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TESE DE DOUTORADO

ASSOCIAÇÃO DE ELETROCONVULSOTERAPIA A TRATAMENTO FARMACOLÓGICO NO TRANSTORNO DEPRESSIVO MAIOR: ANÁLISE DE DESFECHO CLÍNICO, MARCADORES INFLAMATÓRIOS E NEUROTROFINAS.

THIAGO FERNANDO VASCONCELOS FREIRE

ORIENTADOR:
Prof. Dr. Marcelo Pio de Almeida Fleck

CO-ORIENTAÇÃO:
Profa. Dra. Neusa Sica da Rocha

Porto Alegre, outubro de 2016

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Tese apresentada ao Programa de Pós-Graduação em Psiquiatria e Ciências do Comportamento como requisito parcial para obtenção do título de Doutor.

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2016

CIP - Catalogação na Publicação

Freire, Thiago Fernando Vasconcelos
ASSOCIAÇÃO DE ELETROCONVULSOTERAPIA A TRATAMENTO FARMACOLÓGICO NO TRANSTORNO DEPRESSIVO MAIOR: ANÁLISE DE DESFECHO CLÍNICO, MARCADORES INFLAMATÓRIOS E NEUROTROFINAS. / Thiago Fernando Vasconcelos Freire. -- 2016.
137 f.

Orientador: Marcelo Pio de Almeida Fleck.
Coorientador: Neusa Sica da Rocha.

Tese (Doutorado) -- Universidade Federal do Rio Grande do Sul, Faculdade de Medicina, Programa de Pós-Graduação em Psiquiatria e Ciências do Comportamento, Porto Alegre, BR-RS, 2016.

1. Eletroconvulsoterapia. 2. Transtorno Depressivo Maior. 3. Brain Derived Neurotrophic Factor. 4. Interleucina 6. 5. Fator de Necrose Tumoral Alfa. I. Fleck, Marcelo Pio de Almeida, orient. II. da Rocha, Neusa Sica, coorient. III. Título.

Elaborada pelo Sistema de Geração Automática de Ficha Catalográfica da UFRGS com os dados fornecidos pelo(a) autor(a).

“Teus filhos não são teus filhos
São filhos e filhas da vida, anelando por si própria
Vem através de ti, mas não de ti
E embora estejam contigo, a ti não pertencem.
Podes dar-lhes amor mas não teus pensamentos,
Pois que eles tem seus pensamentos próprios.
Podes abrigar seus corpos, mas não suas almas
Pois que suas almas residem na casa do amanhã,
Que não podes visitar sequer em sonhos.
Podes esforçar-te por te parecer com eles,
mas não procureis fazê-los semelhante a ti,
Pois a vida não recua, não se retarda no ontem.
Tu és o arco do qual teus filhos, como flechas vivas, são disparados
Que a tua inclinação na mão do Arqueiro seja para alegria.”

Khalil Gibran.

A Raquel e a meu filho.

AGRADECIMENTOS

Ao professor Marcelo Pio de Almeida Fleck, pela orientação neste trabalho, pelo modelo de professor e médico, pela oportunidade e confiança.

À professora Neusa Sica da Rocha, pela orientação neste trabalho, pela generosidade em dividir seu projeto comigo, pelo entusiasmo, carinho e amizade.

Aos colegas do Grupo de Pesquisa em Qualidade de Vida e Depressão, pelas ricas discussões, pelo apoio e contribuições.

Aos bolsistas de iniciação científica e demais estudantes envolvidos neste projeto, pelo trabalho dedicado e a energia que trouxeram ao grupo.

Aos colegas do Laboratório de Psiquiatria Experimental, pela colaboração que viabilizou a execução deste trabalho.

Aos pacientes que foram colaborativos com todos os procedimentos da pesquisa mesmo durante um período de sofrimento

Aos professores do serviço de Psiquiatria do HCPA, modelos para o ingresso na formação acadêmica.

Ao professor Fábio Gomes de Matos e Souza, pela inspiração para o ingresso no universo da psiquiatria e da pesquisa.

Aos queridos colegas e residentes de Psiquiatria do Hospital Municipal Getúlio Vargas, pela vontade de aprender, pelo entusiasmo, pela oportunidade dos primeiros passos no mundo docente, e pela amizade.

À minha família, em especial aos meus pais Freire (*in memoriam*) e Jacinta, e aos meus irmãos Antônio, Mônica e Verônica, por acreditarem em mim, por seu apoio e confiança.

A Raquel, minha esposa, por dividir a vida comigo, por sua infinita paciência e por seu amor.

Ao meu filho, por tudo que eu ainda vou descobrir.

SUMÁRIO

1. ABREVIATURAS E SIGLAS

ADT Antidepressivos Tricíclicos

ACTH Hormônio Adrenocorticotrópico

BDNF Fator Neurotrófico Derivado do Cérebro (*Brain Derived Neurotrophic Factor*)

CRH Hormônio Liberador de Corticotrofina

DALY Incapacidade Ajustada aos Anos de Vida *(Disability-Adjusted Life Year)*

DMS Doença Mental Severa

ECS *Eletroconvulsive Shock*

ECT Eletroconvusoterapia

FGF-2 Fator de Crescimento Fibroblastico 2

GEE Equações de Estimativa Generalizada

GM-CSF Fator Estimulador de Colônias de Macrófagos e Granulócitos

HDRS *Hamilton Depression Rating Scale*

HDRS-17 *Hamilton Depression Rating Scale 17 items*

HGH Hormônio de Crescimento Humano

HHA Eixo Hipotalâmico-Hipofisário-Adrenal

IC Intervalo de Confiança

IDO Enzima Indolamina 2-3-Dioxigenase

IL-1 Interleucina 1

IL-2 Interleucina 2

IL-4 Interleucina 4

IL-5 Interleucina 5

IL-6	Interleucina 6
IL-10	Interleucina 10
IL-12	Interleucina 12
IL-17	Interleucina 17
IL-1ra	Antagonista de Receptor de Interleucina 1
IMAO	Antidepressivo Inibidor da Monoaminoxidase
IFN-α	Interferon Alfa
IFN-γ	Interferon Gama
LPS	Lipopolissacarídeo
MADRS	*Montgomery-Asberg Depression Rating Scale*
MAO-A	Monoaminoxidase A
MDD	*Major Depressive Disorder*
MINI	*Mini International Neuropsychiatric Interview*
NGF	Fator de Crescimento Neural
NICE	*National Institute of Clinical Excellence*
NMDA	N-Metil-D-Aspartato
NPY	Neuropeptídeo Y
NT-3	Neurotrofina-3
OR	*Odds Ratio*
PCR	Proteína C Reativa
QV	Qualidade de Vida
RNA	Ácido Ribonucléico
STAR*D	*Sequenced Treatment Alternatives to Relieve Depression*
TCLE	Termo de Consentimento Livre e Esclarecido
TDM	Transtorno Depressivo Maior

TE Tamanho de Efeito

TGF-β Fator de Transformação do Crescimento Beta

TNF-α Fator de Necrose Tumoral Alfa

TNF-β1 Fator de Necrose Tumoral Beta 1

TRH Hormônio Liberador de Tireotrofina

TSH Hormônio Tireoestimulante

VEGF Fator de Crescimento Endotelial Vascular

YLD Anos Vividos com Incapacidade *(Years Lived with Disability)*

2. RESUMO

O Transtorno Depressivo Maior (TDM) é um quadro psiquiátrico de alta prevalência, cronicidade e morbimortalidade, apresentando altos níveis de incapacidade e custos associados. Muitos pacientes acometidos não atingem a remissão de sintomas ou obtêm nível de resposta clínica significativa com fármacos antidepressivos. Nestas situações, outros tratamentos, como a eleroconvulsoterapia (ECT), são alternativas possíveis. A quantidade de estudos envolvendo a ECT é limitada, e ainda segue desconhecido o exato mecanismo de ação deste tratamento. Pesquisas envolvendo biomarcadores e ECT, além de auxiliarem na compreensão de seu mecanismo de ação, podem ajudar no esclarecimento da patofisiologia dos transtornos de base para os quais o tratamento é empregado, como a depressão. Questionamentos acerca da validade da clássica teoria monoaminérgica da depressão, e o surgimento de novas evidências como diminuições volumétricas de regiões cerebrais em indivíduos deprimidos, bem como a semelhança do estado comportamental em resposta a doenças sistêmicas infecciosas (*sickenes behaviour*) com estados depressivos, abriram espaço respectivamente para as teorias neurotrófica e inflamatória da depressão. Diversos estudos em modelos animais e humanos têm pesquisado a influência da ECT em neurotrofinas e marcadores inflamatórios no TDM, porém, apesar de resultados positivos, os estudos ainda são poucos, com amostras pequenas, além de outras limitações metodológicas. O objetivo desta tese de doutorado foi avaliar a associação da ECT a tratamento farmacológico no TDM e suas influências nos níveis da neurotrofina *Braind Derived Neurotrophic Fator* (BDNF), nos marcadores pró-

inflamatórios interleucina-2 (IL-2), IL-6, IL-17, fator de necorse tumoral alfa (TNF-α) e interferon gama (IFN-γ), nos marcadores anti-inflamatórios IL-4 e IL-10, bem como suas influências em desfechos clínicos. No primeiro artigo, pudemos observar que não houve diferenças significativas nos níveis de BDNF sérico entre os grupos de tratamento combinado (ECT e farmacoterapia) e de tratamento exclusivamente farmacológico na admissão e nem na alta, e nenhuma variação significativa nos níveis de BDNF ocorreu em qualquer dos grupos durante o estudo. A ECT não restaurou os níveis de BDNF dos pacientes a níveis semelhantes ao de controles saudáveis. No segundo artigo, os pacientes que atingiram remissão dos sintomas depressivos com a ECT tiveram níveis de BDNF antes do tratamento significativamente maiores do que os que não remitiram, e esses valores não variaram significativamente com o tratamento. Concluiu-se que níveis mais elevados de BDNF poderiam prever remissão à ECT antes mesmo de sua realização. Finalmente, no terceiro artigo, a combinação da ECT com a farmacoterapia foi associada com diminuição dos níveis de IL-6 e aumento de TNF-α. Pacientes deprimidos, independetemente do grupo de tratamento ao qual foram submetidos, tiveram uma diminuição dos níveis de IL-6 e IFN- γ após o tratamento. Não foram observados resultados significativos para IL-2, IL-4, IL-10 e IL-17. Esta tese de doutorado traz contribuições acerca da influência da associação da ECT ao tratamento farmacológico do TDM no que concerne a marcadores biológicos e sua relação com desfecho clínico.

3. ABSTRACT

Major Depressive Disorder (MDD) is a psychiatric disorder with high prevalence, chronicity, morbidity and mortality, with high levels of disability and high costs in health. Many affected patients do not achieve remission of symptoms nor get significant clinical response to antidepressant drugs. In these cases, other treatments such as electroconvulsive therapy (ECT) are alternatives. The number of studies involving ECT is limited, and it is still unknown the exact mechanism of action of this treatment. Research involving biomarkers and ECT, in addition to providing understand about its mechanism of action, may explain the pathophysiology of the disorder for which treatment is employed, such as depression. Questions about the validity of classical monoaminergic theory of depression and the emergence of new evidence as volumetric decreases in brain regions in depressed individuals, as well as the similarity of the behavioral state in response to infectious systemic diseases (sickeness behavior) with depressive states allowed the emergence of neurotrophic and inflammatory theories. Several studies in animal models and humans have researched the influence of ECT in neurotrophins and inflammatory markers in MDD, however, despite positive results, studies are still few, with small samples and other methodological limitations. The purpose of this doctoral thesis was to evaluate the association of ECT with pharmacological treatment in MDD and its influences on the levels of *Brain Derived Neurotrophic Fator* (BDNF), proinflammatory (IL-2, IL-6, IL-17, TNF-α and IFN-γ) and anti-inflammatory (IL-4 and IL-10) cytokines, as well as its influences on clinical outcomes. In the first article, we observed that there were no significant differences in serum BDNF levels between the combined

treatment group (ECT and pharmacotherapy) and the group that only used pharmacological treatment on admission and discharge, and no significant change in BDNF levels occurred in any group during the treatment. In patients, ECT did not restore BDNF levels to levels similar to healthy controls. In the second article, patients who achieved remission of the depressive symptoms with ECT had BDNF levels before treatment significantly higher than non-remitters, and these values did not vary with treatment. We concluded that higher levels of BDNF may predict remission with ECT even before its completion. Finally, in the third article, the combination of ECT with pharmacotherapy was associated with decreased levels of IL-6 and increased levels of TNF-α. Depressed patients, regardless of the treatment group that they were assigned, had a decrease in levels of IL-6 and IFN-γ. There were no significant findings for IL-2, IL-4, IL-10 and IL-17. The present doctoral thesis shed light on the association of ECT to pharmacological treatment in MDD in respect of biological markers and their relationship to clinical outcome.

4. APRESENTAÇÃO

Esta tese de doutorado se intitula “Associação de Eletroconvulsoterapia a Tratamento Farmacológico no Transtorno Depressivo Maior: Análise de Desfecho Clínico, Marcadores Inflamatórios e Neurotrofinas” apresentado ao Programa de Pós-Graduação em Psiquiatria e Ciências do Comportamento da Universidade Federal do Rio Grande do Sul em 14 de outubro de 2016.

Os estudos desenvolvidos nesta tese fazem parte do projeto “Avaliação e seguimento dos pacientes com doença mental severa (DMS): fatores diagnósticos, prognósticos e de tratamento e sua associação com marcadores biológicos”, uma coorte naturalística prospectiva realizada entre 2010 e 2014 com pacientes internados na unidade de psiquiatria do Hospital de Clínicas de Porto Alegre.

A motivação inicial para o desenvolvimento desta tese partiu da curiosidade acerca da Eletroconvulsoterapia (ECT). Alvo de histórica polêmica, este tratamento ainda sofre resistências para sua realização nos dias atuais. O contato com o inquestionável sucesso em termos terapêuticos da ECT quando bem indicada geraram o interesse em contribuir com a produção de conhecimento científico relacionado ao tema, aumentando a credibilidade da técnica frente a preconceitos e visões distorcidas.

O exato mecanismo de ação da ECT é ainda desconhecido. Considerando-se o surgimento de novas teorias patofisiológicas da depressão como a neurotrófica e a inflamatória, nossa questão de pesquisa

passou a ser a investigação das influências da ECT nos biomarcadores relacionados a tais teorias, na tentativa de ampliar o conhecimento acerca dos mecanismos pelos quais a ECT provoca melhora do Transtorno Depressivo Maior (TDM).

O primeiro estudo teve por objetivo avaliar se a combinação de ECT a tratamento farmacológico em pacientes deprimidos promoveria aumentos nos níveis da neurotrofina BDNF, restaurando seus níveis a de indivíduos controles não deprimidos. Após inclusão dos sujeitos, pacientes internados com o diagnóstico de depressão foi realizado pelo *Mini International Neuropsychiatric Interview* (MINI) (Sheehan et al., 1998), e a sintomatologia depressiva avaliada na admissão e na alta usando-se a *Hamilton Depression Rating Scale 17 items* (HDRS-17) (Hamilton, 1960). Após a segunda avaliação, os pacientes incluídos foram retrospectivamente divididos entre aqueles submetidos a tratamento psicofarmacológico associado a ECT (31 sujeitos) e aqueles que utilizaram apenas tratamento psicofarmacológico (68 sujeitos). Nenhum indivíduo foi submetido a tratamento exclusivo com ECT. O BDNF sérico foi medido em amostras de sangue coletados na admissão do estudo e na alta. Cem doadores saudáveis sem transtornos psiquiátricos foram incluídos como grupo controle.

A partir do trabalho anterior, o segundo estudo avaliou especificamente os 31 sujeitos submetidos a ECT, com o foco no desfecho clínico remissão de sintomas depressivos. O objetivo foi investigar se a remissão dos sintomas depressivos com a ECT estaria associados a mudanças nos níveis de BDNF.

No terceiro estudo, abordamos os marcadores inflamatórios. Partindo dos grupos de indivíduos submetidos a tratamento psicofarmacológico associado a ECT (31 sujeitos) e aqueles que utilizaram apenas tratamento psicofarmacológico (68 sujeitos), foram realizadas medidas na admissão do estudo e na alta das citocinas pró-inflamatórios IL-2, IL-6, TNF-α, IFN-γ e IL-17, e das anti-inflamatórios IL-4 e IL-10.

Esta tese foi organizada em cinco partes. Na primeira parte, **Introdução**, abordam-se conceitos gerais sobre o TDM e sobre a ECT. A segunda parte, **Revisão de Literatura**, faz-se um apanhado de evidências relativas às teorias patofisiológicas da depressão, bem como as pesquisas associando ECT a neurotrofinas e a citocinas inflamatórias. A terceira parte conta com a **Justificativa**, **Objetivos** e **Considerações Éticas** desta tese. Na quarta parte, encontram-se os textos dos artigos científicos na íntegra: **Artigo 1** (Freire et al., 2015a, publicado no *Journal of Psychiatric Research* em 2015), o **Artigo 2** (Freire et al., 2015b, publicado no *Brain Research Bulletin* em 2015) e o **Artigo 3** (submetido para o *Journal of Psychiatric Research* e atualmente com status *under review*). Na quinta parte, realizam-se as **Considerações Finais**.

5. INTRODUÇÃO

O Transtorno Depressivo Maior (TDM) costuma ser um transtorno crônico, recorrente e debilitante, com significativos prejuízos no funcionamento e na Qualidade de Vida (QV) dos pacientes acometidos (Greden et al., 2001; Ferrari et al., 2013a), bem como sendo responsável por substanciais gastos em saúde (Luppa et al., 2007; Kleine-Budde et al., 2013).

É um transtorno com alta prevalência, com estudos indicando 4,4% da população mundial afetada (Ferrari et al., 2013a), 16,2% dos indivíduos norte-americanos ao longo da vida e 6,2% nos últimos doze meses (Kessler et al., 2003). Em se tratando da população brasileira, a estimativa de prevalência de TDM é semelhante à norte-americana, com 17% da população afetada ao longo da vida e 8% em 12 meses (Silva, Galvao et al. 2014).

O TDM é ainda a segunda maior causa de Anos Vividos com Incapacidade no mundo (em inglês, YLDs) e responsável por cerca de 2,5% da Incapacidade Ajustada aos Anos de Vida (em inglês, DALYs) em 2010 (Ferrari et al., 2013b). A alta prevalência do TDM, aliada aos índices de incapacidade, gastos com tratamento e outros gastos indiretos, resultaram em um custo de 210,5 bilhões de dólares nos Estados Unidos entre 2005 a 2010 (Greenberg et al., 2015).

Em termos de morbimortalidade, projeções futuras são pouco animadoras em relação ao TDM, indicando que será a segunda causa de morbidade em 2020, perdendo apenas para doença cardíaca isquêmica (Murray e Lopez, 1997). O transtorno é ainda fator de risco para duas

importante causas mundiais de morte, o suicídio e a doença cardíaca isquêmica (Ferrari et al., 2013a; Lichtman et al., 2014).

Embora o tratamento com medicações antidepressivas seja efetivo para uma grande parte dos pacientes, muitos deles não toleram seus efeitos colaterais ou não respondem adequadamente (Fleck et al., 2005; Kupfer et al., 2003). Após a primeira tentativa de tratamento com fármacos antidepressivos, somente um terço dos pacientes entra em remissão, 20% alcançam resposta terapêutica (redução de 50% dos sintomas depressivos) e 50% não obtêm nenhuma resposta (Trivedi et al., 2006). Nas tentativas subsequentes, os resultados são ainda piores: em torno de 20% dos pacientes entram em remissão após a segunda tentativa de antidepressivos (Rush et al., 2006) e menos de 20% remitem depois da terceira tentativa (Fava et al., 2006). No maior ensaio clínico já conduzido para se avaliar resposta a tratamento antidepressivo, o STAR*D (*Sequenced Treatment Alternatives to Relieve Depression*), as taxas de remissão para os estágios 1, 2, 3 e 4, respectivamente, foram de 38,8%, 30,6%, 13,7% e 13%, e a taxa cumulativa de remissão foi de 67%. Esse importante estudo corrobora a ideia de que, à medida que ocorrem falhas nos sucessivos estágios de tratamentos empregados para TDM, menores são as taxas de remissão (Rush et al., 2006; Trivedi et al. 2006). Os pacientes que não alcançam a remissão completa dos sintomas apresentam maior risco de recorrência, possuem pior funcionamento psicossocial, pior QV e maior número de sintomas durante o seguimento (Kennedy et al., 2005; Rush et al., 2006; Viinamaki et al., 2008).

Na prática clínica, em caso de falhas no tratamento, as principais alternativas utilizadas incluem associação ou troca de antidepressivo (Rush

et al., 2006, Fava et al., 2006), potencialização com outro agente farmacológico (Nierenberg et al., 2006, Bauer et al., 2014), adição de psicoterapia (Thase et al., 2007) ou o emprego da eletroconvulsoterapia (ECT) (UK ECT Review Group, 2003).

A ECT é um procedimento que consiste na indução de crises convulsivas por meio da passagem de uma corrente elétrica pelo cérebro para fins terapêuticos. A resistência à medicação antidepressiva constitui sua principal indicação (Husain et al., 2004; Braga et al., 2007; Husain et al., 2005). Além disso, a ECT possui maior rapidez de resposta em relação aos medicamentos, o que é necessário em situações graves que necessitam de abordagem de urgência, como a catatonia e o risco de suicídio (McCall et al., 2007).

A eficácia da ECT em tratar sintomas depressivos está estabelecida por meio de inúmeros estudos desenvolvidos durante as últimas décadas (Kho et al., 2003; UK ECT Review Group., 2003; NICE, 2003; Pagnin et al., 2004) . Ela é o tratamento biológico mais efetivo para depressão atualmente disponível, já que nenhum outra intervenção terapêutica até então se mostrou superior à ECT no tratamento da depressão maior em estudos controlados (Prudic et al., 2005).

Um trabalho avaliou a eficácia da ECT na melhora dos sintomas depressivos por meio da meta-análise de 15 estudos. A comparação da eficácia da ECT com tratamentos controles, do *baseline* ao período pós-tratamento, demonstrou um tamanho de efeito (TE) de 0,90 com intervalo de confiança (IC) para 95% de 0,52-1,27. Foi também demonstrado que a ECT é significativamente superior no tratamento da depressão se comparada aos

antidepressivos ou à ECT simulada (quando há indução anestésica, porém nenhuma descarga elétrica é aplicada) (Kho et al., 2003).

O *United Kingdom Electroconvulsive Therapy Revieuw Goup* (UK ECT Review Group., 2003) realizou uma revisão sistemática e meta-análise de 73 ensaios clínicos randomizados controlados que compararam a eficácia da ECT real com ECT simulada, ECT versus farmacoterapia, ou outras técnicas na melhora da depressão. O resultado deste estudo demonstrou que a ECT real foi significativamente mais eficaz que a ECT simulada (seis estudos, 256 pacientes com TE -0,91 e IC 95% -1,27; -0,54) e mais eficaz que farmacoterapia (18 estudos, 1.144 pacientes com TE -0,80 e IC 95% -1,29; -0,29).

