UNIVERSIDADE FEDERAL DE MINAS GERAIS
Faculdade de Medicina
Programa de Pós-Graduação em Ciências da Saúde
Saúde da Criança e do Adolescente

Marina Silva de Lucca

Impacto do metilfenidato no estresse oxidativo e na plasticidade cerebral

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Orientadora: Prof.ª Drª. Débora Marques de Miranda.

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IMPACTO DO METILFENIDATO NO ESTRESSE OXIDATIVO E NA PLASTICIDADE CEREBRAL

MARINA SILVA DE LUCCA

Tese de Doutorado defendida no dia 19 de maio de 2023, como requisito parcial para a obtenção do grau de Doutor em CIÊNCIAS DA SAÚDE, pelo Programa de Pós-Graduação em Ciências da Saúde-Saúde da Criança e do Adolescente e **aprovada** pela Comissão Examinadora designada pelo Colegiado do Programa de Pós-Graduação supramencionado da Universidade Federal de Minas Gerais constituída pelos seguintes Professores Doutores: Márcia Helena Fávero de Souza (UFJF), Leandro Licursi de Oliveira (UFV), Marco Aurélio Romano Silva (UFMG), Bernardo de Mattos Viana (UFMG). As Professoras Débora Marques de Miranda (UFMG) e Silvia Almeida Cardoso (UFV) participaram da sessão na qualidade de orientadora e coorientadora respectivamente.

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Às crianças, em especial, à minha filha, que tentam mostrar de diversas formas do que precisam: presença, cuidado, atenção e respeito.

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RESUMO

Esse estudo avaliou o impacto do tratamento com cloridrato de metilfenidato (MFD) de liberação imediata em crianças com Transtorno de Déficit de Atenção e Hiperatividade. Incluiu metodologia variada, contendo estudo de revisão sobre efeito de metilfenidato sobre BDNF e estudo de coorte experimental. O estudo de revisão seguiu as diretrizes do PRISMA e foi registrado no PROSPERO. No estudo experimental, coorte aberta de centro único foi desenhada, com amostra de conveniência recrutada entre os anos de 2020 e 2022, no ambulatório de ensino da faculdade de Medicina da Universidade Federal de Viçosa (MG). Amostra de 62 crianças, 6 a 14 anos incompletos, sem tratamento prévio, diagnosticadas por psiquiatra infantil segundo os critérios do Manual Diagnóstico e Estatístico dos Transtornos Mentais (DSM5). Média de 8 consultas de acompanhamento clínico realizadas, com coletas de amostras biológicas em 3 delas: tempo inicial, 12 e 24 semanas após uso de MFD. Amostra caracterizada quanto a dados sociodemográficos, sintomas de TDAH, avaliações clínica, psiquiátrica e testagem de inteligência pela psicologia. Dosagens séricas de marcadores oxidativos (níveis de capacidade antioxidante total -FRAP -, atividade de superóxido dismutase – SOD-, catalase – CAT -, glutathione -S-transferase -GST-, níveis de peroxidação lipídica e de proteínas carboniladas) foram nos três tempos da avaliação. MFD foi administrado na dosagem de média de 0,65mg/kg/dia. Usou-se o teste de Shapiro-Wilk e Kolmogorov-Smirnov para análise de normalidade. Frequências absolutas e relativas foram determinadas para as variáveis numéricas que foram descritas por suas médias e desvios padrões. Para comparações múltiplas dos parâmetros oxidativos foi realizado pós teste paramétrico de Tukey e para as demais variáveis análise de variância ANOVA (*f*). Análises dos parâmetros oxidativos foram realizadas no programa GraphPad Prism 7.0 (GraphPad Software, Inc. San Diego, CA, USA) e dos dados sociodemográficos e clínicos no software SPSS (versão 23.0 para Windows). Significância estatística foi considerada com $p < 0.05$. Resultados mostraram: Sexo masculino predominante (71%), idade média 8,58 ± 1,91, mãe e/ou pai biológico como chefe de família e maior frequência de tipo combinado de TDAH. Pressão arterial sistólica, frequência cardíaca e temperatura corporal com alterações significativas, porém sem significado clínica. Índice massa corporal com diferença estatística, 37%, 19,3% e 21% das crianças apresentaram IMC acima do esperado para idade na avaliação 1, 2 e 3 respectivamente. Adesão ao tratamento medicamentoso permaneceu acima de 93,5% na 24ª semana. Durante o tratamento: FRAP não se alterou; atividade de SOD reduziu na 12ª semana em comparação à linha de base; atividade de CAT aumento significativo à 24ª em comparação 12ª semana; aumento significativo dos níveis de peroxidação lipídica à 24ª semana em comparação à 12ª semana. Aumento significativo das proteínas carboniladas na linha de base em comparação aos níveis da 12ª e 24ª semanas. O metilfenidato parece influenciar os parâmetros redox de crianças com TDAH, aumentando o estresse oxidativo, porém com redução de marcador de dano oxidativo permanente. Níveis de BDNF não foram influenciados significativamente por metilfenidato em crianças com TDAH, quando comparados a controles em nossa metanálise.

PALAVRAS-CHAVE: antioxidantes; criança; metilfenidato; oxidantes; plasticidade neuronal; transtorno do deficit de atenção com hiperatividade.

ABSTRACT

This study evaluated the impact of methylphenidate hydrochloride (MFD) treatment (MFD) in children with Attention Deficit Hyperactivity Disorder. The study included varied methodology, including a review study about methylphenidate effects on BDNF and an experimental cohort study. The review study followed the PRISMA guidelines and was registered in PROSPERO. In the experimental study, a single-center open cohort was designed, with a convenience sample recruited between the years 2020 and 2022, at the teaching outpatient clinic of the Faculty of Medicine at Viçosa Federal University (UFV-MG). Sample with 62 children, 6 to 14 years old, without previous treatment, diagnosed by a child psychiatrist according to the criteria of the Diagnostic and Statistical Manual of Mental Disorders (DSM5). Eight clinical follow-up visits were carried out, and biological samples were collected in 3 visits: before MFD beginning and after 12- and 24-weeks medication. Sociodemographic data, ADHD symptoms, clinical and psychiatric assessments were performed, as well as intelligence testing by psychology. Biological samples for oxidative markers serum dosages (total antioxidant capacity levels -FRAP -, superoxide dismutase activity - SOD-, catalase - CAT -, glutathione S transferase -GST-, lipid peroxidation and carbonyl proteins levels) were collected of each child in the 3 evaluation moments. Immediate-release methylphenidate was administered at approximately 0.65mg/kg/day. The Shapiro-Wilk and Kolmogorov-Smirnov test was used for normality analysis. Absolute and relative frequencies were used for numeric variables that were described by their means and standard deviations. Tukey's parametric test and variance analysis ANOVA (f) were performed for multiple comparisons in redox parameters and other variables respectively. Redox parameters analysis was performed using GraphPad Prism 7.0 program (GraphPad Software, Inc. San Diego, CA, USA) and other variables using SPSS software (version 23.0 for Windows). Statistical significance was considered at $p<0.05$. Male was predominant (71%), with a mean age of 8.58 ± 1.91, mother and/or biological father were the householder in most homes. Systolic blood pressure, heart rate and body temperature had significant changes, but without clinical significance. Body mass index showed a statistical difference, with 37%, 19.3% and 21% of the children having a BMI above the expected for their age in assessment 1, 2 and 3 respectively. Combined-ADHD occurred in 58.1% of the children, inattentive in 32.3% and hyperactive/impulsive in 9.7%. Drug treatment adherence was 98.4% (12th week) and 93.5% (24th week). There were no changes in FRAP levels; SOD activity had significant decreased at week 12 compared to baseline activity; CAT activity showed a significant increase at the 24^{th} week compared to 12^{th} week; Significant increase in lipid peroxidation levels at 24^{th} week compared to 12^{th} week. Significant increase in protein carbonyls levels at baseline (before methylphenidate use) compared to levels at 12 and 24 weeks. Methylphenidate can influence the oxidative and antioxidative parameters of ADHD children, increasing oxidative stress. However, buffer brain mechanisms may act and the result of these interactions in brain structure is not completely known. BDNF levels were not significantly affected by methylphenidate treatment in ADHD children and do not differ from controls in our meta-analysis.

KEYWORDS: antioxidants; children; methylphenidate; oxidants; neuronal plasticity, attention deficit hyperactivity disorder.

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SIGLAS

ADHD	-	Attention-deficit/hyperactivity disorder
ASD	-	Autism spectrum disorder
ATP	-	Adenosina trifosfato
BDNF	-	Brain-derived neurotrophic factor; fator neurotrófico derivado do cérebro
BDTD	-	Biblioteca digital brasileira de teses e dissertações
Ca^{+2}	-	Íon cálcio
CADTH	-	Canadian agency for drugs, technologies in health
CAT	-	Catalase
CID 10	-	International classification of diseases
CNPQ	-	Conselho Nacional de Desenvolvimento Científico
DECs	-	Descritores de ciência e saúde
DLD	-	Depressão de longa duração
DSM	-	Diagnostic and statistical manual of mental disorders; Manual Diagnóstico e Estatístico dos Transtornos Mentais
ERONS	-	Espécies reativas de oxigênio e nitrogênio
EROS	-	Espécies reativas de oxigênio
FAPEMIG	-	Fundação de Amparo à Pesquisa do Estado de Minas Gerais
GST	-	Glutationa S transferase
H_2O	-	Águ
H2O2	-	Peróxido de hidrogênio
HOBr	-	Ácido hipobromoso
HOCl	-	Ácido hipocloroso
ICD	-	Classification of Diseases and Related Health Problems
NOS	-	Newcastle-Ottawa scale
NOS	-	Óxido nítrico
O_2	-	Oxigênio molecular singlete
$O2^-$	-	Superóxido
ODD	-	Oppositional defiant disorder
OH^-	-	Radical hidroxila
PLD	-	Potenciação de longa duração
PRISMA	-	Preferred reporting items for systematic reviewsand meta-analyses protocols
PROSPERO	-	International prospective register of systematic reviews
ReBEC	-	REgistro Brasileiro de Ensaios Clínicos
REDCap	-	Research eletronic data capture
RO^-	-	Radical alcoxila
ROO^-	-	Radical peroxila
ROOH	-	Hidroperóxidos orgânicos
SNC	-	Sistema nervoso central
SOD	-	Superóxido dismutase
SPIRIT	-	Standard Protocol Items: Recommendations for Interventional Trials
TDAH	-	Transtorno de Déficit de Atenção e Hiperatividade

SUMÁRIO

1. INTRODUÇÃO

O Transtorno do Déficit de Atenção e Hiperatividade (TDAH) é uma condição prevalente na infância e adolescência (5%), associado ao neurodesenvolvimento, com etiologia multifatorial e complexa, cujo diagnóstico é essencialmente clínico, realizado por meio de entrevistas, exame direto da criança. Relatórios escolares, escalas, questionários e avaliações neuropsicológicas podem auxiliar na avaliação em alguns casos, não havendo exames complementares para o diagnóstico.

