanins and on the antioxidant activity of *Rubus brasiliensis* Mart. fruits

Influência dos métodos de processamento no conteúdo de polifenóis e antocianinas e na atividade antioxidante de frutos de Rubus Brasiliensis *Mart.*

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Abstract

Little is known about the phytochemical composition and the influence of different processing methods on the concentration of bioactive compounds and on the antioxidant activity of the fruits of *Rubus brasiliensis* Mart., a native plant of Brazil. This work aimed to evaluate the influence of different processing methods on the quantification of phenolic and anthocyanin compounds and on the antioxidant activity of *R. brasiliensis* fruits. The plants were processed by different ways - the extracts of fruits were obtained by Spray Dryer (SD) or Lyophilization (LYO), and the fruits were dried directly in an oven (OD) and were also evaluated after freshly thawed (FT). The processing methods were independent. After processing, the polyphenol and anthocyanin contents and antioxidant activity were evaluated. The Total Phenolic Content (TPC) was assessed using the Folin-Ciocalteu technique. The pH differential method was used for quantification of anthocyanin and the antioxidant activity was determined by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. *R. brasiliensis* fruits have a high content of polyphenols and anthocyanins, and an expressive antioxidant activity that can bring benefits to the population. The FT fruits showed the lowest content of total polyphenols. However, the OD fruits showed the most interesting results, since the total polyphenols and anthocyanins contents and the antioxidant activity were similar to the other processing methods performed in this work and were more economically viable. Obtain a bioactive content and adequate antioxidant activity after simple processing such as drying is very interesting when using these fruits for longer, or even, obtaining pharmaceutical formulations. It could

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The role of ozone treatment as integrative medicine. An evidence and gap map

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Introduction: The Brazil has one of the largest public health systems in the world and in the 1980's, Traditional, Complementary and Integrative Medicine were introduced. In 2018, the treatment with ozone became a complementary integrative practice showing several benefits. However, its effectiveness needs to be researched. The objective of this evidence gap map is to describe contributions of Integrative Medicines-Ozone treatment in different clinical conditions, to promote evidence-based practice.

Methods: We applied the methodology developed by Latin American and Caribbean Center on Health Sciences Information based on the 3iE evidence gap map. The EMBASE, PubMed and Virtual Health Library databases, using the MeSH and DeCS terms for the treatment with Ozone were used.

Results: 26 systematic reviews were characterized, distributed in a matrix containing 6 interventions (parenteral oxygen/ozone gas mixture; parenteral ozonated water; systemic routes; topical application ozonated water; topical oxygen/ozone gas mixture; and topical ozonated oil) and 55 outcomes (cancer, infection, inflammation, pain, quality of life, wound healing and adverse effects). 334 associations between intervention and outcome were observed, emphasizing the parenteral oxygen/ozone gas mixture intervention (192 associations, 57%).

Conclusions: The evidence gap map presents an overview of contributions of Ozone treatment in controlling pain, infections, inflammation and wound healing, as well as increasing the quality of life, and it is directed to researchers and health professionals specialized in Ozone treatment. No serious adverse effects were related. Therefore, this treatment may be even more widely known as an integrative treatment, considering its low cost, efficiency and