Outra meta-análise, (Pagnin et al., 2004) foi observada uma resposta significativamente maior da ECT real em relação à ECT simulada e ao placebo (11 estudos, 523 pacientes). A probabilidade de ocorrer uma resposta positiva, em termos de *odds ratio* (OR), foi aproximadamente cinco vezes maior com ECT real do que com ECT simulada ou placebo (OR = 4,77; IC 95% 2,39; 9,49). Na comparação da ECT com antidepressivos em geral (13 estudos, 892 pacientes), foi demonstrada significativa superioridade da ECT, com uma probabilidade de resposta quatro vezes maior em relação aos antidepressivos (OR = 3,72; IC 95% 2,60; 5,32). Comparações individualizadas de ECT *versus* antidepressivos tricíclicos (ADT), e ECT *versus* antidepressivos inibidores da monoaminoxidase (IMAO) também foram realizadas. Foi demonstrada uma eficácia significativamente maior da ECT em relação aos ADT (OR = 2,99; IC 95% 1,91; 4,71) e aos IMAOs (OR = 6,13; IC 95% 3,82; 9,83).

Nas diretrizes do *National Institute of Clinical Excellence* sobre ECT (NICE., 2003), a partir da revisão de 90 ensaios clínicos randomizados (ECR) sobre a eficácia da ECT na depressão, conclui-se também que a ECT tem maior benefício que antidepressivos e que a ECT real é mais efetiva que a simulada.

Com a diminuição dos sintomas depressivos obtida pela ECT, a melhora em algumas funções neurocognitivas tem sido observada, especialmente atenção e concentração, enquanto que alguma mudança foi observada no raciocínio abstrato e criatividade. No entanto, efeitos adversos cognitivos, como desorientação, prejuízo no aprendizado, amnésia anterógrada e retrógrada, podem ser observados após crises convulsivas, incluindo aquelas produzidas pela ECT (Prudic et al., 2008).

Os efeitos colaterais cognitivos são a maior limitação da ECT, ao diminuírem a satisfação do paciente e contribuindo com o estigma associado ao tratamento. Dos efeitos cognitivos, o déficit na memória é o mais importante. Logo após o curso da ECT, a maioria dos pacientes manifesta dificuldade em reter informações recém-aprendidas (amnésia anterógrada) e lembrar de eventos que ocorreram semanas ou meses antes da ECT (amnésia retrograda) (Fraser et al., 2008; Sackeim et al., 1992). Na maioria dos pacientes, a amnésia anterógrada melhora rapidamente após a ECT (em geral, em menos de um mês). Já a amnésia retrógrada é o efeito colateral cognitivo mais persistente, melhorando durante os primeiros meses após o tratamento. A memória de informações autobiográficas é menos afetada que eventos de natureza impessoal (Lisanby et al., 2000).

A decisão quanto a indicar ou não ECT a um paciente, como qualquer outro procedimento, deve se fundamentar nas opções de tratamento disponíveis e nas considerações sobre riscos e benefícios (Figiel et al., 1998). Muitos clínicos e pesquisadores acreditam que a frequência do uso da ECT é muito menor do que deveria ser. Supõe-se que essa baixa utilização se deva, principalmente, a enganos e preconceitos em relação à ECT, devidos, pelo menos em parte, às informações errôneas e distorcidas publicadas pela imprensa leiga e pela mídia (Fink et al., 2001).

6. REVISÃO DE LITERATURA

6.1 Neurotrofismo e depressão

A clássica teoria monoaminérgica para a patofisiologia da depressão surgiu na década de 60 (Schildkraut, 1965). Na época, foi observado que um anti-hipertensivo que depletava monoaminas, a reserpina, provocava sintomas depressivos (Gardner e Boles, 2011). Neste mesmo período, achados experimentais indicaram que a imipramina inibia a recaptação da noradrenalina no cérebro e que era capaz de reverter os efeitos sedativos da reserpina em animais (Herting et al., 1961). Mais adiante, o papel da serotonina na teoria monoaminérgica foi demonstrado com claros correlatos neuroquímicos no sistema nervoso central (Lapin e Oxenkrug, 1969).

Com o desenvolvimento dos antidepressivos observou-se, entretanto, que apesar de causarem um aumento imediato da neurotransmissão monoaminérgica, a resposta antidepressiva ocorria em apenas algumas semanas. Também foi observado que a depleção de monoaminas apenas provocava sintomas depressivos em indivíduos com episódios em remissão ou com história familiar, mas não em indivíduos saudáveis (Ruhé et al., 2007). Instituído o tratamento antidepressivo, muitos indivíduos ainda persistiam com sintomas apesar dos aumentos nos níveis de monoamina sinápticos (Bilgen et al., 2014). Todas essas observações levantam questionamentos sobre o papel da teoria monoaminérgica na etiologia biológica da depressão e teorias alternativas têm surgido.

Estudos recentes têm mostrado que os indivíduos com transtornos depressivos recorrentes, resistentes ao tratamento e de longa duração têm volumes do hipocampo diminuídos (Videbech e Ravnkilde, 2004; Campbell et al, 2004), bem como mudanças estruturais e degeneração no hipocampo, amígdala (Bowley et al., 2002; Hamidi et al., 2004) e córtex pré-frontal (Cotter et al., 2001; Rajkowska et al., 2001). Estas alterações estruturais estão intimamente relacionadas em nível celular com o funcionamento dos fatores de crescimento neuronais, fatores neurotróficos ou neurotrofinas.

Neurotrofinas são moléculas intracelulares implicadas na manutenção de neurônios, permitindo-lhes cumprir as suas funções; ajudam-lhes no seu desenvolvimento e sua reparação (Bilgen et al., 2014). O fator neurotrófico derivado do cérebro (BDNF), que é a mais estudada neurotrofina, desempenha um papel importante na neurogênese e na plasticidade sináptica, auxiliando a manter a viabilidade celular (Lee et al., 2002).

A teoria neurotrófica para a patofisiologia da depressão foi publicada pela primeira vez em 1997 (Duman et al. 1997), a partir da observação dos primeiros estudos mostrando redução no volume de estruturas cerebrais (em especial o hipocampo) em pacientes deprimidos, e também indicando que o estresse induz uma redução na expressão de BDNF que por sua vez leva à atrofia de neurônios hipocampais. Tal teoria foi fortalecida com novas evidências indicando o papel de neurotrofinas no funcionamento do cérebro e do número crescente de ensaios clínicos que estudam a relação entre transtorno depressivo e BDNF (Kotan et al., 2009; Sala et al., 2004; Bilgen et al., 2014).

O primeiro estudo mostrando concentrações reduzidas de BDNF no

sangue periférico de pacientes deprimidos em comparação a controles saudáveis foi publicado em 2002 (Karege et al. 2002). Desde então vários outros trabalhos, incluindo meta-análises, relataram que os níveis de BDNF (séricos ou plasmáticos) são baixos em pacientes depressivos sem uso de fármacos (Shimizu et al, 2003; Fernandes et al., 2011; Bocchio-Chiavetto et al., 2010; Sen et al., 2008), aumentando após tratamento antidepressivo (Shimizu et al., 2003; Aydemir et al., 2005; Huang et al., 2008; Molendijk et al., 2011; Gönül et al., 2005; Brunoni et al., 2008; Sen et al., 2008; Guilloux et al., 2012; Wolkowitz et al., 2011; Matrisciano et al., 2009; Serra-Millàs et al., 2011).

Há também evidências de que os níveis de receptor de BDNF, os receptores Tropomiosina Quinase B (TrkB), estão elevados na depressão (Hung et al., 2010). Estudos genéticos têm demonstrado que o polimorfismo mais comumente estudado do BDNF, o Val66Met (rs6265), tem sido associado com a resposta antidepressiva precoce (Xu et al., 2012) e remissão de sintomas (Taylor et al., 2011).

Apesar destes resultados, ainda não se sabe como BDNF está relacionado a melhorias na depressão. Foi hipotetizado que o BDNF induziria brotamento neuronal no hipocampo e no córtex cerebral, e melhoraria a função sináptica e a conectividade de circuitos neuronais envolvidas na regulação do humor (Duman et al., 1997; Duman e Vaidya, 1998). A Figura 1 ilustra esses efeitos nas células piramidais da região CA3 do hipocampo. À esquerda, temos uma célula saudável, numa situação normal. Ao centro, temos a atrofia celular desencadeada pelo estresse. À direita, temos os

antidepressivos revertendo esse mecanismo de atrofia, promovendo sobrevivência e crescimento celular, retornando à condição normal.

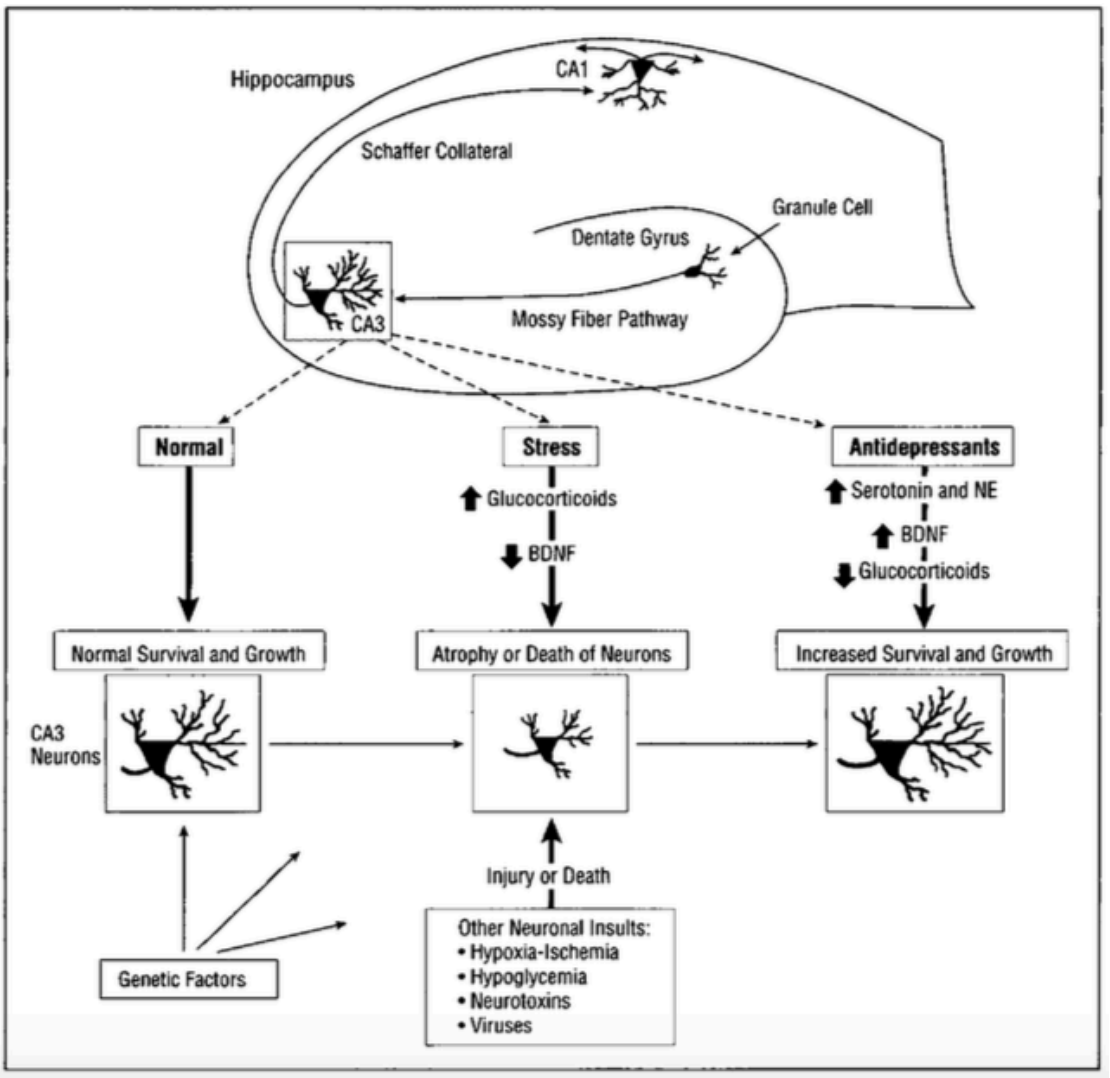

Figura 1. Crescimento e sobrevivência dos neurônios hipocampais, e influências do estresse e do uso de antidepressivos nesses processos. (Duman et al. 1997)

Apesar de tais evidências favoráveis à teoria neurotrófica da depressão, existem alguns estudos com achados controversos. Estudos pré-clínicos não encontraram os padrões descritos de resposta do BDNF aos antidepressivos (Krishnan e Nestler, 2008). Polimorfismos do gene do BDNF responsáveis pela redução da liberação da neurotrofina e à diminuição do volume hipocampal não foram associados a aumento de suscetibilidade para

depressão (López-León et al., 2008). Por fim, em regiões do cérebro como núcleo *accumbens* e área tegmental ventral o BDNF exerce paradoxalmente potentes efeitos depressivogênicos (Eisch et al., 2003).

Algumas questões dificultam o emprego da dosagem de BDNF como ferramenta diagnóstica na prática clínica. Não há, por exemplo, padronização referente às técnicas laboratoriais empregadas, o que provoca uma grande variedade de valores nas dosagens, restringindo a comparação entre os estudos (Teche et al., 2013). Este problema fica evidenciado no fato de que os níveis de BDNF reportados nos controles saudáveis é muito diferente entre os diferentes estudos (Karege et al., 2005). Além disso, o BDNF tem se mostrado um marcador pouco específico, já que outros transtornos psiquiátricos além da depressão apresentam redução de seus níveis, como por exemplo a esquizofrenia, transtorno do pânico, transtornos alimentares e doenças neurodegenerativas como as doenças de Alzheimer e Huntington (Gass e Hellweg, 2010). Várias formas de estresse mostraram-se também capazes de reduzir os níveis de BDNF, ao menos em experimentos com animais (Martinowich et al., 2007; Krishnan e Nestler, 2008). Essa neurotrofina também parece sofrer influências de fatores sazonais. Um estudo com 2851 participantes, incluindo pacientes com depressão, ansiedade e controles saudáveis, identificou uma forte variação dos níveis séricos de BDNF ao longo do ano, com seu nadir (valores mais baixos) no inverno, aumento durante a primavera e pico no verão (Molendijk et al., 2012).

A redução do BDNF durante os episódios depressivos e o retorno a níveis semelhantes aos dos controles após a remissão é uma achado

consistentemente replicado na literatura (Fernandes et al., 2011; Bocchio-Chiavetto et al., 2010; Sen et al., 2008; Brunoni et al., 2008). No entanto, não está bem estabelecido a associação desta redução com outros aspectos relevantes da depressão. Os níveis de BDNF não se associam com a cronicidade da depressão, visto que não diferenciam primeiros episódios depressivos de transtorno recorrente (Molendijk et al., 2011), nem se correlacionam com o tempo de doença (Kenna et al., 2014). Apesar de alguns estudos terem encontrado uma correlação negativa entre a gravidade da depressão e a concentração sérica do BDNF (Gorgulu and Caliyurt, 2009; Satomura et al., 2011), um número maior de estudos não encontrou esta correlação, inclusive uma recente meta-análise (Molendijk et al., 2014; Jevtović et al., 2011; Molendijk et al., 2011; Wolkowitz et al., 2011; Vinberg et al., 2013). Alguns autores chegam inclusive a questionar o modelo teórico que liga o BDNF ao TDM com base nessas inconsistências (Groves, 2007).

Em resumo, as influências que o BDNF sofre do estresse, a falta de padronização laboratorial de suas análises, as variações em múltiplos transtornos psiquiátricos, as interferências sofridas pela sazonalidade e a correlação ainda inconsistente com gravidade e cronicidade da depressão impedem uma ligação definitiva entre BDNF e TDM, dificultando a implementação de sua medida na prática clínica.

6.2 Mecanismos inflamatórios e depressão

O *sickeness behaviour* (Hart, 1988) é um estado comportamental e subjetivo que ocorre em resposta a doenças sistêmicas infecciosas. É caracterizado por fraqueza, mal-estar, baixa ingesta de alimento e de líquidos, redução do interesse pelo ambiente ao redor, e letargia, exercendo uma função protetora para o organismo, já que reorganiza suas prioridades de modo a facilitar a recuperação da infecção (Dantzer e Kelley, 2007). O *sickeness behaviour* resulta da resposta do sistema imune ao patógeno invasor por meio da liberação de citocinas como a interleucina-1 (IL-1), TNF-α e IL-6, e de proteínas de fase aguda, como a proteína C reativa (PCR). Essas substâncias atingem o sistema nervoso central cruzando a barreira hematoencefálica ou através da comunicação aferente do nervo vago, gerando tais sintomas comportamentais (Dantzer et al., 2008).

Exposições experimentais de indivíduos saudáveis a indutores de citocinas pró-inflamatórias foram capazes de induzir *sickeness behaviour* (Reichenberg et al., 2001; Reichenberg et al., 2002). A administração de endotoxinas provocou aumento nos níveis de TNF-α, de IL-16 e do antagonista do receptor de interleucina 1 (IL-1ra), resultando em significativas mudanças na ingesta alimentar (Reichenberg et al. 2002).

Curiosamente, observou-se que os sintomas da *sickeness behaviour* assemelhavam-se aos sintomas depressivos, e passou a ser interrogado um possível papel de mecanismos inflamatórios na patofisiologia da depressão. Essa associação se tornou mais evidente com a demonstração de que citocinas pró-inflamatórias induzem sintomas depressivos em modelos

animais, não restritos ao sintomas do *sickness behaviour* (Frenois et al., 2007). Observou-se também que a injeção de citocinas pró-inflamatórias modula o metabolismo da serotonina, da noradrenalina e da dopamina, diminuindo sua disponibilidade (Felger et al., 2007). A indução experimental de liberação de citocinas em humanos saudáveis também gerou um aumento transitório na ansiedade e no humor depressivo assim como uma diminuição na memória verbal e não verbal (Reichenberg et al. 2001).

Paralelamente, o surgimento de sintomas de humor em pacientes expostos a citocinas inflamatórias com finalidades terapêuticas também passou a sinalizar a ligação entre depressão e inflamação. O interferon é um tratamento bem estabelecido para diferentes doenças, incluindo hepatite C e câncer. O uso do interferon alfa (INF-α) pode produzir uma síndrome depressiva muito semelhante à depressão maior idiopáica (Capuron et al., 2009; Raison et al., 2005). Em doses altas de INF-α, cerca de 50% dos pacientes sem depressão prévia vão preencher critérios para depressão maior dentro dos 3 primeiros meses de tratamento com a droga (Musselman et al., 2001), e uma porcentagem ainda maior (90%) terão pelo menos 1 ou 2 sintomas depressivos significativos, sendo fadiga o mais comum (Capuron et al., 2002). O tratamento com INF-α resulta em aumento nos níveis da citocina pró-inflamatória IL-6 (Raison et al., 2009) e TNF-α (Prather et al., 2009). Essas evidências, somadas à reversão de *sickness behaviour* com o uso de antidepressivos em tratamentos animais levou a elaboração de uma nova hipótese para a patofisiologia da depressão: a inflamatória (Dantzer et al., 2008).

A partir dessa hipótese, diversos estudos têm pesquisado níveis de

citocinas e proteínas de fase aguda em depressão. Entretanto, os dados sofrem de alguma inconsistência devido, entre outras razões, aos inúmeros fatores de confusão a que essas dosagens estão sujeitas, tais como estado metabólico (Huerta e Nadler, 2002), peso corporal (Wellen e Hotamisligil, 2003), ciclo menstrual (O'Brien et al., 2007), distúrbios de sono (Ryan et al., 2005) e os métodos de dosagem e estocagem às quais as amostras laboratoriais são submetidas (Friebe e Volk, 2008).

Apesar dessas limitações, diversos estudos têm confirmado a associação entre depressão e infamação. Duas meta-análises mostraram que os níveis plasmáticos de diferentes proteínas relacionadas à inflamação, incluindo o TNF-α, TNF-β1, IL-6, PCR e interferon estão alterados na depressão maior. A primeira, que incluiu 24 estudos, evidenciou uma significativa elevação nos níveis de TNF-α e IL-6 em pacientes com depressão maior quando comparado aos controles, mas não houve diferenças significativas nas concentrações de outras citocinas pró-inflamatórias investigadas (IL-1, IL-2 , IL-8, e IFN-γ), nem houve diferenças significativas nas citocinas anti-inflamatórias IL-10 e IL-4 (Downlati et al., 2010). Outra meta-análise das concentrações de citocinas em diferentes grupos clínicos encontrou resultados semelhantes, com aumentos de PCR, IL-6, IL-1 e IL-1ra, sendo positivamente associado com depressão maior (Howren et al. 2009).

Níveis mais altos de PCR em pacientes deprimidos do que em controles têm sido repetidamente evidenciados , especialmente em pacientes com depressão grave internados (Lanquillon et al., 2000). Uma correlação significativa entre os níveis de PCR e a gravidade da depressão foi descrita

(Häfner et al., 2008). Níveis mais altos de PCR também foram observados em pacientes em remissão de quadro depressivo prévio, tanto em homens (Danner et al., 2003; Ford e Erlinger, 2004) quanto em mulheres (Cizza et al. 2009; Kling et al., 2007). Altos níveis de PCR (assim como de IL-6) também foram observados como um marcador precoce de sintomas cognitivos em depressão (Gimeno et al. 2008). Na avaliação basal, níveis altos de PCR são um fator de risco independente para o surgimento de um novo episódio depressivo (Pasco et al., 2010).

As evidências sugerem que elevação no sangue periférico da concentração de IL-6 é um achado imunológico comum e um dos mais replicados em depressão (Maes et al., 2014; Liu et al., 2012; Vogelzangs et al., 2012; Dowlati et al., 2010). Níveis mais altos de IL-6 no líquido cefalorraquidiano estão correlacionadas com tendências suicidas, e níveis altos de IL-6 no plasma estão correlacionados com a falta de resposta aos medicamentos antidepressivos convencionais (Bay-Richter et al., 2015). A concentração de citocinas também pode ser usada como preditor de risco para um indivíduo desenvolver depressão como evidenciado em um estudo epidemiológico em que, na avaliação basal, níveis elevados de PCR e IL-6 foram associados com a presença de sintomas depressivos num follow-up após 12 anos (Gimeno et al. 2009).

Níveis mais altos de TNF-α foram observados em pacientes deprimidos comparado com controles (Dantzer et al., 2008; Hestad et al. 2003; Lanquillon et al. 2000; Mikova et al. 2001). Em pacientes com doenças imunológicas, tais como a artrite reumatóide e a psoríase, o tratamento anti-TNF-α alivia o humor deprimido independentemente da melhoria dos

sintomas de doença (Tyring et al., 2006). No entanto, um estudo de eficácia de infliximab (um anticorpo monoclonal contra o TNF-α) para o tratamento da depressão teve resultados negativos (Raison et al., 2013). Por outro lado, uma subanálise desse estudo mostrou que o infliximab foi eficaz em pacientes com valores de PCR>5, sugerindo que estratégias antinflamatórias são mais eficazes em pacientes com aumentos nos níveis de marcadores pró-inflamatórios (Raison et al., 2013). Os níveis aumentados de TNF-α diminuíram em respondedores à farmacoterapia antidepressiva (Lanquillon et al. 2000) e à terapia com ECT (Hestad et al. 2003). Em um estudo comparando níveis de citocinas sanguíneas entre pacientes deprimidos com e sem tentativa de suicídio, foi observado que os pacientes que tentaram suicídio tiveram níveis mais altos de IL-6 e TNF-α do que os que não tentaram e do que os controles saudáveis (Janelidze et al., 2011).