Além de prevalência significativa, apresenta vários desfechos negativos em nível pessoal, familiar, ocupacional e comunitário: prejuízos acadêmicos, baixa autoestima, menor funcionamento adaptativo geral, rejeição por pares, envolvimento com *bullying*, maior risco de infecções sexualmente transmissíveis, suicídio, envolvimento com criminalidade, acidentes e morte prematura principalmente por acidentes. A qualidade de vida dessas crianças sofre impacto negativo, assim como de seus pais e/ou responsáveis. Sendo que a presença de comorbidades pode impactar ainda mais nesses desfechos negativos.

Por outro lado, há evidências de que o tratamento medicamentoso com estimulantes atua não apenas na redução de sintomas apresentados com poder de resposta elevado (aproximadamente 70%), mas também modifica desfechos negativos de morbidade e mortalidade. Sendo assim, sua prescrição tem sido respaldada em evidências científicas de eficácia, segurança e impacto associando tais benefícios à possíveis modificações na plasticidade cerebral. Porém, poderíamos afirmar que realmente o

metilfenidato é capaz de modificar a plasticidade cerebral? E, se sim, seriam modificações apenas benéficas ao indivíduo? Apesar dos benefícios clínicos evidentes, estudos disponíveis até o momento são limitados na duração de tempo, quando comparados ao tempo de evolução do transtorno que tende a persistir ao longo da vida. Na França, por exemplo, a duração média do tratamento entre crianças de 6 anos em 2011 foi de 5,5 anos e as crianças mais novas foram as que receberam tratamento mais prolongados. Um quarto das prescrições e metade das renovações de prescrições foram realizadas fora das indicações governamentais, levantando outra preocupação sobre o uso do cloridrato de metilfenidato (MFD) em populações sem indicação clínica, com uso recreativo ou abusivo.

Portanto, existem lacunas de evidências se os estimulantes possuem o mesmo nível de segurança, quando usados por tempos longos. Assim como se a segurança evidenciada é a mesma para faixas etárias menos prevalentes nos estudos, como pré-escolares, adultos e idosos. Soma-se a isso o fato de o mecanismo de ação do metilfenidato possuir mecanismos parcialmente conhecidos, com estudos indicando possível participação no estado oxidativo e na neuroplasticidade.

Diante dos problemas levantados em relação ao impacto do MFD no estado oxidativo e na plasticidade cerebral, formulamos as seguintes hipóteses para essa pesquisa:

1) Hipótese nula (H0): O uso de metilfenidato por crianças e adolescentes com TDAH não interfere em marcadores oxidativos, neuro-inflamatórios e na neuroplasticidade.

2) Hipótese alternativa (H1): O uso de metilfenidato por crianças e adolescentes com TDAH interfere em marcadores oxidativos, neuro-inflamatórios e na neuroplasticidade.

A presente tese foi estruturada da seguinte forma: introdução, revisão de literatura, objetivos, métodos, resultados/discussão, considerações finais, apêndices e anexos. Os métodos do ensaio clínico foram descritos no primeiro artigo intitulado “BDNF, inflammatory and oxidative levels in treatment-naïve ADHD children treated with methylphenidate: An open cohort protocol”, submetido para a revista *European Journal of Paediatric Neurology*, apresentado item 4.1 de "Métodos”.

Os demais artigos apresentam os resultados desse estudo e estão respectivamente nos itens 5.1, 5.2 e 5.3 de “Resultados”. O artigo “Methylphenidate impacts redox status in ADHD pediatric patients” encontra-se em preparação para submissão, o artigo “Brain-derived neurotrophic factor (BDNF) levels in children and adolescents before and after stimulant use a systematic review and metanalysis” aceito e publicado na revista *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, e o artigo “Can we really modify neuroplasticity using psychostimulants?” formatado para submissão na revista *Brain Sciences*.

2. REVISÃO DE LITERATURA

2.1. Transtorno de Déficit de Atenção e Hiperatividade

O transtorno de déficit de atenção e hiperatividade (TDAH) é um transtorno do neurodesenvolvimento com prevalência aproximada de 5% em crianças e 3% em adultos. (Polanczyk, 2014) Geralmente os sintomas se tornam mais intensos por volta dos 7 anos de idade e ocorre com mais frequência em meninos do que em meninas. É diagnosticado quando os sintomas de hiperatividade, impulsividade e/ou desatenção surgem antes dos 12 anos de idade, apresentam intensidade e duração maiores do que o esperado para a faixa etária avaliada, aparecendo pelo menos em dois ambientes e trazendo prejuízos ao indivíduo, com duração de pelo menos 6 meses. (APA, 2013 e Posner J, 2020)

O TDAH pode se manifestar de formas diferentes entre os pacientes e no mesmo indivíduo ao longo do tempo. A apresentação combinada, a qual preenche os critérios diagnósticos tanto para sintomas de desatenção e hiperatividade/impulsividade é a mais prevalente, seguida pela desatenta e, por último, a hiperativa/impulsiva. Pode ainda ser classificado conforme a gravidade em leve, moderada e grave, dependendo do número de sintomas apresentados e os prejuízos/sofrimento associados. (APA, 2013 e Faraone, 2015)

Trata-se de um transtorno heterogêneo não só em seu fenótipo, mas também na sua evolução ao longo da vida. Pode evoluir com remissão parcial ou total dos sintomas, mas também com persistência dos sintomas em diversos graus. Porém os prejuízos podem persistir mesmo quando há remissão dos sintomas, uma vez que se acumularam ao longo do tempo. (Franke, 2018)

Além disso, é muito comum a comorbidade com outros transtornos mentais e problemas clínicos. Podemos citar, como exemplos, transtorno de oposição desafiante, transtorno do espectro autista, transtornos ansiosos, tiques, enurese, encoprese, transtornos depressivos, obesidade, asma, dermatite atópica e outras alergias, epilepsia e problemas de sono. A desregulação emocional não é um transtorno, mas uma característica que pode estar presente em portadores de TDAH, intensificando os prejuízos, principalmente, de socialização e resolução de problemas. (Gnanavel, 2019; Ventura, 2022)

Além da comorbidade elevada, os desfechos negativos associados ao TDAH são significativos em níveis pessoais, familiares, ocupacionais e comunitários: prejuízos acadêmicos, baixa autoestima, menor funcionamento adaptativo geral, rejeição por pares, envolvimento com *bullying*, maior risco de infecções sexualmente transmissíveis, suicídio, envolvimento com criminalidade, acidentes e morte prematura principalmente por acidentes. A qualidade de vida dessas crianças sofre impacto negativo, assim como de seus pais. Pais de crianças com TDAH tendem a ter relações mais conflituosas do que pais com filhos sem o transtorno. A presença de comorbidades pode impactar ainda mais os desfechos negativos. (Faraone, 2021)

Por outro lado, há evidências de que o tratamento medicamentoso com estimulantes atua não apenas na redução de sintomas apresentados com poder de resposta elevado (aproximadamente 70%), mas também modifica desfechos negativos de morbidade e mortalidade: menor taxa de transtornos de humor, suicídio, criminalidade, transtornos por uso de substâncias, acidentes gerais e de carro, desfechos educacionais, divórcios. (Boland, 2020 e Faraone, 2021)

A etiologia multifatorial do TDAH envolve uma herdabilidade entre 70 e 80%, além de interações entre fatores genéticos, biológicos e ambientais. A hipótese de atraso maturacional para o TDAH continua predominante e considerações da presença de uma desconectividade das redes cerebrais tem crescido. (Dutta, 2022; Kaiser, 2022 e Ohnishi, 2023) (Figura 1)

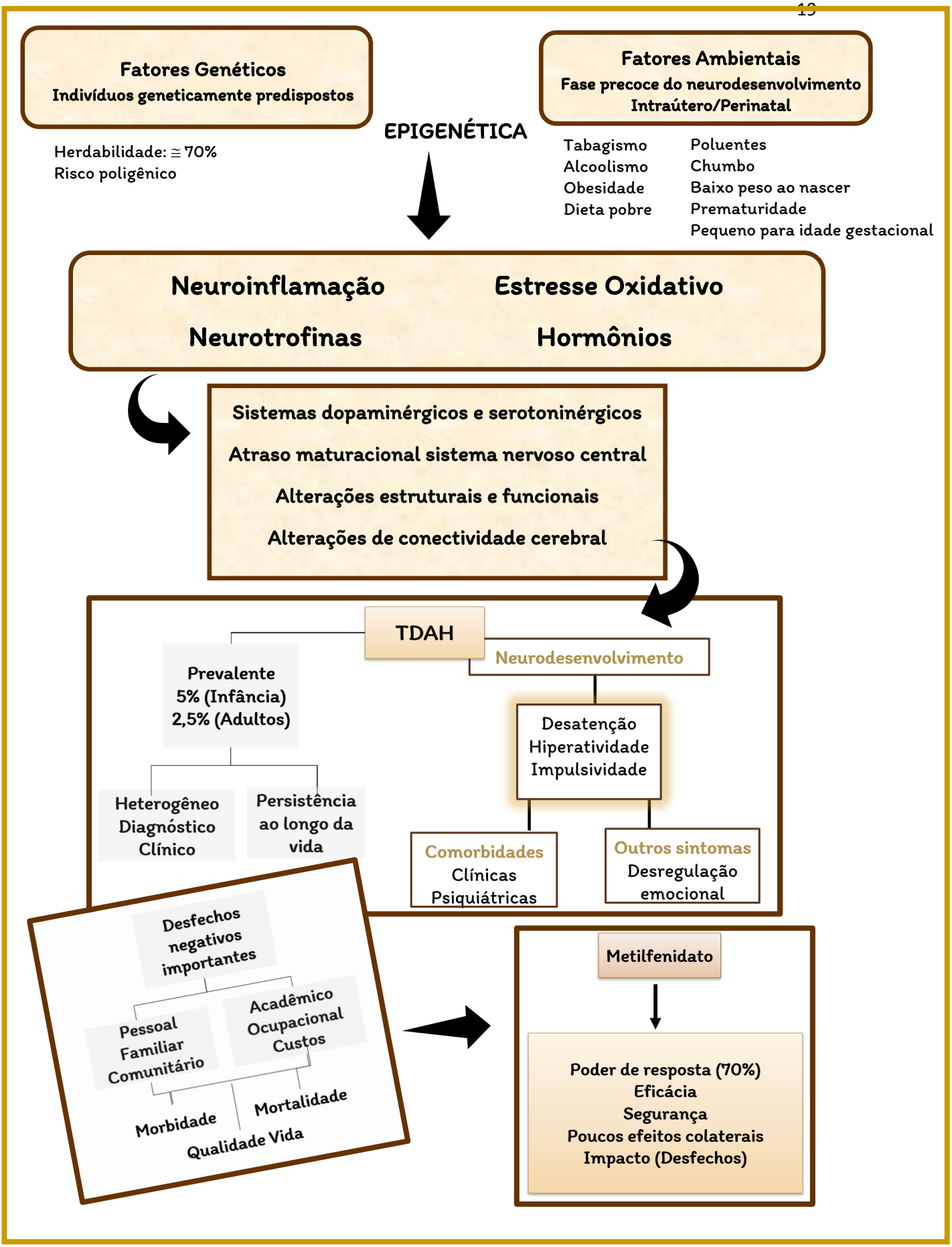

Figura 1: Descrição do TDAH. Fonte: autoria própria.