IL-1ra é um membro da família da interleucina 1 e é um antagonista da IL-1α e da IL-1β. IL-1ra também serve como um marcador da ativação do sistema imune. Em um grupo de pacientes italianos acima de 65 anos, altos níveis de IL-1ra foi preditor de alto risco de sintomas depressivos após um *follow-up* de 6 anos (Milaneschi et al., 2009).

Estudos anteriores com interferon gama (IFN-γ) não encontraram diferenças nos níveis desse marcador entre indivíduos depressivos e controles saudáveis (Spanenberg et al., 2014). No entanto, os níveis foram mais baixos em pacientes depressivos melancólicos em comparação a não-melancólico (Spanenberg et al., 2014; Rothermundt et al., 2001).

Um estudo anterior não encontrou diferenças significativas nas concentrações de IL-17 entre indivíduos deprimidos e controles, e não

encontrou variação dos níveis de IL-17 após o tratamento da depressão (Kim et al., 2013).

Um recente e interessante estudo investigou a relação entre ítens de escala de sintomas depressivos e os níveis de citocinas inflamatórias. Na avaliação basal, no grupo dos pacientes, as citocinas IL-2, IL-4, IL-5, IL-10, IL-12, IFN-γ, TNF-α e de Fator Estimulador de Colônias de Macrófagos e Granulócitos (GM-CSF) foram negativamente correlacionadas com itens individuais da Escala de Depressão de Beck, e tiveram correlações negativas e positivas com os ítens da HAMD-17. Quatro semanas após o início do tratamento antidepressivo e no grupo dos controles não foram observadas tais relações. Os autores sugerem que as citocinas não são genericamente pró-depressivas, mas estão mais relacionadas a uma regulação mais específica de sintomas e à gravidade da depressão (Schimidt et al., 2016).

Efeitos imuno-moduladores de antidepressivos também têm sido descritos. Em alguns estudos, antidepressivos tricíclicos e inibidores seletivos de recaptação de serotonina, assim como os mais novos antidepressivos serotoninérgicos e noradrenérgicos, normalizam os níveis séricos de proteínas inflamatórias e citocinas, incluindo IL-1β, IL-6, TNF-α e IFN-γ (De BD et al. 2010; Janssen et al., 2010). Meta-análises indicaram que o tratamento com antidepressivos reduz os níveis de citocinas pró-inflamatórias (De Berardis et al., 2010; Hannestad et al., 2011) e PCR (Hiles et al., 2012).

Em resumo, a semelhança da *sickness behaviour* com o TDM, bem como a observação de sintomas depressivos secundários ao tratamento com INF-α abriram caminho para o surgimento da teoria inflamatória para a patofisiologia da depressão. Apesar da presença de fatores de confusão e

questões metodológicas nos estudos realizados até o momento, evidências consistentes têm mostrado uma associação entre a síndrome depressiva e marcadores inflamatóricos, como PCR, IL-6 e TNF-α.

6.3 Interface entre teorias patofisiológicas da depressão

Além das teorias monoaminérgica, neurotrófica e inflamatória para a explicar a patofisiologia da depressão, outras tem surgido, como a neuroendocrinológica e a glutamatérgica.

A teoria neuroendocrinológica parte de uma consistente evidência do papel mediador do eixo hipotalâmico-hipófise-adrenal (HHA) na reação ao estresse. O hormônio liberador de corticotrofina (CRH) é liberado no hipotálamo e, ao se ligar a seus receptores na hipófise, estimula a liberação de corticotrofina [hormônio adrenocorticotrófico (ACTH)] da hipófise no plasma. Por fim, o ACTH estimula a liberação de cortisol do córtex adrenal (Belmaker et al., 2008; McEwen et al., 1998). Apesar da ativação aguda do eixo HHA ser uma resposta apropriada ao estresse, a hiperativação crônica desse eixo pode resultar em problemas a longo prazo. A relação entre estresse crônico e depressão é bem documentada (Cohen et al., 2007), tendo os pacientes que sofrem desse transtorno níveis elevados de cortisol no plasma assim como diminuição da sensibilidade à dexametasona externa e ao CRH (Hasler et al., 2004; Ising et al., 2007).

A teoria neuroendocrinológica se aproxima da inflamatória a partir de evidências de que o estresse agudo resulta no aumento da circulação de interleucinas IL-6 e IL-1β (Steptoe et al., 2007), e de que o estresse crônico leva a um robusto aumento de IL-6 (Kiecolt-Glaser et al., 2003). Tais citocinas pró-inflamatórias, por sua vez, estimulam a liberação de CRH e ACTH (Pariante et al., 2001) e diminuem a expressão dos receptores de glicocorticoides (Pace et al., 2009). Citocinas pró-inflamatórias, então,

exercem uma significativa influência na atividade do eixo HHA: podem mediar o hipercortisolismo e diminuir a sensibilidade do eixo HHA frequentemente visto na depressão maior.

A estimulação do eixo HHA é mediada por mecanismos inflamatórios, resultando em aumentos prolongados na circulação de hormônios do estresse no sistema nervoso central (Zunszain et al., 2011). Consequentemente, distúrbios na homeostase de neurotransmissores e na síntese de fatores de crescimento neuronal ocorrem, em particular, nos circuitos neuronais do sistema límbico, que, por sua vez, está implicado no TDM (Miller et al., 2013).

A interface entre o estresse e o neurotrofismo também é discutida em outros trabalhos. Em roedores, é observada atrofia neuronal, redução da densidade sináptica e perda celular em modelos de depressão e estresse, assim como diminuição a neurogênese no hipocampo adulto e redução das células gliais no córtex pré-frontal quando cronicamente expostos ao estresse (Duman e Aghajanian, 2012). Ainda em roedores, a administração de glicocorticoides gera sintomas similares aos depressivos e produz atrofia do hipocampo (McEwen, 2007). A exposição crônica a glicocorticoides é neurotóxica: ocorre liberação de radicais livres, diminuição de transporte de glicose e diminuição de produção de BDNF, que provê suporte trófico aos neurônios. O estresse crônico leva a atrofia dendrítica e, finalmente, morte celular (Willner et al., 2013).

O HHA também se relaciona com a produção de monoaminas. Níveis elevados de cortisol aumentam a atividade da monoaminoxidase-A (MAO-A), com a consequente redução dos níveis de noradrenalina e serotonina (Slotkin

et al., 1998).

As interações entre os mecanismos inflamatórios e neurotróficos também estão consideravelmente bem estabelecidas. As citocinas inflamatórias acessam o cérebro cruzando a barreira hematoencefálica ou através da comunicação aferente do nervo vago e interagem com o metabolismo dos neurotransmissores, influenciando a função neuroendócrina, os circuitos neuronais e a plasticidade sináptica (Borden e Parkinson, 1998). Podem assim induzir alterações relevantes para a patofisiologia da depressão, incluindo a desregulação da glia, prejuízos neuronais e da função cognitiva (Miller et al., 2009). A administração periférica de lipopolissacarideo (LPS), um indutor de citocinas, resultou em disfunção cognitiva e no aumento das concentrações hipocampais de TNF-α e de IL-1β (Wu et al., 2007). Esse estudo também sugeriu que os níveis hipocampais de BDNF foram reduzidos pela administração de LPS.

Mecanismos inflamatórios também afetam o metabolismo do triptofano, o precursor da serotonina. Citocinas pró-inflamatórias, incluindo a IL-2, INF-γ e INF-α, ativam a enzima indolamina 2-3-dioxigenase (IDO) (Muller et al., 2007), a qual cataboliza o metabolismo do triptofano em quinurenina. A quinurenina é posteriormente metabolizada pela enzima quinurenina monooxigenase em ácido quinolínico e ácido quinurenico, dois metabólitos potencialmente neurotóxicos (Muller et al., 2011; Oxenkrug et al., 2010). A enzima quinurenina monooxigenase é também ativada por citocinas pró-inflamatórias. Pacientes com depressão maior com histórico de tentativas de suicídio apresentaram níveis mais altos de quinurenina (Sublette et al., 2011). Aumentos nos níveis de citocinas pró-inflamatórias podem assim

diminuir o triptofano disponível para a síntese de serotonina, diminuindo sua disponibilidade e tornando consequentemente os pacientes mais vulneráveis a depressão maior e a risco de suicídio (Dantzer et al., 2008).

O tratamento com INF-α aumenta a atividade da enzima Indolamina 2-3-Dioxigenase (IDO) e resulta em aumento dos metabólitos tóxicos ácido quinurenico e ácido quinolínico no plasma (Wichers et al., 2005). O tratamento com INF-α também resulta no aumento dessas substâncias no líquido cerebrorraquidiano que se correlacionou com sintomas clínicos de depressão (Raison et al., 2010).

Evidências recentes têm demonstrado que subdoses anestésicas de quetamina, um antagonista do receptor glutamatérgico N-metil-D-aspartato (NMDA), apresenta efeitos antidepressivos. Tais achados levaram à elaboração da mais recente teoria para a patofisiologia da depressão: a glutamatérgica. Pacientes com depressão maior recebendo 0.5mg/kg de quetamina apresentaram melhora dos sintomas depressivos que se manteve significativamente em até 7 dias após sua infusão (Zarate et al., 2006). Um estudo randomizado, duplo-cego, controlado apontou que a infusão de doses subanestésicas de quetamina (0.5mg/kg) em pacientes com depressão bipolar provocou melhora significativa dos sintomas mesmo no 3º dia após infusão (DiazGranados et al., 2010a). O mesmo grupo de pesquisadores observou que infusão de 0.5mg/kg de quetamina reduziu significativamente ideação suicida em pacientes deprimidos em medidas realizadas até 4 horas após a infusão (DiazGranados et al., 2010b). Após essas evidências iniciais, outros experimentos acerca do uso de quetamina em depressão têm sido publicados, inclusive com elaboração de meta-análises com resultados

positivos (Kishimoto et al., 2016; McGirr et al., 2015; Fond et al., 2014).

Evidências sugerem que o BDNF plasmático pode ser um biomarcador periférico relevante para a resposta antidepressiva da quetamina. Camundongos *knockout* para o gene do BDNF tiveram mínimos efeitos à infusão intraperitoneal de quetamina quando submetidos ao teste do nado forçado (Autry et al., 2011). Em humanos, após estudos iniciais sugerirem que os níveis periféricos de BDNF não se correlacionaram com a resposta aintidepressiva da quetamina (Machado-Vieira et al., 2009; Laje et al., 2012), trabalhos subsequentes têm apontado uma associação positiva. Pacientes que apresentaram melhora dos sintomas depressivos com infusão de quetamina tiveram aumentos significativos de BDNF plasmático 240 min após a infusão. Tais níveis de BDNF foram negativamente associados aos escores da Montgomery-Asberg Depression Rating Scale (MADRS) 240 min após a infusão, e também em avaliações realizadas após 24h, 48h e 72h. Nesse estudo, um agente anestésico comparativo (midazolam) não apresentou as mesmas associações (Haile et al., 2014).

Em resumo, além da clássica teoria monoaminérgica para a patofisiologia da depressão, bem como as novas teorias neurotrófica e inflamatória, estudos também têm sugerido a existência das teorias neuroendocrinológica e glutamatérgica. Mais do que concorrentes em explicar os complexos mecanismos biológicos do TDM, essas vias parecem interligadas e complementares.

6.4 Neurotrofismo associado a ECT (modelos animais)

A indução de convulsões através de corrente elétrica em animais (em inglês, *electroconvulsive shock* ou ECS) tem se mostrado um potente indutor gênico nos modelos de tratamento antidepressivo, especialmente de fatores neurotróficos e de crescimento. Estes são o BDNF, o fator de crescimento endotelial vascular (VEGF) e o fator de crescimento fibroblastico-2 (FGF-2) (Nibuya et al., 1995; Newton et al., 2003; Altar et al., 2004).

Uma única sessão de ECS resulta em um aumento de 10 a 20 vezes na indução do gene do BDNF no giro denteado de hipocampo de ratos. A ECS de longo prazo (uma vez ao dia por 7 a 10 dias) também induz o ácido ribonucleico (RNA) mensageiro do BDNF em níveis que se mantêm aumentados por um longo período após ECS de longo prazo comparado com uma sessão de ECS isolada (Nibuya et al., 1995).

Os RNA mensageiros do VEGF e do FGF-2 também se mantêm elevados no hipocampo de roedores após ECS aguda e de longo prazo (Newton et al., 2003; Warner-Schmidt et al., 2007). Esses fatores foram originalmente descobertos como fatores mitogênicos de células não-neuronais, como células endoteliais e fibroblastos, mas hoje são aceitos como fatores neurotróficos e neuroprotetores (Schmidt et al., 2007).

Estudos pré-clínicos têm mostrado que a ECS é a mais robusta indutora de neurogênese no hipocampo de roedores e de primatas não-humanos (Malberg et al., 2000; Perera et al., 2007). Além disso, um estudo

mostrou que a ECS aumenta a neurogênese em áreas cerebrais frontais, especialmente no estriado rostro-medial (Inta et al., 2013).

A administração de fatores neurotróficos e de crescimento diretamente no hipocampo ou no ventrículo lateral de ratos mimetiza efeito antidepressivo em modelos animais. A infusão de BDNF no hipocampo de ratos diminui o tempo de imobilidade no teste do nado forçado de forma similar ao efeito provocado por antidepressivos (Shirayama et al., 2002). Infusões de VEGF nos ventrículos laterais também mimetiza a ação antidepressiva em modelos comportamentais (Shirayama et al., 2002).

A ECS também aumenta a expressão de neuropeptídios como o neuropeptídio Y (NPY), hormônio liberador de tireotrofina (TRH) e VGH (não acrônimo) (Newton et al., 2003; Altar et al., 2004).

O NPY é um peptídeo de 36 aminoácidos que é difusamente distribuído no sistema nervoso central. O efeito antidepressivo do NPY tem sido estudado: sua infusão em ventrículos laterais de roedores produz efeito antidepressivo, e o aumento do RNA mensageiro de NPY no hipocampo foi observado após ECS repetidas (Redrobe et al., 2002; Ishida et al., 2007).

Algumas evidências indicam que o TRH pode aliviar sintomas depressivos (Marangell et al., 1997). O aumento do RNA mensageiro do TRH no hipocampo ocorre após ECS repetidas. Esse aumento é consistente com o efeito da ECS em aumentar o peptídeo prepro-TRH (precursor do TRH) em algumas regiões do cérebro de ratos e com efeitos antidepressivos de TRH no teste do nado forçado (Pekary et al. 2000).

O VGF é um neuropeptídio de 617 aminoácidos precursor de pelo menos 10 peptídeos com expressão restrita ao SNC (Levi et al., 1985). A

infusão de VGF no hipocampo de ratos produz efeitos antidepressivos no teste do nado forçado e no teste de suspensão pela cauda (Hunsberger et al., 2007, Thakker-Varia et al., 2007). A expressão do RNA mensageiro do VGF é aumentada no giro denteado do hipocampo de ratos tanto no curso agudo ou repetido de ECS (Levi et al., 2004).

Em suma, a ECS apresenta-se como um importante indutor gênico de fatores neurotróficos e de crescimento nos modelos animais de tratamento antidepressivo. A administração desses fatores em regiões cerebrais desses animais foi capaz de mimetizar efeito antidepressivo.

6.5 Neurotrofismo associado a ECT (modelos humanos)

O primeiro estudo que associou aumentos dos níveis de BDNF com a ECT em pacientes deprimidos (Bocchio-Chiavetto et al., 2006) mostrou que, apesar de esse aumento não ter surgido entre a avaliação basal (T0) e imediatamente após o curso de ECT (T1), ocorreu significativamente um mês após o término do ECT (T2). Isso sugere que talvez seja necessário um período mais prolongado de tempo para detecção do aumento dos níveis de BDNF no plasma. Reforçando essa ideia, um estudo de correlação entre nível sérico e nível tecidual cerebral de neurotrofinas após ECT em animais demonstrou com evidências substanciais ser justificável medir níveis séricos de BDNF com um atraso de tempo para monitorar as alterações de neurotrofinas em nível tecidual cerebral (Sartorius et al., 2009). Outro trabalho observou que o aumento significativo de BDNF em pacientes deprimidos usando ECT ocorreu numa medida com menor intervalo de tempo que no estudo de Bocchio-Chiavetto, após 2 semanas de conclusão do tratamento (Hu et al., 2010). Ao longo dos últimos anos, diversos estudos têm encontrado associação entre ECT e aumento dos níveis de BDNF, já tendo sido inclusive publicadas três meta-análises confirmando essa relação (Rocha et al., 2016; Polyakova et al., 2015; Brunoni et al., 2014).

Alguns estudos tem sugerido que o BDNF pode contribuir para a eficácia clínica da ECT no tratamento da depressão. Já nos primeiros trabalhos sobre o tema se encontro um significativo aumento do BDNF no plasma sanguíneo após a ECT, o que coincidiu com significativa melhora dos

sintomas depressivos medida pela HDRS-17 (Marano et al., 2007). Um estudo mais recente investigou os efeitos da ECT sobre os níveis séricos de BDNF em pacientes com depressão resistente ao tratamento, constatando que os escores na HDRS-17 foram mais baixos e o nível médio BDNF foi maior após a ECT (Bilgen et al., 2014). Em relação a resposta clínica (melhora ≥ 50% dos sintomas na HDRS-17), encontrou-se um aumento significativo dos níveis séricos do BDNF em pacientes com resposta à ECT (comparando dosagens do marcador antes do início do ECT e dosagens 5 semanas após o início). O mesmo estudo não observou aumento de BDNF em não respondedores ao tratamento (Okamoto et al., 2008). Em relação à remissão dos sintomas (alcance de níveis de HDRS-17 ≤ 7 após o tratamento), observou-se que os pacientes que atingiram a remissão clinica tiveram níveis significativamente maiores de BDNF no *baseline* e uma semana após a conclusão da ECT em comparação aos que não remitiram (Piccinni et al., 2009).

Apesar desses dados mostrando associação entre aumento dos níveis de BDNF e melhora clínica em pacientes deprimidos submetidos a ECT, outros trabalhos não tiveram achados favoráveis. Um estudo recente relatarou que em pacientes com depressão resistente ao tratamento, a ECT reduziu os escores de sintomas depressivos, mas não afetou os níveis séricos de BDNF; não houve correlação entre as alterações no nível de BDNF no soro e mudanças na pontuação em escala de sintomas. Os níveis séricos de BDNF foram baixos tanto no *baseline* quanto após a ECT (Rapinesi et al., 2015). Estes resultados, juntamente com outros estudos (Fernandes et al., 2009; Haghighi et al., 2013) não apoiam a hipótese de que

a melhora da depressão com a ECT seja mediada pelo aumento dos níveis de BDNF.

Alguns trabalhos realizaram pesquisa dos níveis de BDNF em pacientes submetidos a ECT realizando um controle mais apurado dos fármacos antidepressivos em uso. Os resultados, porém, foram conflitantes. Um ensaio clinico randomizado comparou os níveis plasmáticos de BDNF entre um grupo de pacientes deprimidos recebendo unicamente 40 mg/dia de citalopram (grupo controle) e outro recebendo a droga combinada a 12 sessões de ECT (grupo-alvo). O BDNF plasmático aumentou em ambos os grupos ao longo do tempo, com os níveis mais elevados no grupo combinado, sugerindo que a ECT teve o efeito de aumentar o BDNF plasmático independentemente do medicamento associado (Haghighi et al., 2013). Um outro estudo examinou os níveis de BDNF sérico no *baseline* e suas variações durante o tratamento de pacientes deprimidos utilizando o antidepressivo duloxetine, avaliando também a relação desses níveis com a resposta ao tratamento. Os resultados indicaram que níveis mais elevados de BDNF na avaliação basal foram preditores para resultado bem sucedido de tratamento no final do estudo (ao fim de seis semanas), mas um aumento inicial em concentrações de BDNF não foi associado com o resultado do tratamento (Mikoteit et al., 2014). Este trabalho também relatou alguns resultados inesperados, como uma ligação entre a depressão mais grave e níveis mais elevados de BDNF no *baseline*. Além disso, um aumento inicial BDNF entre o início do tratamento e na semana 2 não era estável, caindo de volta aos níveis basais. Estes achados sugerem que alterações nos níveis séricos de BDNF durante o tratamento antidepressivo são complexas e

dinâmicas.

Apesar de diverosos estudos mostrando associação entre aumentos de níveis de BDNF e a realização da ECT (Bocchio-Chiavetto et al., 2006; Marano et al., 2007; Okamoto et al., 2008; Piccinni et al., 2009; Hu et al., 2010; Haghighi et al., 2013; Bilgen et al., 2014; Salehi et al., 2014), incluindo três meta-análises (Rocha et al., 2016; Polyakova et al., 2015; Brunoni et al., 2014), existe uma quantidade considerável de estudos que não encontraram essa associação (Grønli et al., 2009; Fernandes et al., 2009; Gedge et al., 2012; Lin et al., 2013; Stelzhammer et al., 2013). Como vimos anteriormente, esses dados também são conflitantes quando se avalia desfecho clinico e BDNF nos pacientes deprimidos submetidos a ECT, com estudos mostrando associações significativas (Bilgen et al., 2014; Piccini et al., 2009; Okamoto et al., 2008; Marano et al., 2007) e outros sem associação (Rapinesi et al., 2015; Haghighi et al., 2013; Fernandes et al., 2009). Essa variação pode ocorrer devido a diferenças metodológicas, como controle ou não do uso de fármacos durante o estudo, o tempo entre a conclusão da ECT o teste laboratorial, diferenças no protocolo de ECT, entre outras circunstâncias. Diferenças na biodisponibilidade de BDNF se medido em nível plasmático ou sérico também devem ser consideradas. Estudos que mediram BDNF sérico não encontraram aumentos após o tratamento (Fernandes et al., 2009; Grønli et al., 2009; Gedge et al., 2012), diferentemente daqueles que acessaram níveis no plasma (Haghighi et al., 2013; Piccinni et al., 2009; Marano et al., 2007). Levando-se em consideração esses resultados conflitantes, ainda não é possível se chegar a conclusões definitivas acerca da relação entre BDNF, depressão e ECT.

Alguns trabalhos investigando outras neurotrofinas e ECT também têm sido realizados. Com uma pequena amostra de 19 pacientes, um estudo encontrou significativa melhora nos valores da escala *Montgomery-Asberg Depression Score* (MADRS) em pacientes deprimidos após ECT, mas não encontrou variações nos níveis de VEGF com o tratamento. Um mês após a conclusão do tratamento, porém, encontrou-se um aumento significativo de seus níveis, sugerindo provavelmente também ocorrer um atraso para manifestação periférica do aumento de VEGF em nível cerebral (Minelli et al., 2011). Investigando os efeitos da ECT nos níveis séricos do fator de crescimento neural (NGF) em pacientes com depressão resistente ao tratamento, um estudo encontrou que no grupo dos pacientes o NGF não aumentou significativamente. Não houve relação entre a severidade da depressão e os níveis de NGF (Bilgen et al., 2014).