Os fatores ambientais parecem ter um importante papel na expressão do transtorno. Vários fatores de risco já foram descritos, muitos deles envolvendo o período gestacional e perinatal: baixo peso ao nascer, prematuridade, crianças pequenas para a idade gestacional, tabagismo e alcoolismo maternos, exposição a toxinas ambientais (pesticidas organofosforados e chumbo, por exemplo). (Banerjee, 2007; Faraone, 2015; Leffa, 2017 e Heyer, 2017)

Com relação à fisiopatologia, postula-se que a desregulação dos sistemas dopaminérgicos e noradrenérgicos, principalmente em circuitos cerebrais que envolvem o córtex pré-frontal, desempenha um papel crucial na patogênese do TDAH. Além disso, parâmetros oxidativos e inflamatórios, incluindo o envolvimento dos sistemas endócrino e imune, têm sido reconhecidos entre as múltiplas vias de predisposição a transtornos neuropsiquiátricos, concentrando os estudos principalmente em esquizofrenia, transtorno bipolar e transtorno do espectro autista. Alguns fatores de risco - crianças prematuras, filhos de mães tabagistas ou expostas ao chumbo e a pesticidas organofosforados - parecem aumentar o estresse oxidativo. Ou seja, distintos fatores de risco para TDAH podem ter o aumento do estresse oxidativo como via fisiopatológica comum, na predisposição do transtorno. Além disso, uma resposta inflamatória exagerada do sistema nervoso central (SNC) fetal pode estar associada ao TDAH. Inflamação e estresse oxidativo estão interconectados. (Abdollahi, 2004; Noakes, 2007; Ng, 2008; Budziszewska, 2010; Tostes, 2012ª; Ygberg, 2012; Lopes 2016; Frans, 2017; Heyer, 2017; Abdel Ghany, 2017 e Asghari, 2018)

Na gestação e no período perinatal, quando o cérebro está mais vulnerável, (Ikonomidou, 2011), o desequilíbrio redox e a inflamação correlacionada podem associar-se a desfechos negativos tanto gestacionais, quanto neonatais,

(Torres-Cuevas, 2017; Ozsurekci, 2016 e Perrone, 2023), podendo levar a danos funcionais e estruturais no sistema nervoso central (Martini, 2023) e influenciar seu desenvolvimento a curto e longo prazos. (Vasistha, 2020) Portanto, podem deixar um substrato para complicações neuropsiquiátricas ao longo da vida. (Cecile, 2019; Naoise, 2019; Carlsson, 2021 e Woolfenden, 2022)

Na maioria dos casos, o TDAH surge de um conjunto de fatores de risco ambientais e genéticos, que isoladamente têm um pequeno efeito individual e agem juntos para aumentar a susceptibilidade. E os fenótipos heterogêneos apresentados no TDAH são compatíveis com essa diversidade de possíveis fatores de risco (Faraone, 2015), mas que possuem características em comum, como a disfunção das vias dopaminérgicas e noradrenérgicas.

2.2. Tratamento farmacológico do TDAH

O tratamento com metilfenidato é indicado como primeira escolha a partir dos 6 anos de idade, principalmente quando a sintomatologia é moderada ou grave com prejuízos significativos. A eficácia de tratamentos não farmacológicos é consideravelmente menor, quando comparado aos estimulantes nessa faixa etária. (Faraone, 2021)

O metilfenidato foi sintetizado em 1944 e comercializado a partir de 1954 (Lange, 2010), sendo a medicação mais usada para o TDAH e primeira escolha na infância e adolescência. (Cortese, 2018) Embora as estimativas de prevalência global do TDAH possam ter permanecido estáveis nas últimas três décadas (aproximadamente 5% na faixa pediátrica e 2,5% adultos), as taxas de

prescrição aumentaram. Maior reconhecimento do transtorno, maior acesso ao tratamento, diagnósticos equivocados são algumas possibilidades (Polanczyk, 2014). Apesar disso, TDAH continua subdiagnosticado, principalmente em alguns grupos, como mulheres e negros. (Abdelnour, 2022)

O metilfenidato tem se mostrado eficaz, tolerável, seguro em uso a curto prazo e com baixa frequência de efeitos adversos graves. Em recente revisão, mostrou-se seguro do ponto de vista cardiovascular, sendo mais prevalente sintomas de menor gravidade, como perda de apetite e peso, problemas no sono, aumento na pressão arterial, mas com menor implicação clínica, sendo recomendável um acompanhamento próximo do tratamento. (Cortese, 2018)

Seu mecanismo de ação é parcialmente conhecido, modulando diretamente 3 alvos proteicos principais: inibidor dos transportadores de dopamina e noradrenalina e agonista do receptor serotoninérgico, envolvidos na sinalização pré-sináptica, mas com efeito mínimo neste último. Portanto, bloqueia os transportadores de dopamina e noradrenalina pré-sinápticos, aumentando os níveis desses neurotransmissores na fenda sináptica e a transmissão dessas catecolaminas. A disfunção de receptores e transportadores dopaminérgicos são a principal causa de atividade dopaminérgica alterada, podendo participar da fisiopatologia do TDAH. (Quintero, 2022)

O metilfenidato parece estar envolvido em outras vias indiretamente: adrenérgicas, glutamatérgicas. Além disso, tem sido relatada modulação de mediadores intracelulares e fatores de transcrição envolvidos na sinalização neuronal. (Quintero, 2022)

2.3. Estresse Oxidativo

O oxigênio é essencial para o ser humano, participando de sistemas enzimáticos em vários órgãos. Sistemas enzimáticos como citocromo P450, NADPH, flavina monooxigenases, ciclooxigenases, óxido nítrico sintases, prostaglandinas sintases geram espécies reativas de oxigênio (EROS) durante essas reações e promovem um estado pró-oxidante. (Shankar, 2014 e Ortis, 2017)

A respiração celular é uma das principais fontes de produção de espécies reativas de oxigênio. (Gagné, 2014 e Jîtcă, 2022) Além das EROS, outras espécies reativas importantes têm impactos notáveis na biologia redox e, conseqüentemente, no estresse oxidativo: espécies reativas de nitrogênio (por exemplo, óxido nítrico, dióxido de nitrogênio - ambos radicais livres - peroxinitrito e nitrito/nitrato); espécies reativas de enxofre (por exemplo, cisteína, metionina, glutationa, tripanotiona e micotiol), espécies reativas de carbonila (aldeídos e carbonilas eletronicamente excitadas (tripletos), espécies reativas de selênio (resíduos de seleno cisteína e seleno metionina em proteínas) e compostos de natureza quinona. (Sies, 2017 e Jîtcăl, 2022)

As espécies reativas de oxigênio podem ser divididas em: (Khoder-Agha, 2021)

- Radicais livres de oxigênio: superóxido ($O2^{-}$), radical hidroxila (OH^{-}), peroxila (ROO^{-}) e alcoxila (RO^{-}).

- Radicais não livres derivados de oxigênio: peróxido de hidrogênio (H2O2), hidroperóxidos orgânicos (ROOH, oxigênio molecular singlete (O2), ácidos hipocloro (HOCl) e hipobromoso (HOBr).

Eles são produzidos no corpo por estímulos internos (respiração celular, por exemplo) ou externos (radiação ultravioleta, atos respiratórios e alimentares, por exemplo). Os radicais livres de oxigênio são muito instáveis e podem reagir com outras moléculas muito rapidamente. (Ortis, 2017) Figure 2

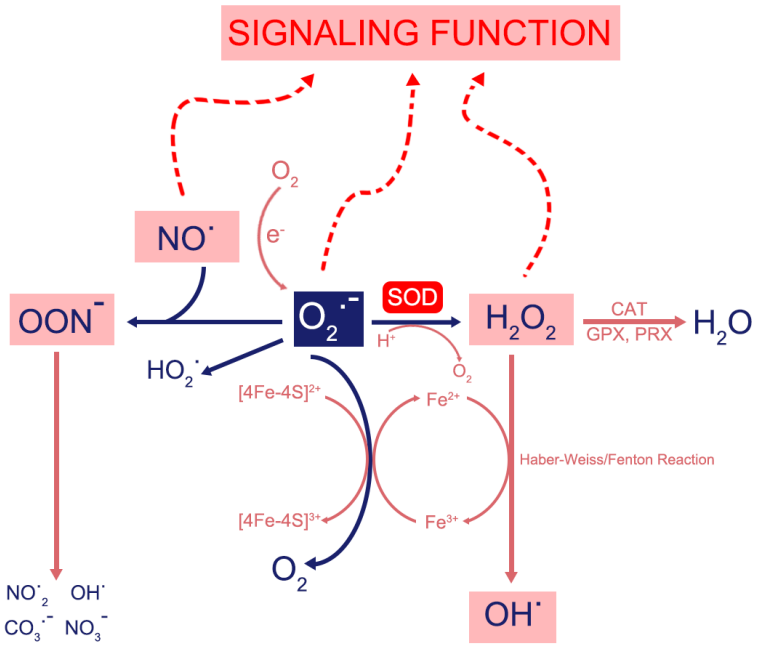

Figura 2: Dismutação do radical superóxido. Fonte: modificada de Wang (2018)

Geralmente, a homeostase intracelular de EROS é finamente controlada, fornecendo níveis extremamente baixos de radicais, sendo importante para vários processos regulatórios normais. Com um aumento do oxidante, os níveis de EROS também podem aumentar. Se a defesa antioxidante for eficiente, os níveis podem retornar ao nível de estado estacionário (intervalo de flutuação). (Ortis, 2017 e Lushchak, 2021). Quando ocorre excesso de

espécies reativas de oxigênio nas células, sobrecarregando a capacidade antioxidante, desenvolve-se estado de estresse oxidativo. (Gagné, 2014)

O estresse oxidativo pode, portanto, ser definido de forma simples como “um desequilíbrio entre oxidantes e antioxidantes em favor dos oxidantes” (Sies, 2000; Sies, 2017; Lushchak, 2021 e Angelo, 2022) Pode ser um aumento transitório (estresse oxidativo agudo) ou de longo prazo (estresse oxidativo crônico) dos níveis estacionários de espécies reativas de oxigênio e nitrogênio (ERONS). Quanto à intensidade, pode ser leve ou forte. (Lushchak, 2021), podendo ou não causar danos em biomoléculas. (Angelo, 2022)

Os processos de oxidação ligados ao oxigênio são a base de vários fenômenos fisiológicos e fisiopatológicos. (Sies, 2000 e Cadet, 2012) Desvios mais pronunciados (por exemplo, em direção à oxidação) podem, em última análise, causar danos às biomoléculas e modular e até interromper a sinalização redox fisiológica. (Sies, 2017)

A distinção entre oxidação prejudicial e benéfica é muitas vezes difícil. (Sies, 2017). Agentes oxidantes endógenos e exógenos também podem danificar o DNA celular, proteínas e lipídios por meio de espécies reativas de oxigênio. (Poulsen, 2012)

Nosso organismo possui mecanismos homeostáticos para compensar a produção de espécies reativas de oxigênio, mantendo seu funcionamento fisiológico. Portanto, mudanças no perfil oxidativo da célula desencadeiam uma cascata de eventos de sinalização para alcançar o equilíbrio novamente. Nesse processo, pode haver aumento ou redução da transcrição de genes. Esses mecanismos homeostáticos incluem defesas antioxidantes. (Shankar, 2014)

Os antioxidantes são substâncias que inibem ou retardam a velocidade das reações de oxidação e atuam impedindo a geração de radicais livres ou interceptando os radicais gerados. (Desai, 2014) Antioxidantes enzimáticos são a primeira linha de defesa ao estresse oxidativo, trabalhando em conjunto para evitar o acúmulo de radical superóxido e possíveis danos. (Ighodaro, 2018) (Figura 3)

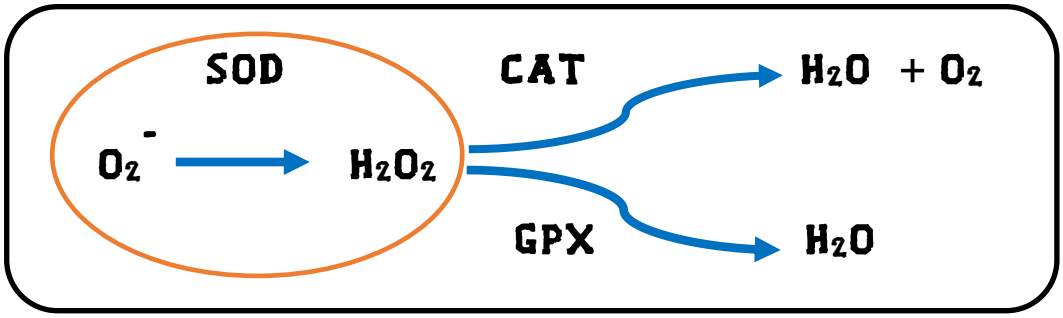

Figure 3. Primeira linha de defesa antioxidante enzimática: SOD, CAT e GPX. Fonte: autoria própria.

Transferência eficiente de elétrons na cadeia respiratória, ligações de metais de transição a proteínas, remoção de peróxidos para evitar a ligação a íons de metais de transição para formar radicais livres reativos são exemplos de defesa preventiva. A catalase (CAT), em organelas celulares especializadas, e a glutationa peroxidase (GSH-Px), nas membranas celulares, também são categorias de defesa preventiva. A catalase desintoxica o peróxido de hidrogênio e a glutationa peroxidase é ativa tanto para o peróxido de hidrogênio quanto para os hidroperóxidos de ácidos graxos. A superóxido dismutase (SOD), no citoplasma celular e mitocôndrias, e a-tocoferol são bons exemplos de

depuradores enzimáticos e não enzimáticos, respetivamente. (Desai, 2014; Gagne, 2014 e Ortis 2017)

Alguns minerais dietéticos são essenciais para a função das enzimas antioxidantes (cobre, zinco, manganês e selênio como cofatores). O sistema de reparo remove as biomoléculas danificadas antes que o metabolismo celular ou a viabilidade celular sejam alterados devido ao acúmulo de espécies reativas de oxigênio. (Desai, 2014; Gagne, 2014 e Ortis 2017)

2.4. TDAH, Metilfenidato e Estresse Oxidativo

O desequilíbrio redox em distúrbios psiquiátricos já foi correlacionado à fisiopatologia da depressão e transtorno bipolar (Kotzaeroglou, 2022), esquizofrenia (Jiao, 2022 e Rambaud, 2022), transtorno do espectro autista (Usui, 2023) e transtorno de atenção e hiperatividade/impulsividade (TDAH) (Ceylan, 2010; Oztop, 2012; Ceylan, 2012; Kul, 2015; Avcil, 2019 e Miniksar, 2023), mas os resultados ainda são indefinidos.

Estudos transversais em humanos sugerem aumento do estresse oxidativo em crianças com TDAH não medicadas quando comparadas com crianças saudáveis. (Ceylan, 2010; Oztop, 2012; Ceylan, 2012; Kul, 2015; Avcil, 2019 e Miniksar, 2023). Miniksar (2023) encontrou aumento do estresse oxidativo em crianças com TDAH medicadas (metilfenidato, atomoxetina) em comparação com crianças com TDAH não medicadas e com sujeitos do grupo controle, mas os resultados são preliminares e não conclusivos. (Tabela 1)

Tabela 1. Achados em estudos com humanos.

Study	Findings
Ceylan M et al., 2010 Cross-sectional study Maybe suggested that oxidative stress is related to ADHD	35 ADHD children and 35 healthy volunteer controls; (7-15 years); without psychotropic drugs in the previous six months. ADHD subtype: 57% (combined), 40% (inattentive) and 3% (hyperactive/impulsive). **MDA level, NO ADHD > Control group.** **GSH-Px activity ADHD < Control group.** CAT and SOD without significant changes.
Ostop D et al., 2012 Cross-sectional May not be direct association between oxidative stress and ADHD children.	30 ADHD children (no medication, no previous diagnoses) and 30 health children control. (6-12 years) **MDA level ADHD < Control group.**
Ceylan MF et al., 2012 Cross-sectional Changes in oxidative metabolism in ADHD children	35 ADHD children and 35 healthy volunteer controls; (7-15 years); without psychotropic drugs in the previous six months. ADHD subtype: 57% (combined), 40% (inattentive) and 3% (hyperactive/impulsive). **NOS level ADHD > Control group (oxidant)** **Xantina oxidase ADHD > Control group (oxidant)** **Adenosine adeaminase ADHD > Control group (oxidant)** **GST ADHD < Control group (antioxidant)** **Paraoxonase 1 ADHD < control group (antioxidant)**
Kul Mulsum et al., 2015 Cross-sectional Increase in oxidant and a decrease in antioxidants levels in ADHD children.	48 ADHD children and 24 health control; (7-18 years); without medication in the previous week and 6 weeks for ADHD e control group respectively. **Total oxidant status ADHD > Control group** **Oxidative stress index ADHD > Control group** **Total antioxidant status ADHD < Control group** **Total antioxidant status ADHD + ODD < ADHD without ODD**
Avcil S et al., 2019 Cross sectional Increased oxidative and nitrosative stress and impaired oxidant-antioxidant balance in ADHD children	103 ADHD drug-naïve children and 73 health control; (±9,5 years) ADHD combined: 59,2% inattentive 33% and hyperactive/impulsive 7,8% **Melatonin ADHD > Control group** **Nitric oxide ADHD > Control group** **Nitric oxide/melatonin ADHD < Control group** **MDA/melatonin ADHD < Control group**
Miniksar D Y et al., 2023 Cross-sectional Oxidative stress increased in patients with ADHD. High MDA level and low SOD activity are predictors of ADHD diagnosis in children and adolescents.	51 ADHD children and 32 health control; (7-18 years). Total antioxidant status ADHD no difference **Total oxidant status ADHD > Control group** **Oxidative status index ADHD > Control group** **MDA ADHD > Control group** **SOD ADHD < Control group** **Oxidative stress ADHD medication (total oxidative status) > ADHD no medication**

Estudos em modelos murinos também avaliaram os efeitos do metilfenidato no desequilíbrio redox. Em geral, encontraram alterações em parâmetros oxidativos principalmente em animais jovens, mas os resultados são

diversos e inconclusivos. (Fagundes, 2007; Gomes, 2007; Gomes, 2009; Schmitz, 2011; Schmitz, 2012; Comim, 2014; Loureiro-Vieira, 2018 e Foschiera, 2022) (Tabela 2) Foschiera et al., (2022) fizeram uma revisão de estudos animais nessa área e concluíram que o metilfenidato é capaz de desencadear estresse oxidativo em modelo animal de TDAH e em murinos saudáveis. Eles sugeriram que o metabolismo das monoaminas e a auto-oxidação da dopamina podem contribuir para o desequilíbrio redox e que os animais jovens são mais propensos a apresentar danos no sistema nervoso central, especialmente no tratamento prolongado com metilfenidato. Ratos adultos podem ser mais sensíveis aos efeitos agudos do tratamento.

Tabela 2. Achados em estudos com modelo animal.

Findings	Examples
Acute and chronic methylphenidate administration produce different responses.	Greater methylphenidate dosage was associated with lipid peroxidation increase in the cerebellum, frontal precortex, hippocampus and striatum in young animals chronically exposed to methylphenidate (Husson I et al., 2004; Martins MR et al., 2006)
Dose-dependent responses	Increased superoxide in cerebellum in acute methylphenidate administration in young rats (all dosages) and in hippocampus only in the highest dosage. Decreased superoxide in cerebellum in chronic methylphenidate Administration in adults' rats (Gomes KM et al., 2009).
Age of methylphenidate exposure	
Brain structure dependente responses	Chronic methylphenidate treatment in young rats: Cerebellum (increased SOD/CAT activity); Striatum and hippocampus, (decreased TBARS); Prefrontal cortex (Increased reactive species formation, increased SOD/CAT ratio, increased lipid peroxidation and protein damage. (Schmitz F et al., 2012[b])
Brain mitochondrial respiratory chain enzymes activation	Brain mitochondrial respiratory chain enzymes activated in brain (cerebellum, cortex prefrontal and striatum) of young rats after chronic exposure to methylphenidate. (Fagundes AO et al., 2007)
Antioxidants and oxidants changes	Acute and Chronic methylphenidate in adults' rats: increased TBARS and carbonyl groups; decreased SOD and CAT activities; alters energetic metabolism in the brain. (Comin et al., 2014)
Lipidic damage biomarkers changes	Chronic methylphenidate treatment in young rats: decreased thiobarbituric acid reactive substances. reactive substances and total non-enzymatic radical-trapping antioxidant, and increased superoxide dismutase and catalase activities (Schmitz F et al., 2012[a])
Protein damage biomarkers changes	Chronic methylphenidate treatment in young rats: Prefrontal cortex: Increased protein damage. (Schmitz F et al., 2012[b])
DNA damage biomarkers changes	Early striatum DNA damage in young and adult rats with chronic treatment greater than in the hippocampus (Andreazza AC et al., 2007)

Existem algumas evidências de que o tratamento com metilfenidato pode modificar os perfis pró-inflamatórios e oxidativos com perda de neurônios dopaminérgicos, ativação da microglia e aumento de marcadores pró-inflamatórios. (Quintero, 2022) As catecolaminas (dopamina, epinefrina e norepinefrina) podem reagir com o O_2 para produzir superóxido e quinonas/semiquinonas que se ligam prontamente às cadeias laterais sulfidrilas e esgotam as já baixas reservas cerebrais de glutationa-S-transferase. (Fraunberger, 2015) Portanto, o metilfenidato pode influenciar o estado oxidativo por seu próprio metabolismo hepático, mecanismo de ação e geração de dopamina e DOPA quinonas altamente reativas. (Miyazaki, 2008 e Oakes, 2019)