Em um pequeno estudo com 15 pacientes deprimidos submetidos a ECT examinou-se os níveis de uma gama maior de neurotrofinas. Os autores não encontraram variações significativas nos níveis de BDNF, de NGF, Neurotrofina-3 (NT3), de Hormônio de Crescimento Humano (HGH) nem de Hormônio Estimulante da Tireóide (TSH) com a ECT. Houve uma mudança estatisticamente significativamente nos níveis de Neuropeptídio Y (NPY), mas de valor clínico incerto pois afetou apenas dois pacientes. Esse estudo não reproduziu os achados em modelos animais (em que todas essas neurotrofinas são aumentadas após ECS), entretanto isso pode ter ocorrido pelo pequeno número de pacientes estudados e por não ter sido feito um atraso na medida sérica dos marcadores em relação ao aumento nos tecido cerebrais (Gronli et al., 2009).

Conclui-se, portanto, que apesar de evidências mostrando aumentos de neurotrofinas após a realização de ECT e associações positivas com desfecho clínico, vários estudos ainda apresentam resultados negativos. Essa variação pode ocorrer devido a diferenças metodológicas e particularidades relativas aos biomarcadores. Mais estudos são necessários para a melhor compreensão dessa associação.

6.6 Inflamação e ECT

Desde o início do emprego da ECT há várias décadas, seu mecanismo de ação é ainda pouco compreendido. Uma das teorias levantadas para o seu funcionamento é a chamada neuroendocrina, baseada na liberação de hormônios hipotalâmicos para o sangue e para o liquido cefalorraquidiano induzidas pela ECT, e pelas evidências de disfunção endocrinológica em pacientes deprimidos graves. Estudos mostram que a libertação de hormonios associados ao HHA é alterada pela ECT (Haskett, 2014; Farzan et al., 2014; Bolwing, 2011) e, como tal, esta é uma das vias mais estudadas envolvidas na ECT. Evidências apontam a normalização da desregulação do eixo HHA após o tratamento com ECT medido pelo teste de supressão de dexametasona (Bolwing, 2011; Fink, 2005; Gibney e Drexhage, 2013). No entanto, os efeitos imunológicos da ECT receberam atenção ainda limitada até o momento. Um estudo de revisão recente destaca que a ECT induz uma ativação imune transitória, enquanto a ECT repetida pode regular negativamente a ativação imune, mas esta evidência é baseada em estudos pequenos (Guloksuz et al., 2014).

O primeiro estudo a explorar o efeito da ECT nas citocinas demonstrou um aumento drástico nos níveis de IL-6 dez minutos após a indução da convulsão (Kronfol et al., 1990). Posteriormente, outro estudo dosou os níveis de IL-1β, de IL-1ar e IL-6 em 9 pacientes deprimidos em alguns momentos do curso da ECT. Esses autores tiveram como resultado que a IL-6 e a IL-1β aumentaram entre 3 e 6 horas após a ECT, indicando que ela é associada a

rápida indução de citocinas inflamatórias no SNC, as quais são mensuráveis no sague periférico (Lehtimäki et al., 2008).

Um estudo realizou dosagens de TNF-α em um grupo de pacientes deprimidos submetidos a ECT, comparando a um grupo de paciente deprimidos não submetidos a ECT e a um grupo controle saudável. Os pacientes deprimidos tiveram no *baseline* níveis marcadamente mais altos de TNF-α do que os controles saudáveis. A melhoria clínica com a ECT foi acompanhada por um declínio gradual e significativo nos nível de TNFα, os quais atingiram os níveis dos controles saudáveis ao final do estudo. Tal declínio não foi visto nos pacientes deprimidos que não receberam ECT, os quais mantiveram níveis altos de TNF-α durante toda a avaliação (Hestad et al., 2003).

Um estudo explorativo realizou dosagem de marcadores inflamatórios após a primeira, quinta e sétima sessões de ECT com 12 pacientes com depressão maior resistente a medicações ou depressão maior com sintomas psicóticos. Uma única sessão de ECT aumentou os níveis de IL-6 e TNF-α, mas não os de IL-10. Sessões repetidas de ECT não provocaram aumento significativo em nenhum desses parâmetros (Fluitman et al., 2011).

Um estudo recente investigou como os níveis de imunomoduladores foram afetados pela ECT em 50 pacientes com depressão resistente ao tratamento em comparação com 30 indivíduos saudáveis. Antes do tratamento, os níveis de IL-1, TNF-α, e IL-10 foram mais baixos no grupo de pacientes que no grupo controle, e os níveis de IL-4 e IFN-γ foram significativamente maiores. Após o tratamento com ECT, houve aumento de IL-1 e IL-10, e diminuição de IL-4, TNF-α e IFN-γ. Não houve variação de IL-

6. Os autores também observaram que a gravidade da depressão diminuiu após a ECT, mas nenhuma relação significativa foi determinada entre a diminuição na gravidade da depressão e variações nos níveis de imunomoduladores (Zincir et al., 2016).

Outro estudo recente investigou marcadores inflamatórios em pacientes com depressão melancólica submetidos a ECT, bem como sua resposta clínica. No início do estudo, os pacientes deprimidos melancólicos tinham níveis significativamente mais elevados de IL-6, e níveis mais baixos de Fator de Transformação do Crescimento-β (TGF-β) que os controles. Houve um aumento significativo de IL-6 uma hora após a primeira sessão de ECT, porém nem os níveis de IL-6 nem os de TGF-β normalizaram após a conclusão do ECT. Setenta por cento dos pacientes atingiram resposta com ECT e 42% atingiram remissão. Não houve correlação entre variações nos níveis de IL-6 e TGF-β e melhora clínica com a ECT. Não ocorreram variações significativas de TNF-α, IL-10 e PCR em relação a depressão melancólica ou resposta à ECT (Rush et al., 2016).

Outros efeitos imunológicos após o curso da ECT incluem um aumento no número de linfócitos ativados (Fischler et al., 1992) e uma redução nas citocinas pró-inflamatórias IL-5 e eotaxina-3 (Rotter et al., 2013). Uma diminuição aguda do número total de linfócitos e de células CD8 e CD16 foi observada uma hora após uma única sessão de ECT em pacientes com TDM (Fischler et al., 1992). Em um estudo com dez pacientes com TDM, foi observado que a ECT corrrigiu a hipofuncionalidade e a pobre imunorreatividade da proteína G de leucócitos. Essa normalização das medidas de proteína G esteve associada a uma melhora clínica dos sintomas

depressivos (Avissar et al., 1998).

Em suma, os resultados desses estudo, embora ainda limitados, indicam que a ECT pode atuar como um agente imunomodulador pela alteração dos níveis de citocinas e outros marcadores imunológicos no sangue periférico.

7. JUSTIFIVATIVA

É grande a relevância do estudo do TDM, considerando-se sua alta prevalência, cronicidade, significativa morbimortalidade, níveis de incapacidade associados e custos elevados em saúde (Ferrari et al., 2013a; Ferrari et al., 2013b; Greenberg et al., 2015; Murray e Lopez, 1997).

Junto a essas características desafiadoras, o cenário do TDM torna-se ainda mais dramático ao se constatar que boa parte dos pacientes submetidos a medicações antidepressivas não atingem a remissão de sintomas ou sequer obtêm qualquer nível de resposta clínica (Rush et al., 2006; Trivedi et al., 2006). Muitas vezes, a aplicação de outras técnicas não farmacológicas como a ECT acabam sendo necessárias (UK ECT Review Group, 2003).

Apesar de ter eficácia estabelecida para tratamento de TDM, assim como oferecer uma rápida resposta em relação às medicações antidepressivas (Kho et al., 2003; UK ECT Review Group., 2003; NICE, 2003; Pagnin et al., 2004), o uso prático da ECT é ainda restrito. Dentre as razões, é possível destacar desde efeitos colaterais cognitivos associados (Sackeim et al., 2007; Lisanby et al., 2000), a enganos e preconceitos frutos de informações errôneas e distorcidas publicadas pela imprensa leiga e pela mídia (Fink et al., 2001).

A quantidade de estudos envolvendo a ECT é ainda limitada, e, apesar da existência de algumas teorias acerca de seu mecanismo de ação, ainda não se conhece exatamente como esse método provoca a melhoria sintomatológica (Bolwig, 2011). Pesquisas envolvendo biomarcadores e ECT, além de auxiliarem na compreensão de seu mecanismo de ação, podem

ajudar no esclarecimento da patofisiologia dos transtornos de base para os quais o tratamento é empregado, como a depressão.

A patofisiologia da depressão, por sua vez, também não está claramente definida. A clássica teoria monoaminérgica tem sido questionada entre ouras razões devido à demora de algumas semanas na ação de fármacos antidepressivos apesar de um aumento imediato na neurotransmissão monoaminérgica, bem como devido à persistência de sintomas depressivos em boa parte dos indivíduos submetidos a esses tratamentos (Bilgen et al., 2014; Ruhé et al., 2007). Outras teorias, em especial a neurotrófica e a inflamatória, têm surgido como modelos patofisiológicos alternativos ou ao menos integrados à teoria das monoaminas.

A teoria neurotrófica decorre da observação de diminuições volumétricas de regiões cerebrais em indivíduos deprimidos (Videbech e Ravnkilde et al., 2004; Campbell et al., 2004), ao número crescente de ensaios clínicos que relacionam transtorno depressivo ao BDNF (Kotan et al., 2009; Bilgen et al., 2014), bem como evidências do aumento desse biomarcador com o tratamento antidepressivo (Fernandes et al., 2011; Bocchio-Chiavetto et al., 2010).

A teoria inflamatória surge da observação da semelhança do estado comportamental e subjetivo que ocorre em resposta a doenças sistêmicas infecciosas (*sickness behaviour*) com estados depressivos (Dantzer et al., 2008; Frenois et al., 2007), bem como do surgimento de sintomas de humor em pacientes expostos a citocinas inflamatórias com finalidades terapêuticas (Capuron et al., 2009; Raison et al., 2005).

Diversos estudos em modelos animais e humanos têm pesquisado a influência da ECT em neurotrofinas e marcadores inflamatórios no TDM (Rocha et al., 2016; Polyakoa et al., 2015; Brunoni et al., 2014. Em relação às neurotrofinas, apesar de existirem trabalhos mostrando associação entre aumentos de níveis de BDNF e a realização da ECT (Rocha et al., 2016; Polyakoa et al., 2015; Brunoni et al., 2014), muitos resultados são negativos (Gedge et al., 2012; Lin et al., 2013; Stelzhammer et al., 2013), em parte justificados por questões metodológicas. Os estudos relativos a marcadores inflamatórios e ECT indicam que esse método pode atuar como um agente imunomodulador (Zincir et al., 2016; Hestad et al., 2011), porém a quantidade de trabalhos publicados é ainda limitada, com amostras pequenas e com pesquisa de biomarcadores diversificados e heterogêneos.

Considerando-se esses dados ainda conflitantes, novos estudos são necessários para a investigação de neurotrofinas e marcadores inflamatórios em indivíduos submetidos a ECT por TDM. Em tais trabalhos, uma avaliação desses fatores biológicos em conjunto com a análise desfechos clínicos deve ser realizada.

8. OBJETIVOS

Objetivo geral

O objetivo desta tese de doutorado é avaliar a associação da ECT a tratamento farmacológico no TDM e suas influências nos níveis da neurotrofina BDNF, nos marcadores pró-inflamatórios IL-2, IL-6, TNF-α, IFN-γ e IL-17, nos marcadores anti-inflamatórios IL-4 e IL-10, bem como suas influências em desfechos clínicos.

Objetivos específicos

Artigo 1: Combining ECT with pharmacological treatment of depressed inpatients in a naturalistic study is not associated with serum BDNF level increase.

(1) determinar se o tratamento antidepressivo (tanto o tratamento exclusivamente farmacológico quanto o tratamento farmacológico combinado à ECT) poderia provocar variações nos níveis de BDNF em um estudo naturalístico;

(2) investigar se a combinação da ECT ao tratamento farmacológico afeta os níveis de BNDF diferentemente do tratamento exclusivamente farmacológico;

(3) comparar os níveis de BDNF nos dois grupos (grupo de tratamento exclusivamente farmacológico e tratamento farmacológico combinado à ECT) antes e depois do tratamento e com níveis de BDNF de um grupo de controles saudáveis;

(4) investigar mudanças nos níveis de BDNF em sujeitos deprimidos com

resposta clínica ao tratamento.

Artigo 2: Remission of depression following electroconvulsive therapy (ECT) is associated with higher levels of brain-derived neurotrophic factor (BDNF).

O objetivo deste estudo naturalístico foi investigar se desfechos clínicos foram associados a mudanças nos níveis de BDNF após a realização de ECT para depressão. Neste estudo, o desfecho clínico medido foi a remissão de sintomas após a ECT.

Artigo 3: Association of electroconvulsive therapy to pharmacological treatment and its influences in inflammatory biomarkers: a naturalistic study.

O objetivo deste estudo naturalístico foi comparar os níveis de marcadores inflamatórios (IL-2, IL-4, IL-6, IL-10, TNF-α, IFN-γ e IL-17) entre a admissão e a conclusão de tratamento em uma população de pacientes deprimidos usando ECT combinado a tratamento farmacológico, comparado uma população de pacientes deprimidos usando apenas tratamento farmacológico.

9. CONSIDERAÇÕES ÉTICAS

Este estudo faz parte do projeto “Avaliação e seguimento dos pacientes com doença mental severa (DMS): fatores diagnósticos, prognósticos e de tratamento e sua associação com marcadores biológicos”, um estudo de coorte naturalístico prospectivo realizado entre 2010 e 2014.

Pacientes com 18 anos de idade ou mais na admissão da internação psiquiátrica do Hospital de Clínicas de Porto Alegre entre maio de 2011 e maio de 2013 foram convidados a participar deste estudo. O termo de consentimento livre e esclarecido (TCLE) foi obtido de todos os participantes. Nos casos em que o paciente se apresentava em condições de assinar o TCLE, o termo era obtido do próprio participante. No caso em que o paciente não se apresentava em condições, um familiar acima de 18 anos era abordado para sua assinatura. Para o grupo controle, doadores de sangue foram convidados a participar do estudo, também tendo sido obtido TCLE desses participantes.

O Comitê de Ética em Pesquisa do Hospital de Clínicas de Porto Alegre aprovou o projeto (Registro no Grupo de Pesquisa e Pós-Graduação 10-0265).

10. ARTIGOS

10.1 Artigo 1

Combining ECT with pharmacological treatment of depressed inpatients in a naturalistic study is not associated with serum BDNF level increase.

Status: Publicado no “Journal of Psychiatric Research”. FI:4.465

Freire, T.F., Rocha, N.S., Fleck, M.P., 2016. Combining ECT with pharmacological treatment of depressed inpatients in a naturalistic study is not associated with serum BDNF level increase. J Psychiatr Res. 76,30-37. doi: 10.1016/j.jpsychires.2016.01.014.

Carta de Aceite:

Em 26/01/2016

Ms. Ref. No.: JPR6444R2
Title: Combining ECT with pharmacological treatment of depressed inpatients in a naturalistic study is not associated with serum BDNF level increase..
Journal of Psychiatric Research

Dear Thiago,

I am pleased to confirm that your paper, "Combining ECT with pharmacological treatment of depressed inpatients in a naturalistic study is not associated with serum BDNF level increase," has been accepted for publication in Journal of Psychiatric Research.

Your accepted manuscript will now be transferred to our production department and work will begin on creation of the proof. If we need any additional information to create the proof, we will let you know. If not, you will be contacted again in the next few days with a request to approve the proof and to complete a number of online forms that are required for publication.

Thank you for submitting your work to our journal. We look forward to future collaboration on submissions and reviews.

Sincerely,

Alan F. Schatzberg, MD
Co-Editor-in-Chief
American Editorial Office
Journal of Psychiatric Research

Versão do manuscrito aceita:

Combining ECT with pharmacological treatment of depressed inpatients in a naturalistic study is not associated with serum BDNF level increase.

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ABSTRACT

Background: BDNF blood levels are reduced in MDD. They can be increased with pharmacologic treatment and ECT, but it is not clear whether the combination of treatments promotes an additional increase. The present study aims to evaluate whether combined treatment promotes an increase in BDNF, restoring the level to that of non-depressed controls. *Methods:* Ninety-nine adult inpatients were invited to participate in this naturalistic prospective cohort study between May 2011 and April 2013. Diagnosis was made by MINI, and the symptoms were evaluated at admission and at discharge by HDRS-17. Those inpatients with a diagnosis of depression were included and divided into two groups: those who underwent combined ECT and medication (31 subjects) and those who used only pharmacotherapy (68 subjects). Serum BDNF was measured in blood samples collected at admission and discharge. One hundred healthy blood donors without any psychiatric diagnosis were included as a control group. *Results:* There were no significant differences in serum BDNF levels between the combined and pharmacological groups at admission and at discharge, and no significant variation in BDNF occurred in any group during the treatment. There were no interactions between time and treatment groups nor significant time effects or treatment group effects for BDNF in the Generalized Estimating Equation Model (GEE). The control group had significantly higher serum BDNF levels in comparison with each of the treatment groups at admission and discharge (p=0.000). *Conclusion:* Combination of ECT with pharmacological treatment did not result in increased serum BDNF levels and did not restore levels to that of controls.

KEYWORDS: ECT; depression; BDNF; pharmacotherapy

INTRODUCTION

Brain-derived neurotrophic factor (BDNF) is the most extensively evaluated neurotrophin involved in mood disorders (Krishnan and Nestler, 2010). Many studies, including meta-analyses, have shown that BDNF blood levels are reduced in major depressive disorders (Huang and Lin, 2015; Krishnan and Nestler, 2008; Brunoni et al., 2008; Sen et al., 2008; Dwivedi et al., 2009; Molendijk et al., 2013) and that the BDNF receptor tropomyosin-related kinase B (TrkB) is increased in this disorder (Hung et al., 2008). The most commonly studied polymorphism of BDNF is Val66Met (rs6265), and previous studies have associated this polymorphism with early antidepressant response (Xu et al., 2012) and remission (Taylor et al., 2011).

There is evidence that pharmacological antidepressant treatment can increase BDNF levels in patients suffering from major depressive disorder (Huang et al., 2008; Sen et al., 2008; Guilloux et al., 2012; Wolkowitz et al., 2011; Matrisciano et al., 2009; Serra-Millàs et al., 2011). Additionally, studies also suggest an increase in serum and plasma BDNF after electroconvulsive therapy — ECT (Bochio-Chiavetto et al., 2006; Marano et al., 2007; Okamano et al., 2008; Piccini et al., 2009; Hu et al., 2010; Haghighi et al., 2013; Bilgen et al., 2014; Brunoni et al., 2014).

Although there is an association of both interventions (ECT and pharmacological) with BDNF levels, few studies evaluate the combined treatments. A previous published RCT compared a group of depressive patients only receiving pharmacological treatment — in this case citalopram — to another group receiving the drug as well as ECT (combined group). Plasma BDNF increased in both groups over time, with higher plasma BDNF levels in the combined group (Haghighi et al., 2013).

Few studies have evaluated the association between BDNF levels and ECT in clinical contexts where there was a response following treatment. Okamano et al. (2008) showed that serum BDNF levels increased significantly in responders to ECT, whereas in non-responders these levels remained unchanged. Other studies had reported negative findings for the correlation between variations in depressive symptoms and BDNF levels (Fernandes et al., 2009; Haghighi et al., 2013).

Although RCTs are the gold standard model for medical studies, often their capacity for generalization is limited in clinical practice, especially in relation to treatment models with particularities such as ECT. Naturalistic studies have the advantage of better representing the 'real world', but the naturalistic design has limitations such as lack of randomization. The results of these two models complement each other. Regarding BDNF analysis in ECT, most previous studies are RCTs; therefore, it is interesting to replicate previous results using a naturalistic study design.

The objectives of this study are (1) to determine if antidepressant treatments (either an exclusive pharmacologic treatment or a pharmacologic treatment combined with ECT) could affect BDNF levels in a naturalistic study; (2) to investigate if combining ECT with pharmacological treatment affects BDNF levels differently from exclusively pharmacological treatment; (3) compare BDNF levels of these two groups before and after treatment with one measure of BDNF from normal controls; and (4) to investigate changes in BDNF levels in depressive subjects with clinical response to treatment.

Our hypotheses are (1) both groups would show an increase in BDNF levels; (2) combining ECT with pharmacological treatment would result in higher BDNF levels compared with pharmacological treatment alone; (3) the BDNF levels of the combined group would be restored to the levels of healthy controls; and (4) BDNF increases would also be present in responders to both treatments.

METHODS

The methodology involved in this study is a naturalistic prospective cohort study analyzing the effects of combining ECT with pharmacological treatment on BDNF levels of depressed inpatients, with a comparison to a healthy control group at discharge.

This study is part of a larger prospective cohort study called "Evaluation and follow-up of patients with severe mental illness (DMS): diagnostic factors, prognosis and treatment and its association with biological markers."

The Ethical and Scientific Committee of the Hospital de Clínicas de Porto Alegre approved the project (Grupo de Pesquisa e Pós-graduação register n° 10-0265) and informed consent was obtained from all inpatients.

SUBJECTS

Inpatients 18 years of age or older at admission to treatment in a psychiatric unit of the Hospital de Clínicas de Porto Alegre, Porto Alegre, Southern Brazil were invited to participate of this study between May 2011 and April 2013.

After informed consent was obtained, psychiatric diagnosis was made by psychiatrists not involved in patient care using the Mini-International Neuropsychiatric Interview (MINI) (Sheehan, et al., 1998), and symptomatology was evaluated using the Hamilton Depression Rating Scale (HDRS-17) (Hamilton, 1960). Inpatients with a diagnosis of a current depressive episode with or without psychotic features, according to MINI, were included in this study.

Inpatients were excluded if they stayed in the hospital less than seven days. Drug or alcohol addiction or dependence were also general exclusion criteria for this study. Inpatients were also excluded if they were pregnant or breast-feeding; if they had acute or chronic infectious, autoimmune, neoplastic or endocrine disease; or if they had suffered myocardial infarction or other major cardiovascular disorders in the last six months because of influences on BDNF levels.

Symptomatology evaluation was repeated one day before discharge by psychiatrists also not involved in inpatient care. A clinical response was considered to have occurred if there was an improvement in the HDRS-17 scores of >50% following treatment.

Some losses in the blood sample collection occurred throughout the study (11 samples at admission and 50 samples at discharge) caused by operational failures on admission or sudden discharges without enough time for sample collection. Such individuals were excluded (see flowchart 1). To ensure that such losses were not biased, comparisons of epidemiological data

(sex and age) and clinical scores (HDRS-17 at baseline) were made between losses and included subjects (see results).

Thirty-one subjects that had ECT combined with pharmacotherapy were included. Sixty-eight subjects receiving only pharmacotherapy were included.

Figure 1 here.

For the healthy control sample, blood donors were invited to participate in the study after screening of their medical history, a physical examination and laboratory tests in a Brazilian center of hematology (Universidade Federal do Rio Grande do Sul). Inclusion criteria for blood donors were: aged between 18 and 67 years; minimum weight of 50 kg; regular and normal blood pulse, not less than 50 nor greater than 100 beats per minute; systolic pressure not higher than 180 mmHg and diastolic blood pressure not higher than 100 mmHg; minimum values of hemoglobin and hematocrit of 12.5 g/dL and 38% for females and 13 g/dL and 39% for males. Exclusion criteria were: being pregnant or breastfeeding; drug or alcohol addiction or dependence; suffering from acute or chronic infections, autoimmunity, neoplasticism and endocrine or cardiovascular disease. After reading and freely signing an informed consent form, psychiatric screening was made by a psychiatrist not affiliated with the hematologic center, using the Mini-International Neuropsychiatric Interview (MINI) (Sheehan, et al., 1998), and the subjects were excluded if they met the diagnostic criteria for any psychiatric disorders or were using psychiatric medication at this interview.

One hundred healthy blood donors met these criteria and were included in the study as a control group. Five milliliters of blood was collected by venipuncture from each subject. This single measurement was repeatedly compared to the depressed patients' samples that were collected twice.

ELECTROCONVULSIVE THERAPY AND PSYCHOPHARMACOLOGICAL MANAGEMENT

Inpatients were referred for ECT by clinical indication of the assistant psychiatrist without interference from the researchers. Clinical indications for ECT were based on the Task Force Report of the American Psychiatric Association (APA, 2001), with primary indications (rapid or a higher probability of response is needed, severely medically ill, risk to harm themselves or others, the patient's medical status, treatment history and treatment preference) and secondary indications (lack of clinical response, intolerance of side effects, deterioration in the psychiatric condition). ECT was performed only after informed consent for the procedure was obtained.

A medical history with physical examination, routine blood examinations and electrocardiogram (ECG) were performed to screen general medical conditions before ECT. All patients received high-dose (six times the convulsive threshold), brief-pulse, right unilateral ECT using the d'Elia position (Prudic et al., 2004; Lane et al., 1989). Patients were anesthetized with thiopental (3 mg/kg intravenously) along with succinylcholine (1 mg/kg intravenously) as a muscle relaxant. The stimulus intensity was determined by the titrated strategy. The ictal response was recorded with an

electroencephalogram, and the cuff method was used to monitor motor convulsive activity. An adequate seizure was defined as a myoclonus of 20 s or longer or an electroencephalographic seizure of 25 s or longer.

ECT procedures were performed as follows: three times a week, in the morning, in an ambulatory surgical center, in the presence of a psychiatrist and an anesthesiologist and with electrocardiographic and electroencephalographic monitoring.

The number of ECT sessions was based on the clinical judgment of the assistant psychiatrist for each patient.

Psychopharmacological management was also based on the clinical decision of the assistant psychiatrist without interference from the researchers and was based on general principles such as the combination of two antidepressants, potentiation with different strategies (e.g., lithium, atypical antipsychotics) or changing to another antidepressant.

BDNF SERUM DETERMINATION

Venous blood samples (10 mL) were collected by venipuncture into a free-anticoagulant vacuum tube from each depressive subject at admission to the psychiatric unit (T0) and at discharge from the unit after the conclusion of treatment (T1). Blood samples from healthy controls were taken at the time of their evaluation. The samples were centrifuged at 4000g for 10 min and serum was stored -80 °C.

Samples were analyzed only after the completion of all collections; all samples were analyzed together, on the same date, at the same time, with the same kits.

BDNF serum levels were measured with a sandwich enzyme-linked immunosorbent assay (ELISA), using a commercial kit (EMD Millipore Corporation, Billerica, MA, USA) in accordance with the manufacturer's instructions. Briefly, microtiter plates (96-well flat bottom) were coated for 24 h at 4 °C with the samples diluted 1:100 in sample diluent and standard curve ranging from 7.8 to 500 pg of BNDF. Plates were then washed four times with wash buffer followed by the addition of biotinylated mouse anti-human BNDF monoclonal antibody (diluted 1:1000 in sample diluent) and then incubated at room temperature for 3 h. After washing, a second incubation with streptavidin-horseradish peroxidase conjugate solution (diluted 1:1000) was carried out at room temperature for 1 h. After addition of substrate and stop solution, the BDNF content was determined (absorbance set at 450 nm). The standard curve demonstrates a direct relationship between optical density (OD) and BDNF concentration. All samples were subjected to a single assay, and the results are expressed in ng/mL.

STATISTICAL ANALYSIS

The Shapiro-Wilk test was used to determine normality. For nonparametric distributions, the Wilcoxon test and the Mann–Whitney U-tests were used to compare nonrelated and related means, respectively. For parametric distributions, dependent or independent t-tests were performed. Pearson and Spearman's correlation coefficients were used to evaluate parametric and nonparametric bivariate correlations, respectively. To access differences between categorical variables, we used chi-square tests.

For repeated multivariate analysis between treatment groups, we used the method of the Generalized Estimating Equation (GEE), rather than ANOVA. The GEE model enables analysis even when the variable does not show normal distribution or sphericity (equal variances at all times and constant correlation between any two moments). Moreover, even when there is missing information of individuals in the sample, it remains possible to include all individuals, which can help to avoid some types of selection bias (Liang et al., 1986).

The Generalized Estimating Equation Model (GEE) was performed to analyse Treatment Groups (the ECT combined with pharmacotherapy group and the only pharmacotherapy group – the control group was not included), Time and BDNF as dependent variables. Potential confounders between groups were included in the analysis as covariates (continuous variables) or factors (categorical variables). Interaction Time*Treatment Groups, Time effect and Treatment Groups effect were analyzed, and if any significance was found, post hoc analyses were performed with the Bonferroni test.

For comparisons between healthy controls and treatment groups for BDNF levels and epidemiological aspects, we performed one-way ANOVA, with Tukey as post hoc analyses.

Data were expressed as the mean±SD and $p \leq 0.05$ was considered statistically significant (2-tailed). Statistical analyses were performed using the SPSS version 20.0 software package.

RESULTS

There were no significant differences between losses and included subjects in terms of HDRS-17 at admission, age or sex, confirming that losses were not subject to bias. (Table 1)

Table 1 here.

Table 2 presents epidemiologic and clinical variables — including the medication used during treatment — of the treatment groups and comparisons between them. A significant difference was found for gender (a significantly higher proportion of women in the combined group), use of selective serotonin reuptake inhibitor and use of benzodiazepines (significantly higher proportions of both in the pharmacological group), HDRS-17 at baseline (scores significantly higher in the combined group) and time of hospitalization (i.e., interval between samples collection) (more days in the combined group). These variables were included as covariates (continuous variables) or factors (categorical variables) in the GEE analysis for correction.

Comparing serum BDNF levels between the combined group and the pharmacological group, the data show that at admission there were no significant differences between the groups (combined group [n=31]: 41.39 ± 17.18 ng/mL; pharmacological group [n=68]: 48.20 ± 21.34 ng/mL; t=1.56; df=97; p=0.12). At discharge (T1), the lack of difference was maintained (combined group [n=31]: 47.01 ± 15.01; pharmacologic group [n=68]: 50.70 ± 21.87; t=0.84; df=97; p=0.39). Both the combined group and the pharmacological group had no significant increase in serum BDNF comparing baseline to discharge (combined group [n=31]: t=-1.24, df=30; p=0.22; pharmacologic group [n=68]: t=-0.68; df=67; p=0.49).

Analysis using GEE for the treatment Groups and time, using serum BDNF levels as the dependent variable, the confounder HDRS-17 at baseline and time of hospitalization (interval between samples collection) as a covariate; and gender, use of selective serotonin reuptake inhibitor and use of benzodiazepines as additional factors, did not reveal a significant interaction of treatment groups * time in a Wald Chi-Square Test (n=186; Wald Chi-Square: 0.30; df=1; p=0.58) and neither a significant time effect (n=186; Wald Chi-Square: 2.64; df=1; p=0.10) nor a treatment group effect (n=186; Wald Chi-Square: 2.88; df=1; p=0.08).

Despite the absence of significant changes in serum BDNF levels, both the combined group and the pharmacological group had significant improvements in their depression symptomatology, as measured by HDRS-17 (HDRS-17 mean in combined group at admission [n=24]: 26.04 ± 6.62; at discharge [n=24]: 8.41 ± 4.51; t=13.29; df=23; p=0.00; HDRS-17 mean in pharmacological group at admission [n=56]: 20.98 ± 7.00; at discharge [n=56]: 7.57 ± 5.26; t=13.69; df=55; p=0.00).

Of the 31 subjects in the combined group, seven had missing response data, 23 (95.8% of valid) were responders, and one (4.2% of valid) was a non-responder. From 68 subjects in the pharmacologic group, 11 had missing response data, 43 (75.6% of valid) were responders and 14 (24.6% of valid) were non-responders. A Chi-square test revealed that there was a significantly higher rate of response in the combined croup than in the pharmacologic group (χ^2=4.65; df=1; p=0.03).

Comparing serum BDNF levels between the combined group and the pharmacological group only for responders, the data shows that at admission there were no significant differences between the groups (combined group [n=23]: 43.54 ± 17.54 ng/mL; pharmacological group [n=43]: 44.91 ± 20.45 ng/mL; t=-0.27; df=64; p=0.78). At discharge, this lack of difference was maintained (combined group [n=23]: 48.65 ± 16.17; pharmacologic group [n=43]: 52.49 ± 21.31; t=-0.68; df=64; p=0.49). Both the combined group and the pharmacological group had no significant increase in serum BDNF comparing baseline to discharge (combined group [n=23]: t=-0.96, df=22, p=0.34; pharmacologic group [n=43]: t=-1.65, df=42, p=0.10).

Analysis using GEE including only responders, for the treatment groups and time, using serum BDNF levels as the dependent variable, the confounder HDRS-17 at baseline and time of hospitalization (interval between samples collection) as a covariate; and gender, use of selective serotonin reuptake inhibitor and use of benzodiazepines as additional factors, did not reveal a significant treatment groups × time interaction in a Wald Chi-Square Test (n=130; Wald Chi-Square: 0.14; df=1; p=0.70), nor a significant treatment group effect (n=130; Wald Chi-Square: 0.61; df=1; p=0.43). However, there was a trend for a time effect (n=130; Wald Chi-Square: 3.55; df=1; p=0.05).

Controls were significantly younger than the combined group (control group (n=100); the combined group (n=31) Mann-Whitney U test p=0.00) than the pharmacologic group (control group (n=100); the pharmacologic group (n=68), Mann-Whitney U test p=0.00); and depressive subjects as a whole group (control group (n=100); and all depressed subjects (n=99), Mann-Whitney U test p=0.0). Controls also had significantly more years of study than the combined group (control group (n=99); combined group (n=31)

Mann-Whitney U test p=0.09) than the pharmacologic group (control group (n=99); and combined group (n=53), Mann-Whitney U test p=0.00) than depressive subjects as a whole group (control group (n=99) and all depressed subjects (n=99) Mann-Whitney U test p=0.00). The combined group had significantly more women than the control group (control group (n of women=56), combined group (n of women=25); χ^2= 6.09; df=1; p=0.01), but there was no difference between the control group and the pharmacologic group (control group (n of women=56); pharmacologic group (n of women=36); χ^2= 0.15; df=1; p=0.75), nor between the control group and depressive subjects as a whole group (control group (n of women=56); all depressed subjects (n of women=61); χ^2= 0.648; df=1; p=0.47).

The control group had a serum BDNF mean of 62.25±20.15 ng/mL. One-way ANOVA results showed that there was a significant difference in BDNF levels between healthy controls, the combined group and the pharmacological group at admission (n=199; F=17.24; df=2; p=0.00) and at discharge (n=199; F=10.24; df=2; p=0.00). At admission, the Tukey post hoc test showed that the control group had significant higher BDNF levels than the combined group (p=0.00) and the pharmacologic group (p=0.00). At discharge, the Tukey post hoc test showed that the control group had significant higher BDNF levels than the combined group (p=0.00) and the pharmacologic group (p=0.00).

Table 2 here.

Figure 2 here.

DISCUSSION

Our naturalistic study found that the combination of ECT with pharmacological treatment was not associated with an increased serum BDNF level. We also found no difference in BDNF levels between admission and discharge either in the pharmacological group or the combined group. Neither group reached the levels of healthy controls.

In an RCT design study, Haghighi et al. (2013) compared plasma BDNF levels between a group of depressive patients receiving only 40 mg/day citalopram (control group) and another receiving the drug plus 12 sessions of ECT (target group). Plasma BDNF increased in both groups over time, with higher plasma BDNF levels in the combined group, suggesting that ECT had the effect of increasing plasma BDNF regardless of the associated medication. In our study, we did not observe this effect.

Some previous observational studies not focused on pharmacological approaches have suggested that there is no effect of ECT on BDNF levels (Grønli et al., 2009; Fernandes et al., 2009; Gedge et al., 2012; Lin et al., 2013; Stelzhammer et al., 2013), but others have found increased BDNF after ECT (Bochio-Chiavetto et al., 2006; Marano et al., 2007; Okamano et al., 2008; Piccini et al., 2009; Hu et al., 2010; Bilgen et al., 2014). A possible explanation for the lack of BDNF increase in the ECT group may be related to the time of measurement. Assessment at a longer time after conclusion of the treatment might be necessary to detect a change in BDNF level (BDNF was

measured one month after ECT conclusion in Bocchio-Chiavetto et al., 2006; two weeks after ECT conclusion in Hu et al., 2010).

Methodological issues such as different degrees of availability of BDNF in plasma and serum also need to be considered. Studies like ours that assessed serum BDNF have not found improvement after ECT treatment (Fernandes et al., 2009; Grønli et al., 2009; Gedge et al., 2012), unlike those assessing plasma BDNF (Haghighi, et al., 2013; Piccinni et al., 2009; Marano et al., 2007). Another way to interpret the absence of variation of BDNF (as the name suggests, a “brain-derived factor”) between treatment modalities is that ECT does not significantly differ from conservative treatments in the way it affects the brain, supporting the idea that ECT does not have harmful effects on the central nervous system.

We also observed that patients who received a combined treatment with ECT were predominantly women with higher HDRS-17 scores at admission and were less likely to be using SSRIs and benzodiazepines. These naturalistic results suggest that in “real life," patients receiving ECT are clinically more severe in terms of their symptoms and more refractory to medications. SSRIs are usually the first drugs used to treat depression, and the lowest use of them in the group that underwent ECT reflects the fact that the treatment was not successful in these patients. The use of fewer benzodiazepines in the group receiving ECT is likely due to these medications having anticonvulsant effects; therefore, their use has been suspended in these patients during ECT treatment.

Evidence suggests that melancholic depression subtype (a more severe clinical presentation of depression characterized by nonreactivity of mood, anhedonia and psychomotor disturbance) is more associated with biological determinants than nonmelancholic depression (Spanenberg, et al., 2014). Considering that patients undergoing ECT have more severe depression as evidenced in our study, researching changes in BDNF levels in people with melancholic depression undergoing ECT would make an interesting future study.

When analyzing the clinical outcomes, both the combined group and the pharmacological group significantly improved depression symptomatology and had high rates of response to treatment. Subjects with combined treatment had higher rates of response in comparison to exclusive pharmacologic treatment, suggesting that although ECT did not aggregate further BDNF increases to pharmacological treatment, it resulted in an adjuvant effect on the response to treatment. This finding is consistent with what is expected in clinical practice, when ECT is often indicated in patients refractory to pharmacologic treatments. Regarding BDNF measures specifically in individuals who responded to treatment, we found no differences between the treatment groups in either time point or significant increases in each group over time. These findings differ from previous studies (Okamano et al., 2008) that found BDNF levels to increase significantly in responders to ECT. Nevertheless, our study has shown that independently of treatment groups, the responder individuals as a whole group had a tendency to increase in BDNF during treatment (time effect).

Mikoteit et al., (2014, 2015) examined baseline serum BDNF levels and their variations during treatment of depressive patients using the antidepressant duloxetine and evaluating its relation with response to therapy.

They found that higher baseline levels of BDNF predicted a successful treatment outcome at the end of the study (after six weeks), but an initial increase in BDNF concentrations during treatment was not associated with treatment outcome. Mikoteit et al., (2014, 2015) also reported some unexpected findings, such as a link between more severe depression and higher baseline BDNF levels. Furthermore, an initial BDNF increase between baseline and week 2 was not stable but fell back to baseline levels. These findings suggest that changes in serum BDNF levels during antidepressant treatment are complex and dynamic. Another study by the same group (Mikoteit et al., 2015) analyzed the association between cognitive performance and serum BDNF levels in the same population. They found that a greater increase in serum BDNF was associated with a greater improvement in alertness at the end of the study.

Detecting biomarkers in psychiatric disorders such as depression is urgently needed in clinical practice. However, there are considerable limitations as regards the feasibility of detection. Thus far, no single molecule or a single pathway has been identified, and more data are clearly needed to fully assess this pathophysiology. BDNF is only one of potentially many biomarkers apparently involved, together with protein assessment, SNPs and epigenetic regulation (Huang et al., 2010; Huang at al., 2013; Huang et al., 2015). Thus, current knowledge does not allow the establishment of a clinical applicability of these markers for diagnosis or for predict treatment response in depression.

Among the limitations of this study, we can highlight the small sample size, although this number is higher than in previous work. Another limitation is the loss of subjects during the study. Nevertheless, there were no differences in the epidemiological characteristics between included and lost subjects, indicating that such losses were not subject to bias. Patients used diverse medications during treatment, but this was expected in a naturalistic study. Despite this, we found differences only in the use of SSRI and benzodiazepine and corrected for these differences in GEE analysis. A limitation of this study, however, is that we could not exclude the medications as a confounding influence on the levels of BDNF, something possible to control in RCTs.

Finally, this study compared BDNF levels between depressed patents and a population of healthy controls. Previous studies have shown that healthy control groups have higher levels of BDNF than depressive subjects at baseline (Bilgen, et al., 2014; Hu, et al., 2010; Piccini, et al., 2009). Our study showed that even after the end of treatment, regardless of the modality of treatment used, serum BDNF levels remain significantly lower in depressed individuals than in healthy controls, suggesting that in such mental disorders there are persistent biochemical differences even after apparent symptomatic improvement.

CONCLUSION

In this naturalistic study, there was no difference in terms of serum BDNF levels when ECT was combined with pharmacological treatment and no differences between admission and discharge in the pharmacological-only group or in the combined treatment group. Significant differences in serum BDNF were not found in treatment responders. The BDNF levels of

depressive inpatients at admission and at discharge were lower than in healthy controls.

More naturalistic works are needed to study biological markers such as BDNF in patients undergoing ECT. In this sense, methodologies addressing subtypes of depression more closely related to biological factors such as melancholic depression are interesting future prospects for research.

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Table 1. Comparison between losses and included subjects.

	Losses (61)	Included Subjects (99)	Test	df	*p* value
Mean age ±SD (years)	46.57 ±14.05 (n=61)	44.91 ±14.30 (n=99)	Mann-Whitney U test		0.34
Gender (female)	n=31 (50.8%)	n=61 (61.9%)	χ^2= 1.80	1	0.18
Mean HDRS-17 at baseline ±SD	23.33 ±5.88 (n=55)	22.15 ±7.16 (n=93)	t=1.00	146	0.31

SD=Standard Deviation; χ^2= Chi Square Test; df= degrees of freedom
HDRS-17=Hamilton Depression Rating Scale 17 Items

Table 2. Comparison of epidemiological and clinical variables between patients submitted to medication, ECT combined with medication and healthy controls.

	ECT and medication (n=31)	Medication (n=68)	Healthy controls (n=100)	Test	df	*p* value	Post Hoc Analysis	Post Hoc *p* value
Mean age ±SD (years)	48.32 ±15.26 (n=31)	43.35 ±13.67 (n=68)	33.65 ±10.93 (n=100)	One-way ANOVA F=21.35	2	**0.00**	Tukey	0.16[a] **0.00**[b] **0.00**[c]
Gender (female)	n=25 (80.6%)	n=36 (52.9%)	n=56 (56%)	χ^2= 6.91[a] χ^2= 6.09[b] χ^2= 0.15[c]	1[a] 1[b] 1[c]	**0.00**[a] **0.01**[b] 0.75[c]		
Mean of years of education ±SD	10.68 ±3.77 (n=19)	8.89 ±4.23 (n=53)	12.23 ±3.85 (n=99)	One-way ANOVA F=12.35		**0.00**	Tukey	0.21[a] 0.26[b] **0.00**[c]
Bipolar by MINI	n=12 (42.9%)	n=27 (41.5%)		χ^2= 0.01	1	0.54		
Psychosis by MINI	n=12 (44.4%)	n=20 (30.8%)		χ^2=1.57	1	0.23		
Mean of number of prior psychiatric hospitalization ±SD	3.42 ±4.55 (n=19)	2.64 ±4.38 (n=53)		Mann-Whitney U test		0.16		
Mean of number of ECT sessions ±SD	7.52 ± 4.80							
Mean HDRS-17 at baseline ±SD	24.59 ±7.50 (n=27)	21.11 ±6.80 (n=63)		t=2.16	91	**0.03**		
Mean in days of hospitalization (interval between samples collection) ±SD	39.61 ±32.92	24.17 ±12.35		Mann-Whitney U test	1	**0.00**		
Medication								
SSRI	n=9 (29.0%)	n=37 (54.4%)		χ^2=5.51	1	**0.01**		
SNRI	n=2 (6.5%)	n=5 (7.4%)		χ^2=0.02	1	0.87		
NDRI	n=1 (3.2%)	n=4 (5.9%)		χ^2=0.31	1	0.57		
TCA	n=2 (6.5%)	n=7 (10.3%)		χ^2=0.38	1	0.53		
TeCA	n=0 (0%)	n=2 (2.9%)		χ^2=0.93	1	0.33		

AAP	n=20 (64.5%)	n=38 (55.9%)	χ^2=0.65	1	0.41
TAP	n=8 (25.8%)	n=26 (38.2%)	χ^2=1.45	1	0.22
MS	n=8 (27.6%)	n=19 (39.6%)	χ^2=1.14	1	0.08
BDZ	n=7 (25.8%)	n=30 (44.1%)	χ^2=3.01	1	**0.00**

ECT= Electroconvulsive Therapy; SD=Standard Deviation; χ^2= Chi Square Test;
[a]=comparison between combined treatment and pharmacologic treatment
[b]=comparison between combined treatment and healthy controls
[c]=comparison between pharmacologic treatment and healthy controls
SSRI=Selective Serotonin Reuptake Inhibitor; SNRI=Serotonin and Norepinephrine Reuptake Inhibitors;
NDRI=Norepinephrine and Dopamine Reuptake Inhibitor; TCA=Tricyclic Antidepressants;
TeCA=Tetracyclic Antidepressants; AAP=Atypical Antipsychotics; TAP=Typical Antipsychotics;
MS=Mood Stabilizers; BDZ=Benzodiazepines
HDRS-17=Hamilton Depression Rating Scale 17 Items; CGI=The Clinical Global Impressions Scale

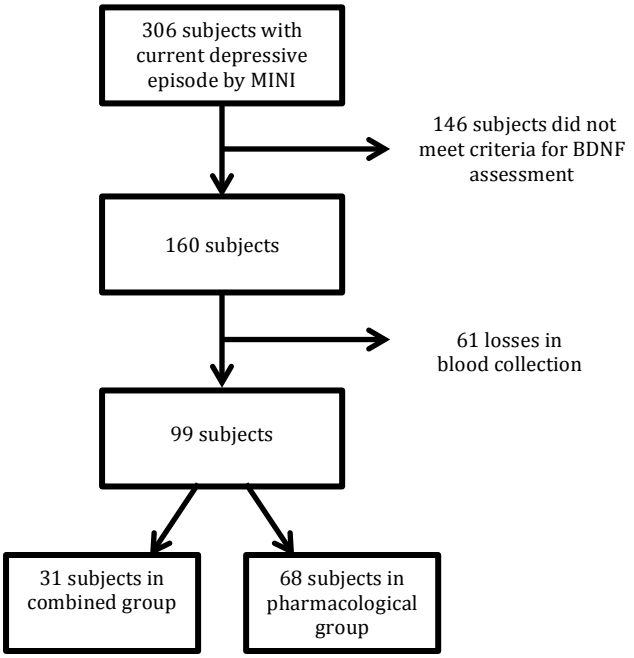
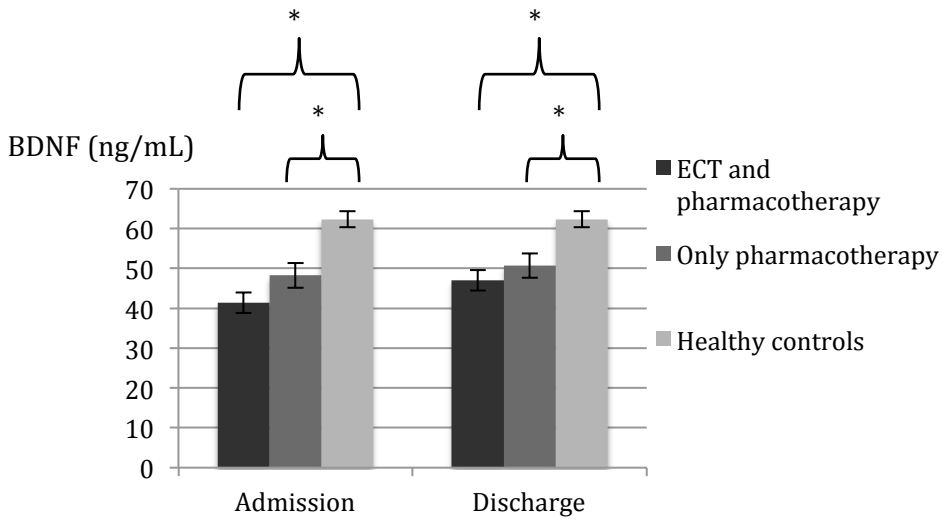


Figure 1. Flowchart of sample selection.