2.5. TDAH, Metilfenidato e Neuroplasticidade

A neuroplasticidade consiste na capacidade do cérebro de alterar a estrutura e a função em resposta a alguns estímulos, sendo chamada de “plasticidade dependente da experiência”. (Kolb, 2010) Apesar da simplicidade do conceito de neuroplasticidade, o mecanismo ainda não está totalmente esclarecido. (Diniz e Crestani, 2023) Alterações plásticas no cérebro podem melhorar as funções motoras e cognitivas e interferir no comportamento. (Kolb, 2010) Os circuitos neuronais sofrem continuamente refinamento ao longo da vida, à medida que novas sinapses se formam, e as sinapses existentes podem aumentar ou retrair, geralmente em resposta a demandas ambientais (por exemplo, desafios intelectuais, situações estressantes. (Raefsky, 2017)

O sistema nervoso central apresenta períodos mais favoráveis ao seu desenvolvimento, durante os quais, estaria mais propenso a mudar tanto por fatores intrínsecos, quanto extrínsecos, podendo levar a alterações permanentes

em vários níveis desde fisiológicos a morfológicos e/ou anatômicos. Para a maioria das substâncias tóxicas, o tempo mais sensível ocorre entre a concepção e o nascimento. Processos chaves do neurodesenvolvimento ocorrem nesse período, o que poderiam explicar porque a exposição em estágios pré-natais está associada com riscos maiores. (Heyer, 2017) O tempo sensível para o risco de TDAH está menos claramente definido. O fato de que não há um período curto e claro para o risco de TDAH pode sugerir que os mecanismos subjacentes também não dependem de eventos com duração breve. As áreas do cérebro mais associadas ao TDAH são o córtex pré-frontal, caudado e cerebelo, cujo desenvolvimento ocorre ao longo da maior parte do período pré-natal e se estende por mais anos após o nascimento. (Heyer, 2017; Sta Maria, 2019) Esses mecanismos poderiam contribuir para a disfunção dopaminérgica que é característica do TDAH. Além disso, tóxicos ambientais também podem atuar diretamente no sistema dopaminérgico em períodos durante o desenvolvimento para perturbar a neurotransmissão de forma semelhante e alterações nos sistemas imune e endócrino podem interferir na função de neurotransmissores. (Heyer, 2017 e Budziszewska, 2010) A neuroplasticidade humana prolongada pode conferir maior risco de psicopatologias do desenvolvimento. (Sydnor, 2021; Diniz, 2023)

Alterações do equilíbrio de circuitos locais do estado inibitório para o excitatório e vice-versa por meio de drogas, experiência sensorial, estresse, nutrição, fatores genéticos, envelhecimento ou epigenéticos pode modular períodos cruciais do neurodesenvolvimento. (Reh, 2020) Isso seria útil se pensássemos na possibilidade de uma droga contribuir para a restauração e/ou redução de danos cerebrais ou estimular cérebros com áreas subdesenvolvidas.

Portanto, devemos ter cuidado com a possibilidade de estimular inadvertidamente a plasticidade patológica, inclusive através dos tratamentos farmacológicos administrados. Por exemplo, estimulantes psicomotores, como anfetaminas, foram associados como estimulador de alterações plásticas no corpo estriado dorsal, núcleo accumbens e córtex pré-frontal. (Robinson, 2004)

Dentre os fatores genéticos associados ao TDAH, pesquisadores têm explorado polimorfismos relacionados à atividade do fator neurotrófico derivado do cérebro (BDNF). (Binder, 2004) O fator neurotrófico derivado do cérebro (BDNF) é um componente crítico da neuroplasticidade e do desenvolvimento, cujos níveis têm o potencial de afetar a atividade cerebral a longo prazo. (Libman-Sokolowska, 2015) A expressão do BDNF pode ser modulada por vários estímulos fisiológicos, como atividade física, ciclo menstrual, exposição à luz, estímulos osmóticos e elétricos (Mitchelmore, 2014). O estresse agudo e o crônico, bem como alterações epigenéticas como a metilação do DNA, podem diminuir sua expressão. (Binder, 2004) Vários estudos investigaram os níveis de BDNF em indivíduos com transtorno de déficit de atenção e hiperatividade (TDAH) em comparação com crianças com desenvolvimento típico, sendo que alguns estudos examinaram alterações nos níveis de BDNF antes e após o tratamento. (Amiri, 2013; Sahin, 2014; Cubero-Millán, 2016; Pekcanlar, 2017; Gumus, 2022) Esses estudos produziram resultados mistos: níveis aumentados, reduzidos ou inalterados foram encontrados no grupo de pacientes com TDAH, quando comparados ao grupo controle saudável ou controle com TDAH não medicado. (Shim, 2008; Sayyah, 2009; Sargini, 2012; Scassellati, 2013; Haimei, 2014; Sahin, 2014; Saadat, 2015; Simsek, 2016; Bilgiç, 2016; Reda, 2016;

Cubero-Millán, 2016; Pekcanlar, 2017; Taha, 2017; Wang, 2019; Yurteri, 2019; Chang, 2020; Ghamry, 2021 e Gumus, 2022)

Como o TDAH é um transtorno do neurodesenvolvimento e o BDNF tem papel central no desenvolvimento e plasticidade cerebrais, alterações em seus níveis poderiam ter efeitos a longo prazo na atividade cerebral e associar-se, portanto, à sua fisiopatologia. (Leffa, 2017) Considerando que o cérebro possui características que o deixam mais susceptíveis ao dano oxidativo e vários processos imunes, metabólicos, infecciosos e inflamatórios podem impactar os parâmetros oxidativos, pode-se atribuir ao desequilíbrio oxidativo uma possível participação na fisiopatologia do TDAH. (Hassan W et al., 2022) Mecanismos oxidativos, inflamatórios, neuroplásticos podem estar interconectados (Asghari, 2018), modulando rotas do desenvolvimento e associando-se a etiologia complexa do TDAH e com possíveis alterações moleculares, estruturais e funcionais em diversas regiões anatômicas cerebrais.

2.6. Estado Oxidativo e Neuroplasticidade

As espécies reativas de oxigênio (EROS) podem direcionar danos aos lipídeos e alterar a fluidez e permeabilidade da membrana lipídica, bem como promover alterações no transporte de íon, inibição de processos metabólicos, lesão mitocondrial, associando-se a sistemas de defesa antioxidante ineficientes. (Desai, 2014) A peroxidação da membrana lipídica pode promover a sobrevivência celular ou induzir a morte celular (apoptose, necrose), dependendo de ser subtóxica (taxas de peroxidação lipídica baixas) ou tóxica (taxas de peroxidação lipídica médias a altas) condições, respetivamente. Mas

ambos os processos eventualmente levam a danos celulares moleculares, facilitando o desenvolvimento de vários estados patológicos e acelerando o envelhecimento. O malondialdeído (MDA) e, em particular, o 4-hidroxi-2-nonenal (4-HNE), produtos da decomposição lipídica da peroxidação, podem participar de reações deletérias secundárias promovendo lesão intramolecular ou intermolecular de proteínas/DNA e produção de espécies mais reativas. (Ayala, 2014 e Desai, 2014)

EROS possuem papel na sinalização intracelular envolvida em processos de neuroplasticidade, porém seu acúmulo excessivo no sistema nervoso central pode causar danos oxidativos. EROS podem reagir com proteínas e ácidos nucléicos, prejudicando vias de transdução entrelaçadas e outras funções neuronais. Modulam, portanto, vias de transdução intracelular e fatores de transcrição (por exemplo, fator nuclear kB, relacionado a transcrição de enzimas antioxidantes) envolvidos na proliferação celular, diferenciação e maturação (várias cascatas da neurogênese). (Beckhauser, 2016; Raefsky,2016; Spaas, 2021 e Ciancarelli, 2022)

A capacidade das sinapses de alterar sua própria força em resposta à estimulação anterior é chamada de plasticidade sináptica. As modificações sinápticas resultam de mudanças na quantidade de neurotransmissores liberados e/ou de mudanças na eficácia com que as células respondem aos neurotransmissores. Tais modificações compreendem a potenciação de longo prazo (PLD), que é um aumento duradouro na eficiência sináptica, e a depressão de longo prazo (DLD), que é uma diminuição duradoura na força da transmissão sináptica. Para manter a longo prazo alterações sinápticas, é necessária a síntese de novas proteínas. O estado redox modula a atividade de quinases

envolvidas nesse processo. Sendo assim, parece que alterações nas concentrações de ROS estão envolvidas na expressão de potenciação e depressão de longo prazo, participando de diferentes etapas do processo de transdução. (Beckhauser, 2016; Raefsky, 2016 e Lejri, 2019)

EROS podem participar dos processos de plasticidade também como segundos mensageiros em várias áreas do SNC, incluindo hipocampo, córtex cerebral, medula espinha, hipotálamo e amígdala. (Beckhauser, 2016). Além disso, em momentos de reduzida defesa antioxidande, EROS podem oxidar dopamina no radical livre tóxico dopamina o-semiquinona que pode estar envolvida na redução da espinha dentrítica. (Raefsky, 2016 e Lejri, 2019)

A função mitocondrial também é crítica para a plasticidade neuronal. (Jeanneteau, 2016) Além da produção de energia na forma de ATP, as mitocôndrias são os principais moduladores da sobrevivência e morte das células cerebrais, controlando o cálcio (Ca2+) e o equilíbrio redox (que por sua vez afeta a liberação de neurotransmissores e a plasticidade neuronal), produzindo EROS e controlando a apoptose celular. O crescimento de neuritos é um processo em que os neurônios em desenvolvimento geram novas projeções à medida que crescem em resposta aos estímulos. Neurotrofinas, principalmente o BDNF) regulam o crescimento dos neuritos. Esses processos de plasticidade requerem muita energia, mostrando a importância das mitocôndrias para a sobrevivência e adaptações neuronais e, consequentemente, da importância do estado redox e suas interligações (Lejri, 2019)

Apesar de achados que sugerem ligações do metilfenidato com o estresse oxidativo, inflamação e neuroplasticidade, ainda persistem várias

lacunas com relação aos seus mecanismos de ação e ações farmacológicas. Sendo um medicamento amplamente usado, por diversas faixas etárias e tempos prolongados, precisa-se aprofundar os estudos nesse campo.

2.7. REFERÊNCIAS

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3. OBJETIVOS

3.1. Objetivo Geral

Avaliar o impacto do tratamento com metilfenidato no perfil oxidativo de crianças e adolescentes diagnosticados com TDAH.