**** Significant differences between control group and each treatment groups were found at admission and discharge (One-way ANOVA; p=0.00)*.***

Figure 2. BDNF serum levels between treatment groups (ECT and pharmacotherapy and only pharmacotherapy) and healthy controls (Means ± Standard Error).

10.2 Artigo 2

Remission of depression following electroconvulsive therapy (ECT) is associated with higher levels of brain-derived neurotrophic factor (BDNF).

Status: Publicado no “Brain Research Bulletin”. FI:2.572

Freire, T.F., Fleck, M.P., da Rocha, N.S., 2016. Remission of depression following electroconvulsive therapy (ECT) is associated with higher levels of brain-derived neurotrophic factor (BDNF). Brain Res Bull. 121,263-269. doi: 10.1016/j.brainresbull.2016.02.013.

Carta de Aceite:

Em 12/02/2016

Ms. Ref. No.: BRB-D-15-00220R2
Title: Remission of depression following electroconvulsive therapy (ECT) is associated with higher levels of brain-derived neurotrophic factor (BDNF)
Brain Research Bulletin

Dear Mr. Freire,

I am pleased to confirm that your paper "Remission of depression following electroconvulsive therapy (ECT) is associated with higher levels of brain-derived neurotrophic factor (BDNF)" has been accepted for publication in Brain Research Bulletin.

Comments from the Editor and Reviewers can be found below.

Your accepted manuscript will now be transferred to our production department and work will begin on creation of the proof. If we need any additional information to create the proof, we will let you know. If not, you will be contacted again in the next few days with a request to approve the proof and to complete a number of online forms that are required for publication.

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With kind regards,

Ian S. Zagon, Ph.D.
Section Editor
Brain Research Bulletin

Comments from the Editors and Reviewers:

Reviewer #1: Dear Editor,
Firstly, thank you so much for invite me to review an article of BBB. The authors of manuscript BRB-D-15-00220R2 made the changes and corrections solicited in first version. teh atual version have a significant improvement, making possible the atual version to publication. Thus, in my opinion, the manuscript is able to be accepted for publication in BBB.
Thank you

Versão do manuscrito aceita:

Remission of depression following electroconvulsive therapy (ECT) is associated with higher levels of brain-derived neurotrophic factor (BDNF)

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ABSTRACT

INTRODUCTION: Research on the association between electroconvulsive therapy (ECT) and increased brain derived neurotrophic factor (BDNF) levels has produced conflicting result. There have been few studies which have evaluated BDNF levels in clinical contexts where there was remission following treatment. The objective of this study was to investigate whether remission of depression following ECT is associated with changes in BDNF levels. **METHODS:** Adult inpatients in a psychiatric unit were invited to participate in this naturalistic study. Diagnoses were made using the Mini-International Neuropsychiatric Interview (MINI) and symptoms were evaluated at admission and discharge using the Hamilton Rating Scale for Depression (HDRS-17). Thirty-one patients who received a diagnosis of depression and were subjected to ECT were included retrospectively. Clinical remission was defined as a score of less than eight on the HDRS-17 at discharge. Serum BDNF levels were measured in blood samples collected at admission and discharge with a commercial kit used in accordance with the manufacturer's instructions. **RESULTS:** Subjects HDRS-17 scores improved following ECT ($t=13.29$; $p=0.00$). A generalized estimating equation (GEE) model revealed a remission*time interaction with BDNF levels as a dependent variable in a Wald chi-square test [Wald $\chi^2=5.98$; $p=0.01$]. A post hoc Bonferroni test revealed that non-remitters had lower BDNF levels at admission than remitters ($p=0.03$), but there was no difference at discharge ($p=0.16$). **CONCLUSION:** ECT remitters had higher serum BDNF levels at admission and the level did not vary during treatment. ECT non-remitters had lower serum BDNF levels at admission, but levels increased during treatment and were similar to those of ECT remitters at discharge.

KEYWORDS: ECT, depression, BDNF, clinical remission

1 INTRODUCTION

Depression is a highly debilitating psychiatric condition that can severely reduce quality of life and may have other serious consequences, such as increasing the risk of suicide (Antunes et al., 2009). Approximately 50 percent of patients endure chronic illness and up to 20 percent have an insufficient response to drug treatments (Fava et al., 2003; Hussain and Cochrane, 2004).

Persistence of symptoms despite increases in synaptic monoamine levels following treatment with antidepressants raises questions about the 'monoamine hypothesis' of the biological etiology of depression and alternatives have emerged (Bilgen et al, 2014). Recent studies have shown that subjects with recurrent or treatment-resistant, long-lasting depressive episodes have lower hippocampal volumes (Videbech and Ravnkilde, 2004; Campbell et al., 2004), and structural changes and degeneration in hippocampus, amygdala (Bowley et al., 2002; Hamidi et al., 2004) and prefrontal cortex (Cotter et al., 2001; Rajkowska et al., 2001). These structural changes are closely related to the functioning of nerve growth factors or neurotrophic factors at cellular level.

Neurotrophic factors are intracellular molecules involved in sustaining neurons enabling them to fulfill their functions; they assist in the development and repair of neurons (Bilgen et al., 2014). Brain-derived neurotrophic factor (BDNF), which is the most studied neurotrophin, plays an important role in neurogenesis and synaptic plasticity and helps to support cell viability (Lee et al., 2002). BDNF is a protein which is mainly expressed in the central nervous system but mainly stored in blood platelets (Binder and Scharfman, 2004); serum levels are 100 times higher than plasma levels (Yamamoto and Gurney, 1990).

The 'neuroplasticity hypothesis' of depression emerged from evidence for the role of neurotrophins in brain functioning and there the number of clinical trials studying the relationship between depressive disorder and BDNF is increasing (Kotan et al., 2009; Sala et al., 2004; Bilgen et al, 2014). Several studies, including a meta-analysis, have reported that BDNF levels (serum or plasma) are low in drug-free depressive patients (Karege et al., 2002; Shimizu et al., 2003; Fernandes et al., 2011; Bocchio-Chiavetto et al., 2010; Sen et al., 2008) and increase following antidepressant treatment (Shimizu et al., 2003; Aydemir et al., 2005; Huang et al., 2008; Molendijk et al., 2011; Gönül et al., 2005; Aydemir et al., 2005; Brunoni et al., 2008; Sen et al., 2008; Guilloux et al., 2012; Wolkowitz et al., 2011; Matrisciano et al., 2009; Serra-Millàs et al., 2011). There is also evidence that BDNF receptor tropomyosin-related kinase B (TrkB) levels are elevated in depression (Hung et al., 2007). Genetic studies have demonstrated that the most commonly studied polymorphism of BDNF is Val66Met (rs6265), and this polymorphism has been associated with early antidepressant response (Xu et al., 2012) and remission (Taylor et al., 2011). Despite this research it remains unclear how BDNF is related to improvements in depression. Duman et al. (1997) hypothesized that BDNF induces neuronal sprouting in the hippocampus and cerebral cortex and improves synaptic function and the connectivity of neuronal circuits involved in mood regulation (Duman et al., 1997; Duman and Vaidya, 1998).

Electroconvulsive therapy (ECT) is the most effective treatment for

refractory depression, with effectiveness rates of between 80 and 90 percent (UK ECT Review Group, 2003), although its mechanism of action is still unknown. Rodent studies have demonstrated significant increases in BDNF mRNA (Duman et al., 1997; Nibuya et al., 1995) and BDNF protein (Altar et al., 2004) following administration of ECT to several brain areas.

Although there have been several studies in humans suggesting that BDNF levels increase following ECT (Bocchio-Chiavetto et al., 2006; Marano et al., 2007; Okamano et al., 2008; Piccini et al., 2009; Hu et al., 2010; Haghighi et al., 2013; Bilgen et al., 2014; Salehi et al., 2014), and a recent meta-analysis reached the same conclusion (Brunoni et al., 2014), there is also a considerable body of data in which there was no association (Grønli et al., 2009; Fernandes et al., 2009; Gedge et al., 2012; Lin et al., 2013; Stelzhammer et al., 2013). The differences in results may be due to methodological differences including use of medication during the study, the time lapse between the conclusion of ECT treatment and blood testing, whether BDNF was measured in serum or plasma, differences in ECT protocols etc. Given the conflicting results it is not yet possible to draw firm conclusions about the relationships between BDNF, depression and ECT. Few of the studies which reported an association between BDNF levels and ECT evaluated BDNF levels in clinical contexts where there was remission following treatment. Piccini et al. (2009) found that remitters had higher BDNF levels than non-remitters at baseline and one week after the end of ECT treatment. In relation to clinical measures other than remission, Okamano et al. (2008) found that in responders to ECT serum BDNF levels increased following treatment, whereas those of non-responders were unchanged. Other studies have, however, found no correlation between scores on depressive symptom scales and BDNF levels (Fernandes, et al., 2009; Haghighi et al., 2013). A recent investigation of the association between serum BDNF levels and clinical improvement following ECT in treatment-resistant depression (Rapinesi et al., 2015) found that in patients who had reduced symptom score following ECT serum BDNF levels were low at baseline and did not change following ECT.

The research discussed above suggests that BDNF is a potential biomarker for depression, but the evidence on whether levels are modified by pharmacotherapy and ECT and the relationship between BDNF levels and clinical outcome is unclear. The objective of this naturalistic study was to investigate whether clinical outcome are associated with changes in BDNF levels following ECT for depression. In this study the clinical outcome measured was remission of symptoms after ECT treatment. We hypothesized that patients who showed remission following ECT would have higher BDNF levels than non-remitters at baseline and at the conclusion of treatment.

2 METHODS

This was a naturalistic, prospective cohort study which analyzed differences between the serum BDNF levels of ECT remitters and non-remitters. It forms part of a larger study called ‘Evaluation and follow-up of patients with severe mental illness: Diagnostic factors, prognosis and treatment and its association with biological markers’. It received approval from the Ethical and Scientific Committee of the Hospital de Clínicas de Porto Alegre (Grupo de Pesquisa e Pós-graduação register n° 10-0265). Informed

consent to participate of the study was obtained from all participants. In cases where the patient was competent to provide consent written informed consent was provided by him or her; in the cases that patient was not competent to provide consent, a relative over 18 years old was approached to provide written consent to the patient's participation.

2.1 SUBJECTS

Patients aged at least 18 years old who were admitted to a psychiatric unit (Hospital de Clínicas de Porto Alegre, Porto Alegre, Southern Brazil) for inpatient treatment between May 2011 and April 2013 were invited to participate in this study.

After consent to participation was obtained, a psychiatric diagnosis was made by psychiatrists not involved in patient care using the MINI (Sheehan et al., 1998), and symptoms were evaluated using the 17-item version of the Hamilton Depression Rating Scale (HDRS-17; Hamilton, 1960). Inpatients diagnosed with depression, with or without psychotic features, were monitored until discharge.

Prior to discharge, inpatients were re-evaluated and excluded from the study if their hospital stay had lasted less than seven days or they were dependent on drugs or alcohol. Patients were also excluded from the study if they were pregnant or breast-feeding, or had suffered from an acute or chronic infection, autoimmune condition, neoplastic or endocrine disease, myocardial infarction or other major cardiovascular disorders in the last six months because these conditions have the potential to influence BDNF levels. Finally, we reviewed the treatment patients had received and included only those who undergone ECT.

At this point we re-evaluated the symptoms of subjects included in the study. Clinical remission was defined as a score of less than eight on the HDRS-17 (Frank et al., 1991).

2.2 ELECTROCONVULSIVE THERAPY

ECT was prescribed by the assistant psychiatrist, who was not part of the research team, and was only administered in cases where informed consent for ECT had been obtained. Where patients were not competent to provide informed consent, written consent was sought from a relative aged over 18 years.

Indications for ECT were based on the Report of the American Psychiatric Association Task Force (APA, 2001). Primary indications include the need for a rapid response or a higher probability of response than is offered by pharmacotherapies, severe medical illness, risk of harm to self or others, the patient's medical status, treatment history and treatment preference. Secondary indications are lack of clinical response, intolerance of side effects and a deterioration in psychiatric condition.

Before ECT patients were screened for general medical conditions using a physical examination, routine blood tests and an electrocardiogram (ECG).

The ECT procedure was performed as follows: Three times a week, with high-dose (six times the convulsive threshold), brief-pulse, right unilateral using the d'Elia position (Prudic et al., 2004; Lane et al., 1989). Thiopental (3

mg/kg intravenously) was used as an anesthetic and succinylcholine (1 mg/kg intravenously) as a muscle relaxant. The ictal response was recorded using electroencephalography (EEG) and an adequate seizure was defined as an EEG seizure lasting at least 25 s with a myoclonus of at least 20 s.

The number of ECT sessions for individual patients was determined by the assistant psychiatrist on the basis of individual response to treatment. Patients received concomitant psychopharmacotherapy based on the general principle of combining two antidepressants with different mechanisms of action, potentiation with different strategies (e.g. atypical antipsychotics) or changing to a different antidepressant from that the patient had been receiving prior to admission. Decisions about drug treatment were made on a purely clinical basis by an assistant psychiatrist who was not part of the research team.

2.3 BDNF SERUM LEVELS

Ten milliliters of blood were withdrawn by venipuncture into a free-anticoagulant vacuum tube from each subject on admission to the psychiatric unit (T0) before the ECT procedures described above, including the administration of anesthetic and muscle relaxant drugs; blood was also collected at discharge from the psychiatric unit after ECT treatment (T1).. The blood was centrifuged at 4000g for 10 min and serum was stored -80 °C. All samples from both time points were analysed together, on the same date, at the same time, using the same kit.

BDNF serum levels were measured with sandwich enzyme-linked immunosorbent assay (ELISA), using a commercial kit (Milipore, USA) in accordance with the manufacturer's instructions. In brief, microtiter plates (96-well; flat-bottom) were coated for 24 h at 4 °C with the samples diluted 1:100 in sample diluent and standard curve ranging from 7.8 to 500 pg of BNDF. Plates were then washed four times with wash buffer followed by the addition of biotinylated mouse anti-human BNDF monoclonal antibody (diluted 1:1000 in sample diluent), and then incubated at room temperature for 3 h. After washing, a second incubation with streptavidin-horseradish peroxidase conjugate solution (diluted 1:1000) was carried out at room temperature for 1 h. After addition of substrate and stop solution the BDNF content was determined (absorbance set at 450 nm). The standard curve demonstrates a direct relationship between optical density (OD) and BDNF concentration. All samples were subjected to a single assay and the results are expressed in ng/mL.

2.4 STATISTICAL ANALYSES

The Shapiro-Wilk test was used to determine normality. Where variables were not normally distributed the Mann–Whitney U-test was used to compare means. Dependent or independent t-tests were used to compare the means of normally distributed variables. Categorical variables were analyzed using the chi-square test.

A generalized estimating equation (GEE) model was used to analyze effects of time (admission, T0; discharge, T1), remission (binary variable; defined in terms of HDRS-17 score), and the interaction between them, with BDNF levels as the dependent variable. The Bonferroni test was used for post

hoc analyses.

Data were expressed as mean ± SD and results with $p \leq 0.05$ (2-tailed) were considered significant. Analyses were carried out using the Statistical Package for the Social Sciences (SPSS) version 20.0.

3 RESULTS

Thirty-one subjects were included in the study. Following treatment the sample showed an improvement in symptoms of depression, as measured by HDRS-17 (T0: M = 26.04 ± 6.62, T1: M = 8.41 ± 4.51; t = 13.29; p = 0.00).

Eleven subjects (35.48 percent) had missing remission data. Eleven of the remaining 21 subjects (52.38%) were remitters and 10 (47.62%) were non-remitters. In order to establish whether there were differences between participants for whom remission information was and was not available, we compared the two groups in terms of age, sex and baseline HDRS-17 score. There were no group differences in age (missing data group: M = 48.00 years, SD = 14.85; full data group: M = 48.75 years, SD = 15.40; t = 0.11; p = 0.90), or sex (missing data group: women = 5 (62.5%); full data group: women = 19 (90.5%); χ^2 = 3.17, p = 0.11). Subjects for whom remission data were not available did, however, have lower baseline HDRS-17 scores (missing data group: M = 17.80, SD = 6.61; full data group: M = 26.45, SD = 6.83; t = 2.54, p = 0.01).

There were no difference between remitters and non-remitters in terms of age, gender, diagnosis (unipolar or bipolar depression; psychosis), use of medication, baseline HDRS-17 score or number of ECT sessions (see Table 1).

Table 1 here

In ECT remitters BDNF levels at baseline and discharge were similar (T0 = 47.30 ± 20.31; T1 = 42.09 ± 11.69; t = 0.66; p = 0.52); however in non-remitters levels changed between T0 and T1 (T0 = 35.02 ± 10.99; T1 = 51.56 ± 15.98; t = -2.82; p = 0.02).

A GEE model was calculated, with time (T0; T1) and remission in terms of HDRS-17 score after ECT (yes; no) as factors; the dependent variable was BDNF level. There was a remission*time interaction for BDNF levels in the Wald chi-square test (Wald χ^2 = 5.98; p = 0.01).

A post hoc Bonferroni test confirmed that that non-remitters had lower baseline BDNF levels than remitters (p = 0.03), however at T1, i.e. following completion of treatment, levels in the two groups were similar (p = 0.16). Per group GEE analysis corroborated the results of the t-tests, indicating that BDNF levels were different at T0 and T1 in non-remitters (p = 0.00) but not in remitters (p = 0.43) (see Figure 1).

Figure 1 here

4 DISCUSSION

This naturalistic study showed that the depressive symptoms of patients who underwent ECT improved following treatment. There was a

remission by time interaction with respect to BDNF levels. Remitters had a higher mean BDNF level than non-remitters at baseline, and it did not vary during treatment. Although non-remitters had a lower mean BDNF level at baseline, it improved during treatment and at discharge was similar to that of remitters.

Only one previous study has analyzed BDNF levels and remission following ECT and like this study it found that ECT remitters had higher baseline BDNF levels than ECT non-remitters, suggesting that higher plasma BDNF levels are associated with a positive remission to treatment (Piccini et al., 2009). Taking together our results and the Piccini et al. (2009) results, a possible hypothesis could be that higher levels of BDNF at baseline could predict remission for ECT.

Piccini et al. (2009) also found that remitters' BDNF levels increased during the course of the study and thus remained different from those of non-remitters at the end of the study. In this study, however, we found that remitters' BDNF levels did not change over time although those of non-remitters did increase during treatment and were similar to those of remitters by the time of discharge. The mechanism underlying this increase in BDNF levels in the group which had a less favorable clinical outcome is unclear.

As there are few published studies of remission and BDNF levels in patients who undergo ECT for depression this discussion refers to research using other measures of clinical outcome. Analysing clinical response (improvement of 50% of symptoms in depression rating scales), Okamano et al., 2008 found that BDNF levels increased in responders to ECT in comparison to non-responders. A similar study of patients who received the antidepressant duloxetine alongside ECT (Mikoteit et al., 2014) found that higher baseline serum BDNF level predicted a positive clinical outcome at the end of the study (after 6 weeks), whereas increase in BDNF level during treatment was not associated with treatment outcome. The other unexpected finding of this study was that more severe depression was associated with higher baseline BDNF levels.

Haghighi et al. (2013) compared plasma BDNF levels in a group of patients with depression receiving citalopram at 40mg/day and another group receiving drug treatment plus 12 sessions of ECT. Plasma BDNF increased over time in both groups; the combined treatment group had a higher difference in the increase of plasma BDNF. Both groups showed similar reductions in scores on the Beck Depression Inventory scores and HDRS-17 scores following treatment.

A study which investigated the effects of ECT on serum BDNF levels in patients with treatment-resistant major depression found that HDRS-17 scores were lower and mean BDNF level was higher after ECT (Bilgen et al., 2014). Rapinesi et al. (2015) reported that in patients with treatment-resistant depression ECT reduced symptom scores, but did not affect serum BDNF levels; there was no correlation between change in serum BDNF level and changes in symptom scale scores. Serum BDNF levels were low both at baseline and after ECT. These results do not support the hypothesis that improvements in depression following ECT are mediated by increases in serum BDNF levels. Recent evidence suggests that ketamine infusions have a rapid antidepressant effect that may be tracked by changes in peripheral BDNF levels (Duncan et al., 2013; Haile et al., 2014). A study comparing

serum BDNF levels and clinical response after ketamine infusions and ECT (Allen et al., 2015) found that ketamine transiently increased serum BDNF in symptomatic responders one week after the first infusion, but subsequent infusions did not produce a similar increase nor any other response that could be related to changes in symptoms. Improvement in symptoms following ECT was not associated with changes in BDNF serum levels.

Taken together these studies fail to provide clear evidence of an association between ECT, BDNF levels and clinical outcome. BDNF is only one of a number of molecules which have been suggested to be involved in depression. The association between changes in BDNF levels and changes in symptoms is not linear (Haghighi et al., 2013); it is much more complex than was previously thought and may involve other psychoneuroendocrine mechanisms and genetic factors such as promoter methylation of the BDNF gene (Kleimann et al., 2014) or BDNF gene polymorphisms (Bousman et al., 2014). An investigation of the links between neuroendocrine pathways and psychological processes (Hibel et al., 2013) showed that cortisol secretion was not associated with symptom improvement in depressive patients. These studies suggest that if there is a link between a biological pathway and clinical outcome in depression, it is complex and is probably neither unique to depression nor universally applicable.

As one would expect in a naturalistic study, the patients in our sample were prescribed a diverse range of medications alongside their ECT. Despite evidence that antidepressants influence BDNF levels (Sen et al., 2008) we found no differences between remitters and non-remitters with respect to type of medication used (see Table 1).

An important limitation of this study is the small sample size. Previous similar studies have used similar or larger samples. To confirm the finding that remission following ECT is correlated with higher baseline BDNF level further research using a larger sample is required.

The lack of increase in BDNF levels in remitters following ECT might be due to the timing of the second evaluation. Bocchio-Chiavetto et al. (2006) found that the increase in BDNF levels only reached significance one month after completion of ECT, whereas Hu et al. (2010) demonstrated this difference after two weeks. In our study the post-treatment assessment of BDNF levels was carried out at the time of discharge and, like Grønli et al. (2009), we found no increase in BDNF levels among remitters. Stelzhammer et al. (2013) assessed BDNF levels six hours after ECT, Fernandes et al. (2009) and Lin et al. (2013) measured BDNF levels one day after the end of ECT, and Gedge et al. (2012) made their assessment one week after treatment was completed. None of these studies reported an increase in BDNF levels.

The lack of long-term, post-treatment measurements of BDNF levels are a further limitation. The missing data is a weakness of the study; subjects with missing information had lower baseline HDRS-17 scores at baseline than those for who we obtained complete data sets, but this does not appear to have affected the main findings.

5 CONCLUSION

Patients who underwent ECT for depression subsequently showed an improvement in symptoms. Patients who achieved remission of depression

following ECT had higher baseline serum BDNF levels than those who did not; their BDNF levels did not change during treatment. Although non-remitters had lower baseline BDNF levels, their levels improved during treatment. These results suggest that the relationship between BDNF, depression and clinical response to ECT is complex.

ACKNOWLEDGEMENTS

We thank the Molecular Psychiatry Laboratory in the Hospital de Clínicas de Porto Alegre for carrying out the molecular analyses. We also thank the psychiatric inpatients of the Hospital de Clínicas de Porto Alegre. We are grateful to the Grupo de Pesquisa e Pós-Graduação (GPPG) in the Hospital de Clínicas de Porto Alegre for undertaking the statistical analyses and for funding this study. We thank CAPES (Coordenação de Aperfeiçoamento Profissional de Nível Superior), CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico), FIPE/HCPA (Fundo de Incentivo à Pesquisa e Eventos do Hospital de Clínicas de Porto Alegre) and FAPERGS (Fundo de Amparo à Pesquisa do Estado do Rio Grande do Sul).

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Table 1. Demographic and clinical characteristics of remitters and non-remitters to ECT

	Remitters (n=11)	Non-remitters (n=10)	Test	*p* value
Mean age ±SD (years)	43.90±11.81	54.66±17.81	t=-1.61	0.12
Gender (female)	9 (81.81%)	10 (100%)	χ^2=0.15	0.47
Diagnosis (MINI)				
Unipolar Depression	5 (45.45%)	6 (60%)		
Bipolar Depression	5 (45.45%)	4 (40%)	χ^2=0.20	1.00
Psychotic Depression	6 (54.54%)	4 (40%)	χ^2=0.46	0.65
Medication				
SSRI	3 (27.3%)	4 (40.0%)	χ^2=0.38	0.65
SNRI	0	1 (10%)		
NDRI	1 (9.1%)	0		
TCA	0	1 (10%)		
AAP	7 (63.6%)	7 (70.0%)	χ^2=0.09	1.00
TAP	2 (18.2%)	3 (30.0%)	χ^2=0.40	0.63
MS	4 (36.4%)	2 (20.0%)	χ^2=0.68	0.63
BDZ	3 (27.3%)	4 (40.0%)	χ^2=0.38	0.65
Number of ECT sessions	8.81±2.27	8.80±2.34	Mann-Whitney U=54,00	0.94
Mean HDRS-17 in baseline ±SD	24.80±7.28	28.10±6.27	t=-1.08	0.29

ECT= Electroconvulsive Therapy; SD=Standard Deviation; χ^2= Chi Square Test;
SSRI=Selective Serotonin Reuptake Inhibitor; SNRI=Serotonin and Norepinephrine Reuptake Inhibitors;
NDRI=Norepinephrine and Dopamine Reuptake Inhibitor; TCA=Tricyclic Antidepressants;
AAP=Atypical Antipsychotics; TAP=Typical Antipsychotics;
MS=Mood Stabilizers; BDZ=Benzodiazepines
HDRS-17=Hamilton Depression Rating Scale 17 Items; CGI=The Clinical Global Impressions Scale

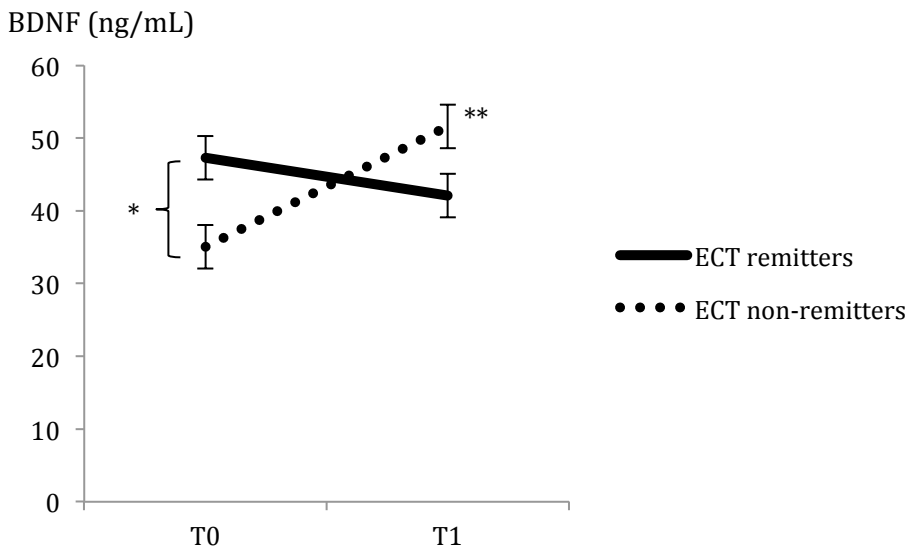

Figure 1. BDNF serum levels between ECT remitters and non-remitters - Means ± Standard Error (*p=0.03, **p=0.00)

10.3 Artigo 3

Association of electroconvulsive therapy to pharmacological treatment and its influences in inflammatory biomarkers: a naturalistic study.

Status: Submetido, e atualmente *Under Review* no “Journal of Psychiatric Research”. FI:4.465

Carta de submissão:

Em 18/07/2016

Dear editor of Journal of Psychiatric Research,

We present you the study “Association of electroconvulsive therapy to pharmacological treatment and it influences in inflammatory biomarkers: a naturalistic study”.

This is a naturalistic prospective cohort study, analyzing the effects of combining ECT with pharmacotherapy, as well as pharmacotherapy alone, on pro-inflammatory cytokines IL-2, IL-6, TNF-α, IFN-γ, and IL-17, and anti-inflammatory IL-4 and IL-10 levels of depressed inpatients.

This is the first study that suggests that the combination of repeated ECT sessions with pharmacologic treatment is associated with a significant reduction of IL-6. This biomarker also decreased with the general treatment of depression. In agreement with previous literature, IL-6 appears to be a useful marker for depression, with a close relation to ECT.

We also found an increase of TNF-α in patients undergoing ECT, and an increase of IFN-γ in depressed patients as a whole group.

Yours sincerely,

Thiago Freire
Corresponding author

Confirmação de submissão:

Em 18/07/2016

Dear Thiago,

Your submission entitled "Association of electroconvulsive therapy to pharmacological treatment and its influences in inflammatory biomarkers: a naturalistic study." has been received under the section "Original article" in Journal of Psychiatric Research

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Journal of Psychiatric Research

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Versão do manuscrito submetida:

Association of electroconvulsive therapy to pharmacological treatment and its influences in inflammatory biomarkers: a naturalistic study.

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ABSTRACT

INTRODUCTION: A growing body of evidence shows that disturbances in the immune system are involved in the pathogenesis of depression. Although immune-modulating effects of antidepressants have been described, few studies have addressed the functioning of the immune system in relation to electroconvulsive therapy (ECT). This study aims to investigate if the addition of ECT to pharmacotherapy is associated with changes in cytokines levels. **METHODS:** Adult inpatients were invited to participate in this study at admission in a psychiatric unit. Those with a diagnosis of depression by Mini-International Neuropsychiatric Interview were included. At treatment discharge, patients were retrospectively divided into those who used combined ECT and pharmacotherapy (31 subjects) and those who used only pharmacotherapy (68 subjects). Pro-inflammatory cytokines IL-2, IL-6, TNF-α, IFN-γ, and IL-17, and anti-inflammatory IL-4 and Il-10 were measured in blood samples collected at admission and discharge. Generalized Estimating Equation Model and post hoc Bonferroni test were performed for statistical analysis. **RESULTS:** Combination of ECT with pharmacotherapy was associated with a decrease of IL-6 and increase of TNF-α. Depressive inpatients, as a whole group, had a decrease of IL-6 and an increase of IFN-γ. No significant results were found for IL-2, IL-4, Il-10 and IL-17. **CONCLUSION:** As the clinical relevance of this study, we highlight that, in agreement with previous literature, IL-6 appears to be a useful marker in depression, and we show, for the first time, that its reduction is closely related to use of ECT.

KEYWORDS: ECT; depression; immunology; IL-6; TNF-α; IFN-γ.

INTRODUCTION

Electroconvulsive therapy (ECT) remains the most effective treatment option for several psychiatric conditions and is mainly used in treatment-resistant depression (UK ECT Review Group, 2003). Numerous studies show that the release of hormones associated with the hypothalamic-pituitary-adrenal (HPA) axis is altered by ECT (Haskett, 2014; Farzan et al., 2014; Bolwing, 2011) and, as such, this is one of the studied pathways underlying ECT. Studies report normalization of HPA-axis deregulation, as measured by the dexamethasone suppression test, after successful ECT treatment (Bolwing, 2011; Fink, 2005; Gibney and Drexhage, 2013).

Disturbances in certain aspects of the immune system are involved in the pathogenesis of depression (Gibney and Drexhage, 2013; Raison et al., 2006). Large-scale epidemiological studies and meta-analyses have found increased levels of pro-inflammatory mediators, in particular C-reactive protein (Wium-Andersen et al., 2013; Howren et al., 2009), interleukin-6 (IL-6) (Miller et al., 2013; Downlati et al., 2010; Kenis et al., 2002), interleukin-1 (IL-1) (Miller et al., 2013), and tumor necrosis factor α (TNF-α) (Downlati et al., 2010), in depressed patients.

Pro-inflammatory cytokines increase the catabolism of tryptophan (the precursor for serotonin synthesis), causing an accumulation of neurotoxic metabolites in the brain (De Berardis et al., 2010). Pro-inflammatory cytokines also stimulate the HPA axis, resulting in prolonged increases in circulating stress hormones in the central nervous system (Zunszain et al., 2011). In these ways, disturbances in neurotransmitter homeostasis and neuronal growth factor synthesis occur, with consequences, particularly, in the neuronal circuits of the limbic system (Miller et al., 2013).

Immune-modulating effects of antidepressants have been described (Kenis et al., 202; De Berardis et al., 2010). Recent meta-analyses indicated that treatment with antidepressants reduces the levels of pro-inflammatory cytokines (De Berardis et al., 2010; Hannestad et al., 2011) and C-reactive protein (Hiles et al., 2012). Some studies have shown acute effects of ECT in inflammatory markers. IL-6 activity was increased after a single session of ECT (Kronfol et al., 1990). Fluitman et al. (2011), likewise, demonstrated that a single session of ECT was associated with increased production of IL-6, IL-10, and TNF-α, as well as decreased production of IFN-γ, by immune cells. After a single session of ECT, plasma IL-1β and IL-6 concentrations increased over the following 3-hour time period, returning to baseline concentrations in 24 hours (Lehtimäki et al., 2008).

Although there is an acute increase of citokynes after initial application of ECT, different results have been found after repeated sessions. Hestad et al (2003) showed that, although ECT indeed increases TNF-α 1 h after the first session, repeated treatments gradually reduce TNF-α and reached levels comparable to that in healthy controls at the end of the study, whereas TNF-α concentrations during the study period remained stable in patients who received only drug treatment.

Although randomized controlled trials are the gold standard design for studies involving interventions, it is a difficult decision to do randomization in treatments such as ECT with particularities in indications (as refractoriness) and procedures. Naturalistic studies can be considered alternatives in these

cases, with better generalization capacity, representing the 'real world'. Considering that only a limited number of studies have addressed the functioning of the immune system in relation to ECT for depression, while also differentiating effects of different therapeutics strategies as pharmacologic therapy and ECT in these biomarkers, the objective of this study is to compare levels of inflammatory markers between admission and conclusion of treatment of a population of depressed inpatients using either ECT combined with pharmacological treatment or only pharmacological treatment. We choose a naturalistic design to perform this study. .

METHODS

This is a naturalistic prospective cohort study analyzing the effects of combining ECT with pharmacotherapy with those of using pharmacotherapy alone on pro-inflammatory cytokines IL-2, IL-6, TNF-α, IFN-γ, and IL-17 levels and anti-inflammatory IL-4 and IL-10 levels of depressed inpatients.

This study is part of a larger prospective cohort study called “Evaluation and follow-up of patients with severe mental illness: diagnostic factors, prognosis and treatment and its association with biological markers”.

The Ethical and Scientific Committee of Hospital de Clínicas de Porto Alegre approved the project (Grupo de Pesquisa e Pós-graduação register n° 10-0265).

SUJECTS

Between May 2011 and April 2013 inpatients of 18 years of age or more at admission of treatment in a psychiatric unit of the Hospital de Clínicas de Porto Alegre, Porto Alegre, Southern Brazil were informed about the study, invited to participate, signing informed consent if they agreed to participate.

Psychiatric diagnosis was made by psychiatrists not involved in patient care, using the Mini-International Neuropsychiatric Interview (MINI) (Sheehan et al., 1998), and, if diagnosed with depression with or without psychotic features, patients were included. Symptomatology was evaluated at admission and discharge, using Hamilton Depression Rating Scale (HDRS-17) (Hamilton, 1960).

Subjects were excluded if they stayed in the hospital for less than 7 days; were pregnant or breast-feeding; had factors that could influence cytokines levels, such as acute or chronic infectious, autoimmune, neoplastic or endocrine disease, had myocardial infarction, or other major cardiovascular disorders in the last 6 months; or had drug or alcohol addictions or dependence.

Included subjects were retrospectively subdivided into a combined group (inpatients submitted to combined ECT and pharmacological treatment) and a pharmacological group (inpatients who used only pharmacological treatment). None of the included individuals had exclusive use of ECT without medication.

ELECTROCONVULSIVE THERAPY AND PSYCHOPHARMACOLOGICAL MANAGEMENT

Patients were referred for ECT based on the clinical judgment of the assistant psychiatrist without interferences of researchers. Clinical indications of ECT included: rapid or a higher probability of response was needed, risk to patients harm themselves or others, the patient's medical status, treatment history, lack of clinical response to other treatments, intolerance of side effects with other treatments, and deterioration in the psychiatric condition (American Psychiatric Association, 2001). ECT was only performed after informed consent for the procedure was obtained.

Before ECT, a medical history, with physical examination, blood routine examinations and electrocardiogram (ECG), was requested. Firstly, patients were anesthetized with thiopental (3 mg/kg intravenously) and muscle relaxed with succinylcholine (1 mg/kg intravenously). After that, patients received a right unilateral d'Elia position high-dose (6 times the convulsive threshold) brief-pulse ECT (Prudic et al., 2004; Lane et al., 1989). Seizures were considered adequate if they involved myoclonus of at least 20 s or an electroencephalographic seizure of at least 25 s. ECT was performed three times a week, and the number of sessions was based on the clinical judgment of the assistant psychiatrist. Concomitant psychopharmacological drug use was also based on the assistant psychiatrist's decision, and was based on the general principles of potentiation with different strategies (e.g., lithium, atypical antipsychotics), combination of two antidepressants or changing to another antidepressant.

CYTOKINES SERUM DETERMINATION

Venous blood samples (5mL) were collected from each subject at psychiatric unit admission and at unit's discharge by venipuncture into a free-anticoagulant vacuum tube.

The samples were centrifuged for 4 h after the venipuncture at 3000 rpm for 5 min, and serum was kept frozen at −80 ◦C until assayed.

Serum cytokine concentrations were determined by flow cytometry using the BD™ cytometric bead array Th1/Th2/Th17 Human Cytokine kit (BD Biosciences, San Diego, CA, USA). The cytometric bead array kit allows discrimination of IL-2, IL-4, IL-6, IL-10, TNF-α, IFN-γ, and IL-17. A FACSCalibur flow cytometer (BD Biosciences) was used for sample processing and data analyses. Results were generated in graphical and tabular format using FCAP Array™ cytometric bead array analysis software (BD Biosciences).

STATISTICAL ANALYSES

A Shapiro-Wilk test was used to determine normality. For nonparametric distribution, a Wilcoxon test and Mann–Whitney U-tests were used to compare, respectively, nonrelated and related means. For parametric distributions, dependent or independent t-tests were performed. Data were expressed as mean±SD for parametric analyses, and median and interquartile range (IQR) for nonparametric tests. In order to access differences between

categorical variables, we used chi-square test. P≤0.05 was considered statistically significant (2-tailed).

Generalized Estimating Equation Model (GEE) was performed to investigate multivariate repeated measurements analyzing the variables time of blood assessment (pre and post-treatment) and treatment groups (combined ECT to pharmacotherapy, and only pharmacotherapy), using cytokines levels as dependent variable.

We used GEE rather than classical ANOVA because of the inclusion of subjects with missing data in analyses. Even when there is missing information of individuals in the sample, it remains possible to include all individuals in GEE, which can help to avoid some types of selection bias (Liang and Zeger, 1986).

GEE initially calculates the interaction between the two variables together, then calculates the effect of each variable individually. Because it is a longitudinal study, in which the change in the cytokine values over time is critical for interpretation of results, we only considered analysis including the variable time (interaction between variables including variable time and effect of variable time individually).

The Post-hoc analyses were performed with a Bonferroni test.

Statistical analyses were performed using the SPSS version 20.0 software package.

RESULTS

Thirty-one subjects submitted to ECT in combination with pharmacotherapy and sixty-eight subjects submitted only to pharmacotherapy were included.

Combined group and pharmacologic group had no differences in terms of diagnosis of bipolar depression, diagnosis of psychosis or mean of number of prior psychiatric hospitalizations, however, they had differences in gender, HDRS-17 at baseline and mean in days of hospitalization (interval between samples collection). From all medication used in the subjects, the only significant difference between groups was found in the use of selective serotonin reuptake inhibitor (SSRI) and benzodiazepines (BDZ) (see Table 1). Gender, HDRS-17 at admission, mean in days of hospitalization, use of SSRI and BDZ were included in GEE analyzes as covariates for correction.

Table 1 here.

Combined group and the pharmacological group had significant improvements in their depression symptomatology (HDRS-17 mean in combined group at admission [n=24]: 26.04 ± 6.62; at discharge [n=24]: 8.41 ± 4.51; t=13.29; p=0.00; HDRS-17 mean in pharmacological group at admission [n=56]: 20.98 ± 7.00; at discharge [n=56]: 7.57 ± 5.26; t=13.69; p=0.00).

Significant interaction treatment groups*time was found for IL-6 (p=0.05) and TNF-α (p=0.02). A post hoc Bonferroni test showed that subjects submitted to ECT combined to pharmacotherapy had significant reduction of IL-6 (p=0.02), while subjects only submitted to pharmacotherapy had no

significant variations of IL-6 (p=0.37) (see Figure 1). The post hoc Bonferroni test also showed that there was a significant increase of TNF-α in subjects submitted to ECT combined with pharmacotherapy (p=0.01), while subjects only submitted to pharmacotherapy had no significant variations of TNF-α (p=0.19).

Figure 1 here.

Analyzing variable time individually, independently of the other studied variables (time effect), there were significant results for IL-6 (p=0.00) and for IFN-γ (p=0.02). A post hoc Bonferroni test showed that all subjects (independently of the treatment used) had significant reduction of IL-6 with the treatment (p=0.00) (see Figure 2), and a significant increase of IFN-γ (p=0.03).

No significant results were found for IL-2, IL-4, IL-10 and IL-17.

Figure 2 here.

DISCUSSION

Our study is the first to show that the combination of repeated ECT sessions with pharmacologic treatment is associated with a significant reduction of IL-6. Combined group also had a significant increase of TNF-α in comparison to pharmacotherapy alone. Depressive inpatients (independently of treatment groups) had a decrease of IL-6 and an increase of IFN-γ as a result of the treatment.

Evidences suggests that elevation in peripheral blood IL-6 concentration is a common, and one of the more highly replicated, immunological finding in depression (Maes et al. 2014; Liu et al., 2012; Vogelzangs et al., 2012; Dowlati et al., 2010), and our findings show that these levels reduce with treatment. Higher IL-6 levels in cerebrospinal fluid is correlated with suicidality, and elevated IL-6 levels in plasma is correlated with non-responsiveness to conventional antidepressant drugs (Bay-Richter et al. 2015). The absence of response to antidepressants is a common indication of ECT. Intriguingly, in our study, patients who underwent ECT had more significant variations of IL-6 than patients who used only pharmacologic treatment.

Previous studies with ECT showed that, depending on the moment of the measurement of the cytokines, there may be induction or reduction of the biomarker. There is an acute and transient induction of IL-6 after a single ECT session (Hestad et al., 2003; Lehtimäki et al., 2008; Fluitman et al., 2011), however, there was no previous evidence suggesting the association of repeated ECT sessions and IL-6 reduction.

Evidence suggests that TNF-α signaling is involved in mood disorders (Dantzer et al. 2008). In patients with immunological diseases, such as rheumatoid arthritis and psoriasis, anti-TNFα treatment alleviates depressed mood independently of improvement in sickness symptoms (Krishnan et al. 2007; Tyring et al. 2006). Nevertheless, an efficacy trial of infliximab (a

monoclonal antibody against TNF-α) for depression treatment had negative results (Raison et al. 2013).