3.2. Objetivos Específicos

- Caracterizar clínica e sociodemograficamente crianças com TDAH e suas e famílias.
- Avaliar o potencial de alteração redox promovido pela implementação do uso de metilfenidato.

  - Quantificar a capacidade antioxidante sérica total.
  - Quantificar a atividade de enzimas antioxidantes.
  - Determinar os níveis dos marcadores de dano oxidativo.

- Avaliar as alterações de plasticidade potencialmente produzidas pelo metilfenidato.

4. MÉTODOS

A metodologia do ensaio clínico referente ao presente estudo foi apresentada em um protocolo de ensaio clínico do tipo coorte aberta de centro único. Seguindo as diretrizes internacionais do SPIRIT: Standard Protocol Items: Recommendations for Interventional Trials, foi submetido à publicação para a revista "*European Paediatric Neurological Disorders*".

As metodologias empregadas são específicas de cada artigo apresentado e amplamente descritas no respectivo artigo.

4.1. Artigo 1: "BDNF, inflammatory and oxidative levels in treatment-naïve ADHD children treated with methylphenidate: An open cohort protocol."

European Journal of Paediatric Neurology

BDNF, inflammatory and oxidative levels in treatment-naïve ADHD children treated with methylphenidate: An open cohort protocol.

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Abstract:	Background: The attention-deficit hyperactivity disorder (ADHD) has a complex etiology, involving the interaction between biological, genetic, and environmental factors. The ADHD pathophysiology remains unknown even though there are hypotheses that inflammatory, hormonal, oxidative and neurotrophic factors are associated. This clinical trial aims to evaluate the contribution of brain derived neurotrophic factor (BDNF), inflammatory and oxidative levels before and after 12 and 24 weeks of methylphenidate use. Methods: Patients will be screened upon their entry into Child and Adolescent Psychiatry Teaching Outpatient Clinic of the Medical Course at the Federal University of Viçosa in Minas Gerais, Brazil. One hundred and fifty ADHD treatment-naïve children of both sexes, between 6–14 years old, will be invited to participate, after the ADHD diagnosis by an experienced psychiatrist and the child fulfilling the inclusion criteria. Children and their caregivers will answer questionnaires regarding mental health and the children will undergo neuropsychological tests, physical, nutritional and activity assessment, in addition to blood sampling at baseline, 12 and 24 weeks of methylphenidate use respectively. Discussion: This clinical trial intends to verify how the pharmacological treatment changes the plasma BDNF, inflammatory and oxidative levels in treatment-naïve Brazilian children diagnosed for ADHD. Trial Registration: Submitted for registration on Brazilian Registry of Clinical Trials (ReBEC). Trial identifier: 13612. Registry name: Níveis de neurotrofina, perfil inflamatório e oxidativo em crianças com TDAH tratadas com metilfenidato.
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BDNF, inflammatory and oxidative levels in treatment-naïve ADHD children treated with methylphenidate: An open cohort protocol.

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Abstract

The attention-deficit hyperactivity disorder (ADHD) has a complex etiology, involving the interaction between biological, genetic, and environmental factors. The ADHD pathophysiology remains unknown even though there are hypotheses that inflammatory, hormonal, oxidative and neurotrophic factors are associated. This clinical trial aims to evaluate the contribution of brain derived neurotrophic factor (BDNF), inflammatory and oxidative levels before and after 12 and 24 weeks of methylphenidate use.

Methods

Patients will be screened upon their entry into Child and Adolescent Psychiatry Teaching Outpatient Clinic of the Medical Course at the Federal University of Viçosa in Minas Gerais, Brazil. One hundred and fifty ADHD treatment-naïve children of both sexes, between 6–14 years old, will be invited to participate, after the ADHD diagnosis by an experienced psychiatrist and the child fulfilling the inclusion criteria. Children and their caregivers will answer questionnaires regarding mental health and the children will undergo neuropsychological tests, physical, nutritional and activity assessment, in addition to blood sampling at baseline, 12 and 24 weeks of methylphenidate use respectively.

Discussion

This clinical trial intends to verify how the pharmacological treatment changes the plasma BDNF, inflammatory and oxidative levels in treatment-naïve Brazilian children diagnosed for ADHD.

Trial Registration

Submitted for registration on Brazilian Registry of Clinical Trials (ReBEC).

Trial identifier: em processo de avaliação pelo ReBEC

Registry name: Níveis de neurotrofina, perfil inflamatório e oxidativo em crianças com TDAH tratadas com metilfenidato.

Administrative information – Spirit 2013 Checklist

Title {1}

BDNF, inflammatory and oxidative levels in treatment-naïve ADHD children treated with methylphenidate: An open cohort protocol.

Trial registration

Registry {2a}

Submitted for registration on Brazilian Registry of Clinical Trials (ReBEC).

Trial identifier: 13612.

Registry name: Neurotrophin level, inflammatory and oxidative profile in ADHD children treated with methylphenidate.

Data Set {2b}

The Universal Trial Number (UTN) is U1111-1285-2908.

Date recruitment began: 23/10/2020; Approximate date when recruitment will be completed: 29/11/2022.

Protocol version {3}

First version.

Submitted for registration on Brazilian Registry of Clinical Trials (ReBEC).

Trial identifier: 13612.

Funding {4}

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Roles and Responsibilities {5}

Contributorship {5a}

Marina Silva de Lucca. Master in Physical Education and PhD student in Health Sciences, Faculty of Medicine, UFMG.

Laira Lopes Tonon. Medicine student (UFV). Research volunteer member.

Bárbara Silva Cabral. Medicine student (UFV). Research volunteer member.

Jordânia Alves Ferreira. Medicine student (UFV). Scientific initiation scholarship

Cleuberton Kenedy Oliveira Raimundo. Medicine student (UFV). Research volunteer member.

Silvia Almeida Cardoso. Master and Doctor in Immunology (USP). Associate Professor of Nursing and Medicine, Faculty of Medicine, UFV.

Débora Marques de Miranda. Master and Doctor in Biochemical and Molecular Pharmacology (UFMG). Associate Professor of Pediatrics, Faculty of Medicine, UFMG.

ML is the Chief Investigator; she conceived the study, led the proposal and protocol development. LLT, BSC, JAF and CKOR contributed to development of the proposal and methodology. SC e DM contributed to study design and to development of the proposal and methodology. All authors read and approved the final manuscript.

Sponsor contact information {5b}

Not applicable.

Sponsor and Funder {5c}

This funding source had no role in the design of this study and will not have any role during its execution, analyses, interpretation of the data, or decision to submit results.

Committees {5d}

Principal Investigator and Research Physician

Design and conduct the clinical appointments: Marina Silva de Lucca.

Preparation of protocol and revisions: Marina Silva de Lucca, Sílvia Almeida Cardoso, and Débora Marques de Miranda.

Organizing steering committee meetings: Marina Silva de Lucca and Débora Marques de Miranda.

Publication of study reports: Marina Silva de Lucca, Sílvia Almeida Cardoso, and Débora Marques de Miranda.

Steering committee

The composition of the trial committee includes all authors.

Agreement of final protocol: Marina Silva de Lucca, Sílvia Almeida Cardoso, and Débora Marques de Miranda.

All lead investigators will be steering committee members.

Recruitment of patients: Laira Lopes Tonon, Bárbara Silva Cabral and Jordânia Alves Ferreira, Cleuberton Kenedy Oliveira Raimundo.

Reviewing progress of study and if necessary, agreeing changes to the protocol and/or investigators brochure to facilitate the smooth running of the study: Marina Silva de Lucca and Débora Marques de Miranda.

Trial Management Committee (TMC)

Study planning; Organization of steering committee meetings: Marina Silva de Lucca and Débora Marques de Miranda.

Data verification: Marina Silva de Lucca.

Endpoint adjudication: Marina Silva de Lucca and Débora Marques de Miranda.

Data Management team

Data entry, data verification: Laira Lopes Tonon, Bárbara Silva Cabral, Jordânia Alves Ferreira, Cleuberton Kenedy Oliveira Raimundo and Marina Silva de Lucca.

Lead Investigators

Marina Silva de Lucca, Sílvia Almeida Cardoso, and Débora Marques de Miranda.

Schedule of enrolment, interventions, and assessments

Introduction

Background and rationale {6a}

Attention deficit hyperactivity disorder (ADHD) is a neurodevelopmental disorder characterized by symptoms of inattention, hyperactivity, and impulsivity that appear in childhood and usually persist into adulthood, causing some degree of dysfunction in daily activities [1,2,3]. The heterogeneous clinical presentations of ADHD are a hallmark of this disorder [4] important negative outcomes are reported, both in terms of morbidity and mortality [3].

ADHD has a complex etiology, involving the interaction between genetic, biological and environmental factors. However, its pathophysiology remains unknown [5]. There are hypotheses that inflammatory, oxidative, hormonal, and neurotrophic factors are associated, and they have mutual interactions. [3,6].

Different ADHD risk factors, such as prematurity, paternal smoking, exposure to pesticides and lead may be associated with common pathophysiological pathways, such as inflammation, oxidative stress, and neurotrophic factors. Likewise, intrauterine pro-inflammatory factors during the gestational period may be associated with restricted intrauterine growth, miscarriage, premature birth, placenta abruption, neurological damage, some of which are risk factors for ADHD [7,8,9, 10, 11, 12]. Neuroinflammation in physiological and pathological conditions may activate microglia, astrocytes, oligodendrocytes, and ependymal cells. In addition, it may increase proteases, glutamate, reactive oxygen species, nitric oxide, chemokines, toxic cytokines, prostaglandins, and may induces infiltration of T and B neutrophils, monocytes/ macrophages and dendritic cells. When activated, these cells release pro-inflammatory cytokines that increase neuroinflammation [13]. Some cytokines are elevated in ADHD, some of which are related to the severity of symptoms [13, 14,

15]. A systematic review on the level of cytokines in peripheral blood showed increased levels of cytokines-interleukin-6 (IL-6) in patients with ADHD, especially in those younger than eighteen years and not on medication, when compared to a healthy control group [13, 16]. However, another systematic review found no difference in IL-6 levels when comparing children with and without ADHD [17]. Furthermore, patients with ADHD, especially in those younger than eighteen years and not on medication, had lower levels of tumor necrosis factor-alpha human (TNF-alpha) compared to healthy controls [16, 17]. The results regarding cytokines-interleukin-10 (IL-10) were contradictory and may be increased or unchanged in children with ADHD compared to children without the pathology [13, 16, 17]. ADHD treated patients seem to have a decrease in interferon gamma (IFN-gamma) and interleukin-13 (IL-13) levels, compared to those naïve to treatment [13].

Therefore, neuroinflammation could alter the blood-brain barrier, neurotransmitter metabolism, increase oxidative stress, and neurodegeneration [14, 15]. Animals and humans with ADHD show, in different brain structures, an increase in reactive oxygen species without an adequate antioxidant response, resulting in an increase in oxidative stress. This negative imbalance in ADHD may be explained in the central nervous system due to the large consumption of oxygen by neurons associated with an antioxidant defense system of modest action and a constitution rich in lipids. As a result, the brain has difficulty regulating excess reactive oxygen species, making it more susceptible to damage [15, 18, 19, 20, 21, 22]. Therefore, there is evidence of increased oxidative stress in ADHD patients [19, 20, 21 22, 23, 24].