In our study, the combination of ECT with pharmacotherapy was associated with a significant increase of TNF-α in comparison to pharmacotherapy alone. Plasma TNF-α levels were correlated with depression severity and resistance to antidepressants in meta-analyses of clinical studies (Dowlati et al. 2010; Miller et al. 2009). Non-responsiveness to conventional antidepressant drugs is a common indication of ECT, and this could partly explain the higher levels of TNF-α in this group of patients, as found in our results. However, it is unclear why the ECT would have further increased the levels of this biomarker. Previous studies suggest that ECT acutely induces a transient increase in TNF-α (Hestad et al., 2003; Lehtimäki et al., 2008; Fluitman et al., 2011), but that repeated treatments gradually reduce these levels, most pronounced 1 week after the last ECT session (Hestad et al., 2003). In our study, TNF-α levels were higher after repeated ECT treatment, opposite to previous studies. A possible hypothesis for our result would be that TNF-α was accessed prior to discharge, immediately after ECT treatment conclusion, and, perhaps, an acute induction of TNF-α was more significant at this time point. Performance of assessments after longer periods following ECT conclusion (for example, after 1 week) would be required to develop a better understanding of TNF-α variation. Further studies are needed to better understand this finding.

Previous studies with IFN-γ found no differences in this level between depressive subjects and healthy controls (Spanenberg et al., 2014), however, there were lower levels in melancholic depressive patients in comparison to non-melancholic (Spanenberg et al., 2014; Rothermundt et al., 2001). In the only previous study investigating IFN-γ and ECT, Fluitman et al. (2011) found that acute ECT down-regulated pro-inflammatory IFN-γ production by T cells. There were no previous studies about the influences of repetitive ECT on IFN-γ levels. In our study we found that depressive inpatients, as a whole group, had an increase of IFN-γ with the treatment (with or without ECT).

Depression is associated with stimulus in the HPA axis (Zunszain et al., 2011), and it results in hypercortisolemia and an increase in plasma adrenocorticotropic hormone and increase catecholamines (the major stress hormones). This increase may systematically inhibit the Th1 pro-inflammatory response via IFN-γ (Calgani et al., 2006). This could explain why previous studies found lower levels of IFN-γ in melancholic depression, suggesting a decrease in cell-mediated immunity induced by hypercortisolemia (Spanenberg et al., 2014; Rothermundt et al 2001). Although we have not divided our patients into melancholic and non-melancholic groups, there is likely to be a higher prevalence of individuals with melancholy in the group that underwent ECT, due to the greater severity of their symptoms. Thus, we hypothesize that the IFN-γ increase observed in our study may have been associated with disinhibition of Th1 proinflammatory response due to depression treatment (regardless of the therapeutic method used).

Our study did not find significant variations of IL-2, IL-4, IL-10 and IL-17 in relation to treatment for depression (pharmacotherapy combined or not to ECT). A previous meta-analysis, which investigated concentrations of specific cytokines in patients diagnosed with a major depressive episode in comparison to controls, reported significantly higher concentrations of the

proinflammatory cytokines TNF-α and IL-6, but there were no significant differences in the concentrations of other proinflammatory cytokines investigated (IL-1β, IL-2, IL-8, and IFN-γ), and no significant differences in the anti-inflammatory cytokines IL-10 and IL-4 (Dowlati et al, 2010). A previous study did not find significant differences in the concentrations of IL-17 between depressive subjects and controls, and did not find variation of IL-17 levels after treatment for depression with antidepressants (Kim et al., 2003). An interesting recent study investigated the relationship between separate symptoms/single depression items and cytokines levels. At baseline, in the patient group, cytokines IL-2, IL-4, IL-5, IL-10, IL-12, IFN-γ, TNF-α and granulocyte-macrophage-colony-stimulating-factor (GM-CSF) were negatively correlated with individual Beck Depression Inventory items, factors and severities and showed both negative and positive correlations with HAMD-17 items. Four weeks following initiation of antidepressant treatment and within the controls, no such relationships were observed. The author suggest that cytokines are not generally pro-depressive but rather relate to more specific regulation of symptoms and severities in major depression (Schimidt et al., 2016).

In a recent study, Zincir et al. (2016) investigated how levels of serum immunomodulators were affected by ECT on 50 patients with treatment-resistant major depression compared with 30 healthy individuals. Before treatment, the levels of IL-1, TNF-α, and IL-10 were lower in the patient group than in the control group, and IL-4 and IFN- γ were significantly higher than in the control group. After treatment with ECT, the mean levels of IL-1 and IL-10 increased, and the mean levels of IL-4, TNF-α, and IFN- γ decreased. There was no significant difference in the levels of IL-6 before and after the treatment when compared to the control group. The authors also found that severity of major depression decreased with ECT, but no significant relationship was determined between the decrease in the severity of major depression and variations in the levels of serum immunomodulators.

Inflammatory cytokines access the brain and interact with neurotransmitter metabolism, neuroendocrine function, neural circuitry and synaptic plasticity (Borden and Parkinson, 1998), and can induce abnormalities relevant to the pathophysiology of depression, including dysregulation of glial/neuronal interactions and cognitive function (Miller et al., 2009). In a previous study, peripheral administration of lipopolysaccharide (LPS)—a cytokine inducer—resulted in cognitive impairment and increased hippocampal concentrations of TNF-α and IL-1β (Wu et al., 2007). This study also suggested that hippocampal protein levels of Brain Derived Neurothrophic factor (BDNF) were reduced by the LPS administration, and that LPS-stimulated IL-1β may be involved in the synthesis of BDNF. Lapchak et al. (1993) have showed that intraperitoneal administration of IL-1β reduced hippocampal BDNF mRNA expression in rats. Considering that there is growing evidence regarding the influence of BDNF in the pathophysiology of depression (Brunoni et al., 2008; Sen et al., 2008; Molendijk et al., 2013), our group previously investigated the BDNF levels of the subjects included in the current study (ECT combined to pharmacotherapy versus pharmacotherapy alone), also comparing this with a group of healthy controls. Combination of ECT with pharmacological treatment did not result in increased serum BDNF levels, and healthy controls had higher serum BDNF levels than depressive

subjects, even after intervention (Freire et al., 2016a). In a second study to specifically analyze BDNF levels and clinical outcomes in the patients who underwent ECT (Freire et al., 2016b), ECT remitters had higher serum BDNF levels at admission than ECT non-remitters, but there was no difference between groups at discharge.

Considering that all psychotropic medications, particularly antidepressants, have an effect on immune markers, distinguishing the impact of ECT from that of psychotropic medications on immune markers is fundamental. In this study, no significant differences in terms of psychotropic medications were found between treatment groups, except for the use of selective serotonin reuptake inhibitor, which was significantly higher in the group that used only pharmacologic treatment compared to the group that used ECT combined with pharmacologic treatment; and use of benzodiazepines, which was significantly lower in the ECT combined group. Thus, it is not possible to ignore an influence of these drugs on levels of inflammatory markers. Although GEE analysis was corrected for the use of selective serotonin reuptake inhibitor and benzodiazepines as covariates, this analysis would be more enlightening if individuals undergoing ECT without the concomitant use of drugs could be assessed. However, this is limited in clinical practice with refractory patients, where the concomitant use of psychotropic medications is often required. Factors intrinsic to the methodology of ECT may also influence inflammatory markers, such as the use of anesthetic agents, although the results available so far are scarce (Wang et al., 2011; Mitchell et al., 1990). Animal models of treatment-resistant depression treated with electroconvulsive shock (ECS) could better control the influence of such pharmacological factors in inflammatory markers, and, thus, can be seen as future prospects.

A growing body of evidence suggests that immune-mediated pathological pathways underlie the cognitive impairment related to neuropsychiatric diseases such as Alzheimer disease, and cytokines, such as IL-6 and TNF-α, have been associated with cognitive decline and dementia (McAfoose and Baune, 2009). Whereas cognitive impairments are a side effect of ECT (McClintock et al., 2014), it would be intriguing to also investigate, in future, whether ECT-induced cognitive problems are related to immune changes.

LIMITATIONS

Regarding the limitations of our study, we would like to highlight the small sample size that could result in limited statistical power, and the realization of a single measurement of inflammatory markers after the treatments conclusion. Specifically in relation to ECT, previous studies showed different results if the measures were performed 15-30 minutes after the ECT session or one week after treatment conclusion.

CONCLUSION

This is the first study that suggests that the combination of repeated ECT sessions with pharmacologic treatment is associated with a significant reduction of IL-6. This biomarker also decreased with the general treatment of

depression. In agreement with previous literature, IL-6 appears to be a useful marker for depression, with a close relation to ECT.

We also found an increase of TNF-α in patients undergoing ECT associated with pharmacologic treatment, and more studies are needed to better understand this finding. Depressive inpatients, as a whole group, had an increase of IFN-γ, probably associated with disinhibition of Th1 proinflammatory response due to depression treatment.

Further studies involving ECT and inflammatory markers should be performed, ideally with larger sample sizes, better controlling the influence of pharmacological factors and investigating cognition.

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Table 1. Comparison of clinical and epidemiological characteristics between patients submitted to pharmacologic or ECT combined treatment.

	ECT and pharmacological treatment (n=31)	Only pharmacological treatment (n=68)	Test	*p* value
Median of years (IQR)	46.00 (24)	42.00 (19)	Mann-Whitney U test	0.13
Gender (female)	25 (80.6%)	36 (52.9%)	χ^2= 6.91	**0.00**
Bipolar by MINI	n=12 (42.9%)	n=27 (41.5%)	χ^2= 0.01	0.54
Psychosis by MINI	n=12 (44.4%)	n=20 (30.8%)	χ^2=1.57	0.23
Mean of number of ECT sessions ±SD	7.52 ± 4.80			
Mean HDRS-17 at baseline ±SD	24.59 ±7.50 (n=27)	21.11 ±6.80 (n=63)	t=2.16	**0.03**
Median of days of hospitalization - interval between samples collection (IQR)	31.00 (18)	21.50 (17.8)	Mann-Whitney U test	**0.00**
Medication				
SSRI	n=9 (29.0%)	n=37 (54.4%)	χ^2= 5.51	**0.01**
SNRI	n=2 (6.5%)	n=5 (7.4%)	χ^2= 0.02	0.87
NDRI	n=1 (3.2%)	n=4 (5.9%)	χ^2=0.31	0.57
TCA	n=2 (6.5%)	n=7 (10.3%)	χ^2= 0.38	0.53
TeCA	n=0 (0%)	n=2 (2.9%)	χ^2= 0.93	0.33
AAP	n=20 (64.5%)	n=38 (55.9%)	χ^2= 0.65	0.41
TAP	n=8 (25.8%)	n=26 (38.2%)	χ^2= 1.45	0.22
MS	n=8 (27.6%)	n=19 (39.6%)	χ^2= 1.14	0.08
BDZ	n=7 (25.8%)	n=30 (44.1%)	χ^2= 3.01	**0.00**

ECT= Electroconvulsive Therapy; SD=Standard Deviation; χ^2= Chi Square Test; IQR=Interquartile Range
SSRI=Selective Serotonin Reuptake Inhibitor; SNRI=Serotonin and Norepinephrine Reuptake Inhibitors;
NDRI=Norepinephrine and Dopamine Reuptake Inhibitor; TCA=Tricyclic Antidepressants;
TeCA=Tetracyclic Antidepressants; AAP=Atypical Antipsychotics; TAP=Typical Antipsychotics;
MS=Mood Stabilizers; BDZ=Benzodiazepines
HDRS-17=Hamilton Depression Rating Scale 17 Items;

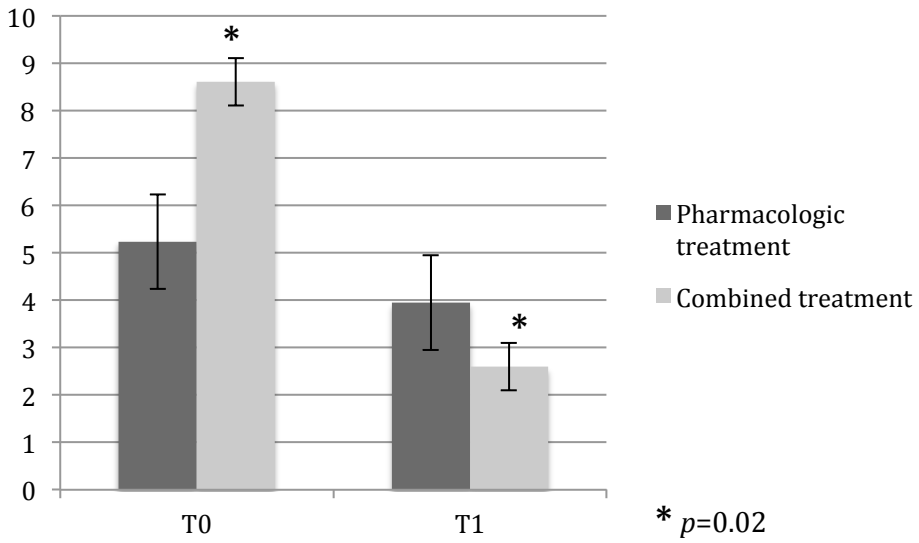

Figure 1. Comparison of IL-6 levels between treatment groups and time points – Interaction Treatment groups*Time (Values in Means ± Standard Error)

Pharmacologic treatment: subjects using only medication; Combined treatment: subjects using medication plus ECT; T1: admission; T2: discharge; Test: GEE Bonferroni post-hoc.

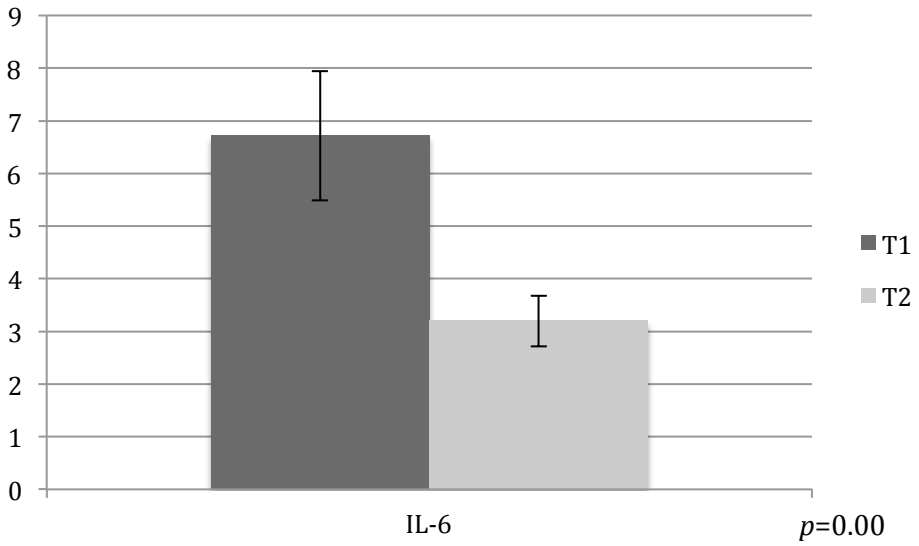

Figure 2. Comparison of IL-6 levels between time points in all subjects included disregarding treatment groups and remission - Time Effect (Values in Means ± Standard Error)

**T1: admission; T2: discharge; Test: GEE Bonferroni post-hoc.*

11. CONSIDERAÇÕES FINAIS

Esta tese de doutorado teve como resultado a produção de três artigos científicos, dois dos quais se encontram publicados e o terceiro encontra-se submetido e sob revisão.

No primeiro artigo, pudemos observar que não houve diferenças significativas nos níveis de BDNF sérico entre os grupos de tratamento combinado (ECT e farmacoterapia) e o tratamento exclusivamente farmacológico na admissão e nem na alta, e nenhuma variação significativa nos níveis de BDNF ocorreu em qualquer um dos grupos durante o tratamento. Não houve interações significativas entre a variável tempo e os grupos de tratamento, nem foi observado um efeito tempo ou efeito grupo no modelo de Equações de Estimativa Generalizada (GEE). Com isso, podemos concluir que, diferentementemente do que se esperava com base no que é sugerido pela literatura (Rocha et al., 2016; Polyakova et al., 2015; Brunoni et al., 2014), a combinação de ECT ao tratamento farmacológico não resultou em aumento dos níveis séricos de BDNF, não restaurando os níveis de BDNF a níveis semelhantes ao de controles saudáveis.

Apesar de os achados principais terem sido negativos, este estudo teve achados secundários interessantes. Ao se analisar o desfecho clínico, tanto o grupo combinado quanto o grupo farmacológico melhoraram significativamente a sintomatologia depressiva e tiveram altas altas taxas de resposta ao tratamento. Indivíduos com tratamento combinado tiveram maiores taxas de resposta ao tratamento em comparação aos que realizaram tratamento farmacológico exclusivo, sugerindo que embora a ECT não

agregue aumentos adicionais de BDNF ao tratamento farmacológico, proporcionou um efeito adjuvante na resposta ao tratamento. Este resultado é consistente com o que é esperado na prática clínica, quando ECT é frequentemente indicada em pacientes refratários aos tratamentos farmacológicos. No tocante às medidas de BDNF especificamente em indivíduos que responderam ao tratamento, não foram encontradas diferenças entre os grupos de tratamento em qualquer tempo ou aumentos significativos em cada grupo ao longo do tempo. No entanto, o nosso estudo mostrou que, independentemente dos grupos de tratamento, os indivíduos com resposta clínica tiveram uma tendência a apresentar aumentos de BDNF (efeito tempo).

Ainda no artigo 1, os pacientes que receberam tratamento combinado com ECT eram predominantemente mulheres com pontuações maiores na HDRS-17 na admissão, com menos uso de inibidores seletivos da recaptação de serotonina (ISRS) e de benzodiazepínicos (BDZ). Estes resultados naturalísticos sugerem que na "vida real", os pacientes que recebem ECT são clinicamente mais graves em termos de sintomas e mais refratários a medicamentos. Os ISRS são geralmente as primeiras drogas utilizadas para tratar a depressão, e o menor uso dessa classe antidepressiva no grupo submetido a ECT reflete o fato de que esses pacientes provavelmente já apresentaram falhas terapêuticas às opções iniciais. O uso de menos BDZ no grupo que recebeu a ECT provavelmente se deveu a efeitos anticonvulsivantes dessas medicações, justificando sua suspensão para a realização da ECT.

No segundo artigo, o modelo de GEE revelou uma interação entre as

variáveis remissão e tempo, com os níveis de BDNF como uma variável dependente. Os pacientes que atingiram remissão dos sintomas depressivos com a ECT tiveram níveis de BDNF antes do tratamento significativamente maiores do que os não remitiram, e esses valores não variaram significativamente com o tratamento. Tomando esses resultados em conjunto com os de estudos prévios (Piccini et al., 2009), uma possível hipótese seria que níveis mais elevados de BDNF poderiam prever remissão à ECT antes mesmo do sua realização.

Ainda no artigo 2, embora antes do tratamento os pacientes que não atingiram remissão tenham tido níveis significativamente inferiores de BDNF que os que tiveram remissão, esses níveis aumentaram significativamente com o tratamento, atingindo na alta níveis de BDNF semelhantes ao dos pacientes que remitiram. Estudos prévios mostram que, em pacientes que não remitem ao tratamento, os níveis de BDNF não sofrem variações significativas com o tratamento (Piccini el al., 2009). O mecanismo subjacente a este aumento observado no nosso estudo justamente nos pacientes que tiveram um resultado clinico desfavorável é incerto. Uma hipótese possível é que variações nos níveis de BDNF não estejam tão intimamente relacionados com melhoras em variáveis clínicas como previamente sugerido.

Finalmente, no terceiro artigo, a combinação da ECT com a farmacoterapia foi associada com diminuição dos níveis de IL-6 e aumento de TNF-α. Pacientes deprimidos, independetemente do grupo de tratamento ao qual foram submetidos, tiveram uma diminuição dos níveis de IL-6 e IFN-γ. Não foram observados resultados significativos para IL-2, IL-4, IL-10 e IL-17.

Níveis elevados de IL-6 configuram um dos achados imunológicos mais comuns e altamente replicados na depressão (Maes et al. 2014; Liu et al., 2012; Vogelzangs et al., 2012; Dowlati et al., 2010), e nossos resultados mostram que tais níveis diminuem com a melhora da depressão. Curiosamente, os pacientes que foram submetidos a ECT tiveram variações mais significativas de IL-6 do que os pacientes que utilizaram apenas o tratamento farmacológico. Este é o primeiro estudo que apresenta uma relação entre ECT e diminuição de IL-6.

Não está claro porque os níveis de TNF-α aumetaram com a relização da ECT. Estudos prévios têm indicado aumentos deste biomarcador na depressão (Dantzer et al., 2008; Hested et al. 2003; Lanquillon et al. 2000; Mikova et al. 2001), e o esperado seria que o tratamentos como a ECT provocasse sua redução. Há evidências mostrando, entretanto, que o TNF-α está correlacionado com a gravidade da depressão e com resistência a antidepressivos (Dowlati et al. 2010; Miller et al. 2009), a qual é uma indicação comum de ECT, e isso poderia parcialmente explicar os níveis mais altos do marcador nesses pacientes. Estudos prévios sugerem ainda que a ECT induz agudamente o aumento de TNF-α (Hestad et al., 2003; Lehtimäki et al., 2008; Fluitman et al., 2011), mas sessões repetidas de ECT gradualmente reduzem esses níveis, mais pronunciadamente após 1 semana da conclusão do tratamento (Hestad et al., 2003). Uma hipótese possível aqui é que o TNF-α tenha sido medido imediatamente após a conclusão da ECT, e talvez a indução aguda tenha sido mais pronunciada neste momento. Medidas após períodos maiores de tempo (por exemplo, após 1 semana)

poderiam ser necessárias para o melhor entendimento da variação do TNF-α. Mais estudos são necessários para compreender esse achado.

A depresão está associada e estímulos no eixo HHA (Zunszain et al., 2011), o que resulta em hipercortisolemia, aumento do hormônio corticotrópico no plasma e aumento de catecolaminas (hormônios relacionados ao estresse). Esse aumento pode sistematicamente inibir a resposta pró-inflamatória das células Th1 via INF-γ (Calgani et al., 2006). Isso pode explicar porque estudos prévios encontraram níveis mais baixos de INF-γ em depressão melancólica, sugerindo uma diminuição na imunidade celular induzida pela hipercortisolemia (Spanenberg et al., 2014; Rothermundt et al 2001). Apesar de neste estudo não termos dividido os pacientes entre aqueles com depressão melancólica e não melancólica, é provável que exista uma maior prevalência de indivíduos com depressão melancólica entre aqueles submetidos a ECT, devido à maior gravidade de seus sintomas. Assim, levantamos a hipótese de que os aumentos de IFN-γ observados em nosso estudo podem ter sido associados a desinibição da resposta pró-inflamatória Th1 devido ao tratamento da depressão (independentemente do método de tratamento utilizado).

Por fim, esta tese de doutorado traz contribuições acerca da influência da associação da ECT ao tratamento farmacológico no que concerne aos marcadores biológicos e sua relação com o desfecho clinico no TDM. Apesar da ECT não ter agregado aumentos significativos de BDNF nem ter restaurado os valores de controles saudáveis, pudemos observer que os níveis desse marcador podem funcionar como preditor de desfecho clinico favorável ao tratamento. Além disso, em consonância com a literature prévia,

a IL-6 aparece como um biomarcador útil na depressão, sendo a redução de seus níveis relacionada à ECT.

12. REFERÊNCIAS BIBLIOGRÁFICAS

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