ADHD may be associated with a decrease in ascorbate, catalase, albumin and bilirubin and an increase in uric acid. So, it may contribute to oxidative stress increase [25]. On the other hand, treatment with methylphenidate seems to increase catalase levels. Other study demonstrated that the activity of superoxide dismutase (SOD), an important antioxidant defense enzyme, is lower in patients with ADHD [25].

Increased oxidative stress may be associated with reduced levels of BDNF, as well as reduced oxidative stress with increased BDNF [26]. Therefore, changes in the BDNF expression at critical moments during development may promote a cascade of events interfering with the brain maturation of some regions, being a substrate for altered response to stress in adulthood and development of neuropsychiatric disorders [26]. Another evidence of intense oxidative stress is the modulation of telomere size and shortening during life [27] and telomere shortening is present in ADHD children [28]. So, telomere length may be a potential biomarker of the ADHD symptoms burden in families affected by this neurodevelopmental disorder [29].

Studies with rats have been trying to observe the results of chronic use of methylphenidate in brain cells [30, 31, 32]. Chronic use of methylphenidate in healthy rats caused oxidative damage in the brain of young rats [30]. Furthermore, the chronic use of methylphenidate by adult rats induced oxidative stress and inflammation in the hippocampus, causing cellular damage in this area [31]. Increased oxidative stress was also observed in spontaneously hypertensive adult rats (animal version of ADHD) [32].

In ADHD children and adolescents' study, low levels of nitric oxide were found in baseline, which did not change significantly with the use of methylphenidate over ten weeks [33]. Guney et al. (2015) [34] found that methylphenidate may reduce oxidant levels and increase antioxidant levels in children and adolescents.

Studies have evaluated BDNF levels in individuals with ADHD, comparing them with typical children [17, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51] and after stimulant treatment [40, 45, 50, 51, 52], with divergent results.

Due to the controversial studies and the importance of treating ADHD, this protocol study aims to explain the methodology that will evaluate BDNF, inflammatory and oxidative levels, their associated factors in ADHD treated children for 6 months with methylphenidate.

Choice of comparators {6b}

BDNF, inflammatory markers and oxidative stress levels of the ADHD children will be compared before and after methylphenidate use. Therefore, the same group of children will be compared in three different times: before starting methylphenidate and after 12 and 24 weeks of methylphenidate use. All patients will be in follow-up at the child and adolescent psychiatry outpatient clinic, receiving pharmacological treatment for ADHD. Local health system provides free methylphenidate (generic medication) in the presentation of 10mg, immediate-release tablets.

Control groups (ADHD children without methylphenidate use) will not be possible, because it would be unethical to deprive them of effective treatment, capable of reducing not only morbidity, but also mortality.

Objectives {7}

The objectives of this open cohort clinical trial will be:

1. Evaluate the BDNF, inflammatory and oxidative levels before and after 12 and 24 weeks of methylphenidate use in treatment-naïve ADHD children.
2. Investigate if there is a moderating effect of the sociodemographic data, ADHD presentation, comorbidity presence, caregiver psychopathology, parenting styles, emotional regulation level on BDNF, cytokines and oxidative stress level, telomere length, and other variables from the survey instruments detailed throughout this protocol.

Trial Design {8}

This trial is designed as an open cohort, single center, with convenience sample.

Methods: Participants, Interventions, Outcomes

Study setting {9}

All subjects will be invited to the child psychiatry teaching outpatient clinic at the Federal University of Viçosa (UFV), in the state of Minas Gerais, Brazil.

Eligibility criteria {10}

Participants inclusion criteria: {10}

Treatment-naïve ADHD children, of both sex in outpatient treatment; age between complete 6 and incomplete 15 years old. Children must have criteria for ADHD diagnosis according to the DSM-5 and must not have chronic diseases or use of any other medications that may interfere with the immune system or BDNF. Children who need iron or vitamin replacement may be included. The following comorbidities may be included: oppositional defiant disorder (ODD), tic disorder, enuresis, encopresis, skin picking disorder. Autism spectrum disorder (ASD) level 1, Classification of Diseases and Related Health Problems (ICD) 10 F84.5, could be included if the diagnosis occurs during the study. All parents must sign the free and informed consent form, just like children should also sign the assent form. The terms will be delivered during the initial assessment of the participants, if they wish to participate voluntarily in the research.

Participants exclusion criteria: {10}

Families and children that refusal to participate in the research or refusal to use stimulant medications; presence of autoimmune, neurodegenerative diseases, and immunodeficiencies; intellectual disability, severe clinical or psychiatric comorbidities, except autism spectrum disorder level 1 (ICD 10 F84.5), ODD, tics, trichotillomania, skin picking disorder, enuresis, and encopresis; previous or current use of stimulants or other psychiatry medications; current use of medications for chronic diseases or that interfere in immune

system or BDNF levels (antidepressants for example); girls who have menstruated; stimulant contraindication use.

Participant timeline: {13, 14, 15, 18}

Participants will be included starting in October 2020, with the aim of reaching 150 children, based on sample calculation with analysis power between 80% and 95% of accuracy. This sample calculation was based on an article of Akay et al., 2017 [37].

They will be forwarded by physicians, schools and psychologists from the city and health region. To reach the sample number, the health units, as well as the municipal education department were communicated about the research and how the children could be referred for screening. There was also publicity on the local radio and the website of the Federal University of Viçosa.

Children who are eligible for the study will be categorized by ADHD presentation, disorder severity, with or without Oppositional Defiant Disorder (ODD), with or without autism spectrum disorder (ASD), at any emotional dysregulation level. Their caregivers will be sorted by parenting style.

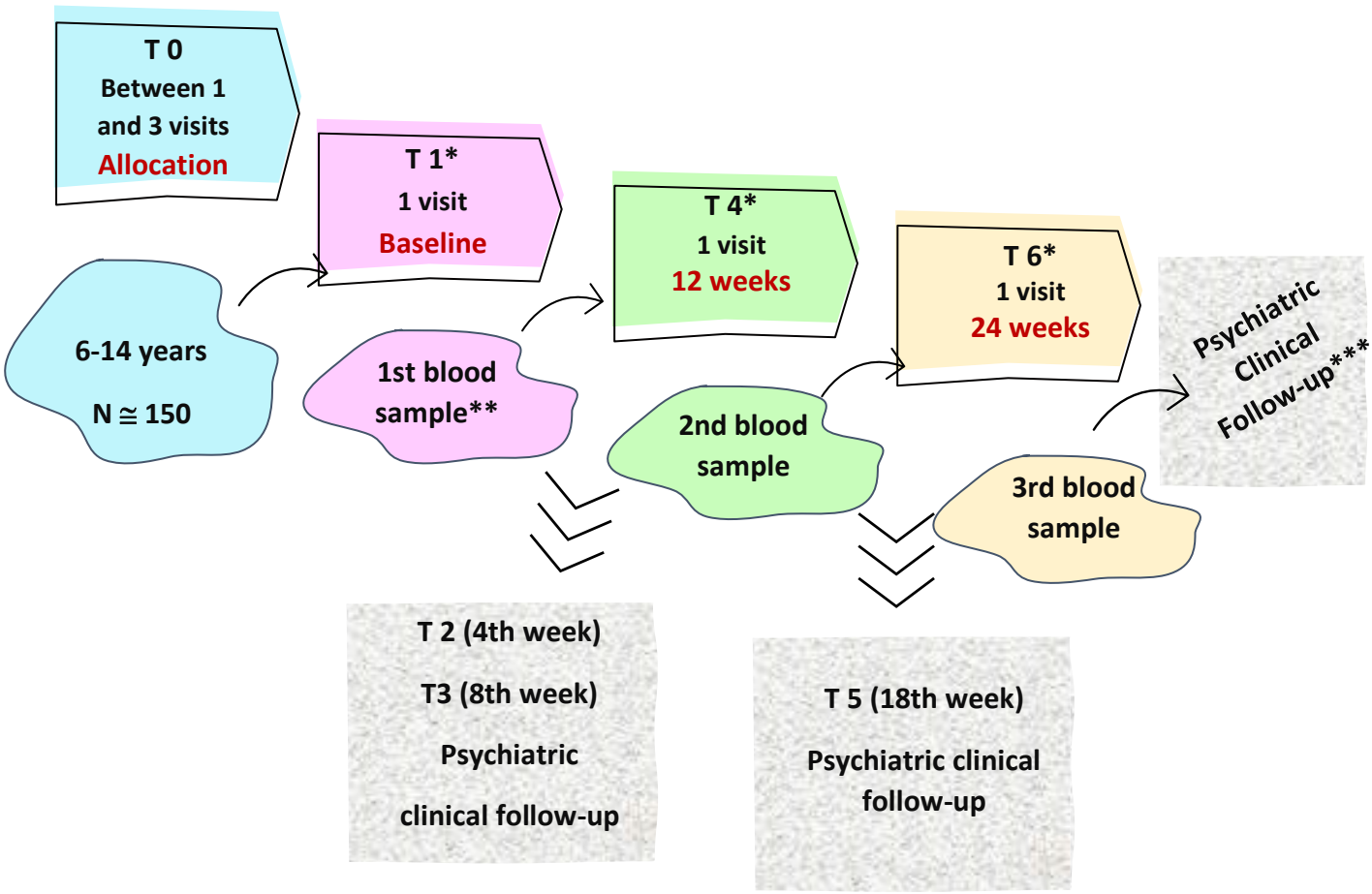
Overall, each participant will make 8 to 10 visits during the study period.

The research procedure for participants at each visit are described in Fig. 2.

The initial assessment includes the semi-structured interview Kiddie-Schedule for Affective Disorders and Schizophrenia – Present and Lifetime Version (K-Sads-PL 2013) [53].

To assess the severity of ADHD symptoms, the SNAP-IV scale, Swanson, Nolan e Pelham version [54], and the Assessment Scale of Childhood and Adolescent Behaviors in ADHD in a Family Environment (ETDAH-parents) [55] and ADHD Self-Assessment Scale-Version for Children and Adolescents (ETDAH-Criad) [56] were answered by parents and children. The SNAP-IV scale has 26 items, which also investigates symptoms of a common comorbidity that is Defiant and Oppositional Disorder. [54]. The ETDAH scale has one version for parents and another for the children. [55,56]. The parent subscale has four subscales that investigates symptoms of inattention, hyperactivity/impulsivity, emotional regulation, and adaptive behavior. The children subscale has 2 subscales that investigates inattention and hyperactivity/impulsivity symptoms.

Fig. 2. The participants visit.



* Assessment points (Psychiatric clinical follow-up, Nutrition assessment, Neuropsychological assessment, Sample Blood, Questionnaires and Scales).
** Methylphenidate prescription after first blood sample.
***Children who finished the trial times will be transferred to the general outpatient clinic of child psychiatry at the UFV medical school.

The diagnosis will be confirmed by an interview done by a Child and Adolescent Psychiatrist. Sociodemographic data was obtained from the Brazil Economic Ranking Criterion and “Background information” part of the K-Sads-PL instrument (K-Sads-PL 2013) [53, 57, 58]. A standard record for the clinical history was constructed with data on pregnancy, childbirth, neuropsychomotor development, and the child's previous and family history.

For the care of children at the outpatient clinic, an anamnesis model was built. This anamnesis also includes a habitual physical activity questionnaire [59], Brazilian food safety scale [60], SISVAN food consumption markers questionnaire [61], food frequency questionnaire [62, 63], bullying questionnaire (Kidscape) [64], measurement questionnaire adherence to treatment [65], CRAFFT/CESARE instrument (Car; Relax; Alone; Forget; Family/Friends; Trouble acronym) [66].

Situations of violence and/or health identified during the research will be duly forwarded to the necessary assistance network, such as justice, other medical specialties, social services, and others. The caregiver is screened for symptoms of depression, anxiety, ADHD, and alcohol use [67, 68, 69, 70]. In case of positive screening, evaluation by an adult psychiatrist will be offered.

Interventions {11a, 18}

Screening visits

Medical students trained by an experienced child and adolescent psychiatrist and under her supervision, will apply in the first assessment (between one and three visits): Fig. 1. Schedule of enrolment, interventions, and assessments.

Multimodal Treatment Assessment Study – Swanson, Nolan e Pelham (MTA-SNAP-IV) scale, that evaluates symptoms of attention deficit/hyperactivity disorder and oppositional defiant disorder in children and adolescents [54]

Kiddie Schedule for Affective Disorders and Schizophrenia for School Aged Children – Lifetime Version (K-Sads-PL 2013) [53]
Sociodemographic questionnaire, including the Brazil Economic Ranking Criterion and the background information of the K-Sads-PL 2013 [53,57,58]
ADHD Behavior Assessment Scale – Parents Version (ETDAH-parents) [55]
Bullying questionnaire [64]
Physical activity questionnaire [59]
SISVAN food consumption markers [61]
Brazilian Food Security Scale (EBIA) [60]
Scale of Sleep disorders in children [72]
Behavior Inventory for Children and Adolescents (CABI) [73]
Parenting Styles and Dimensions Questionnaire – Short Version (PSDQ) [74]
Epidemiological Studies Center Depression Scale (CES-D) [67]
State-Trait Anxiety Inventory (IDATE) [68]
Adult Self-Report Scale (ASRS-18) [69]
Alcohol Use disorders identification test (AUDIT) [70]
Standardized psychiatric clinic interview with standard record.
Physical examination.

Psychology students, under supervision of an experienced psychologist in neuropsychology will apply:

Scale for screening drug use CRAFFT/CESARE [66]

Social Skills Test for Children and Adolescents in School Situation (THAS-C) [75]

ADHD Self-Assessment Scale – Version for Children and Adolescents (ETDAH-CriAd) [56]

Standardized and validated psychological tests for the Brazilian population.

- Non-Verbal test of Children's Reasoning (TNVRI) [76]
- Battery for Attention Assessment (BPA) [77, 78]
- Five digits teste (FDT) [79]
- Brief Child Neuropsychological Assessment Instrument (NEUPSILIN-inf) [80]

Nutrition’s student under supervision of an experienced nutritionist will apply:

24-hour food recall [81, 82]

Fig 1. Schedule of enrolment, interventions, and assessments.

	STUDY PERIOD							
	Enrolment	Allocation	Post-allocation					Close-out
TIMEPOINT**	$-t_1$	t_0	t_1 *baseline*	t_2 *4 sem*	t_3 *8 sem*	t_4 *12 sem*	t_5 *18 sem*	t_6 *24 sem*
ENROLMENT:								
Eligibility screen	X							
Informed consent	X							
K-Sads-PL	X							
Sociodemographic data	X							
Allocation		X						
INTERVENTIONS:								
Child Questionnaires								
ADHD Self-Assessment Scale – Version for Children and Adolescents (ETDAH-Cri Ad)			X			X		X
ADHD Behavior Assessment Scale - Parental Version (ETDAH-parents)			X			X		X
SNAP-IV			X			X		X
Psychiatry interview (standard record)			X	X	X	X	X	X
Physical Examination			X	X	X	X	X	X
Measure Treatment Adherence (MTA) and pill count			X	X	X	X	X	X
24-hour food recall			X			X		X
SISVAN food consumption markers			X			X		X
Bullying questionnaire			X			X		X

(Kidscape)								
Physical activity questionnaire			X			X		X
Scale of sleep disorders in children			X			X		X
Social Skills Test for Children and Adolescents in School Situation (THAS-C)			X			X		X
CRAFFT/CESARE (scale for screening drug use)			X			X		X
CABI (Behavior Inventory for Children and Adolescents)			X			X		X
Caregivers Questionnaires								
Parenting Styles and Dimensions Questionnaire – Short Version (PSDQ)			X			X		X
Brazilian Food SecurityScale (EBIA)			X			X		X
Epidemiological Studies Center Depression Scale (CES-D)			X			X		X
State-Trait Anxiety Inventory (IDATE)			X			X		X
Audit (Alcohol Use Disorder Identification Test)			X			X		X
Asr18 (Adult Self-Report Scale)			X			X		X
Neuropsychological Assessment								
TNVRI			X			X		X
BPA			X			X		X
FDT			X			X		X
Neupsilin			X					
Pharmacological Intervention								
Methylphenidate (short acting)			X	X	X	X	X	X
Sample Blood								
BDNF			X			X		X

Cytokines			**X**			**X**		**X**
Oxidative stress			**X**			**X**		**X**
ASSESSMENTS:								
First trial assessment			**X**					
Second trial assessment						**X**		
Third trial assessment								**X**

Assessment points {11a, 18a}

The assessment points occur in three timepoints: baseline (before methylphenidate initial use) and after 12 and 24 weeks of methylphenidate use. Fig 1 and 2.

In the first assessment point the blood sample is collected, the family receives psychoeducation about the disorder and its treatment, and the methylphenidate is started.

In the second and third assessment points, all instruments will be applied in screening are reapplied, except K-Sads-PL, sociodemographic questionnaire, standardized psychiatric clinic interview with standard record and NEUPSILIN-inf. In these two timepoints, we will use standardized follow-up anamnesis. Participants will also be clinically evaluated to verify the effect of the treatment (the Clinical Global Impressions (CGI) scale) [83, 84, 85], dose adjustment, the presence of adverse effects, adherence to treatment, clarification of doubts regarding the research, the disorder, and the treatment and reinforce the guidelines regarding the next steps of the study.

Follow-up visits {11a, 18a}

Participants will also be clinically evaluated at 4, 8 and 18 weeks to verify the effect of the treatment, dose adjustment, the presence of adverse effects, adherence to treatment, clarification of doubts regarding the research, the disorder, the treatment and reinforce the guidelines regarding the next steps of the study.

The number of visits could vary, depending on the patient's individual needs.

Pharmacotherapy {11b}

Pharmacotherapy will be conducted by experienced child and adolescent psychiatry with primary responsibility for childcare.

Pharmacotherapy starts with a first-line stimulant medication methylphenidate. [86]. The initial dose will be prescribed as follows: 5mg in the morning and after lunch in the first week, with adjustment to 10mg at the same time until the next evaluation at 4 weeks. From that moment on, the dose will be titrated to optimize the desired therapeutic effect and minimize undesirable adverse effects. The average dose will be 1 mg/kg/day.

If the child does not tolerate the methylphenidate [87, 88] or does not respond to medication, lisdexamfetamine will be offered. [88] Other formulations may be used such as methylphenidate hydrochloride extended-release capsules or OROS methylphenidate if necessary. In these cases, equivalent doses will be prescribed.

Blood sample {11a}

A trained nursing professional will collect three samples of venous blood from each patient/volunteer: before medication treatment, 12 and 24 weeks after starting treatment. Blood samples will be kept at a suitable temperature [89] until they are sent to the Biochemistry laboratory of the Department of Biochemistry (UFV). The BDNF [90] and the cytokines levels [91, 92, 93, 94] will be evaluated in blood plasma and the oxidative stress [95,96] level will be evaluated in serum. Telomeres measurements will be made by DNA extraction [27, 28, 29].

Adherence to intervention protocols {11c, 18b}

To improve adherence to medical visits and research evaluations, nursing technique will confirm the consultation by telephone (calls or WhatsApp messages) the day before. In case of participant absence, contact by phone will be made to offer appointment rescheduling.

The assessment methylphenidate adherence will be by pill count and the Treatment Adherence Measure (MAT) questionnaire [65] in all scheduled visits [97].

Modifications {11b}

The participant will be excluded from the survey if he does not attend the assessment points (blood collection times). If the patient reports the impossibility of attending the assessment visit and is available to reschedule it within a maximum of 15 days, the patient can proceed with the intervention. The patient

may miss the visits number 4, 8, 18 and proceed with the intervention, if the regular use of medication continues.

The child will be excluded in case of not tolerating the medication, family withdraws from participation, diagnosis change during follow-up.

The pharmacological intervention may have adverse effects and the child will receive all the necessary and standard care following strictly the clinical protocol. Most adverse effects are usually mild and occur early in treatment [87]. They will be informed and medication will be adjusted accordingly the need.

Participants whose diagnosis on screening differs from the diagnosis of the psychiatry team will be excluded from the study.

Concomitant care {11d}

The treatment can also include speech therapy and occupational therapy if there is a clinical indication. The psych treatment was not available.

Outcomes {12}

Primary outcomes:

The BDNF levels [37], cytokines and level of oxidative stress (oxidative and antioxidant substances) after treatment with methylphenidate.

Secondary outcomes:

Behavioral symptoms of Attention Deficit/Hyperactivity Disorder and/or Oppositional Defiant Disorder, emotional regulation level determined by the Multimodal Treatment Assessment Study - Swanson, Nolan e Pelham (MTA-SNAP-IV) scale, which assesses symptoms of attention deficit/hyperactivity disorder and Oppositional Defiant Disorder in children and adolescents, and ETDAH - parents, which assesses emotional regulation.

Cardiovascular parameters on physical examination, as well as body mass index will be recorded.

Retention {18b}

The intervention has the potential to bring great benefits to the child and whole family. The stimulant treatment in ADHD children is associated with reduced morbidity and mortality.

WhatsApp contact will be possible for participants to clarify their doubts, reschedule the appointment, as well as be reminded of the appointments during the period in which they participate in the study.

Moreover, a report containing the child's neuropsychological assessment will be given to the parents. In addition, all children will be under medical supervision at the outpatient clinic, even after the end of the study (UFV).

Post-intervention measures will not be necessary after the twenty-fourth week. The analysis will be performed with the information obtained up to the time of dropping out in case of child drops out of the study.

Data Management {19}

A part of the data will be entered electronically and registered in REDCap database. A random sampling checking will be done to quality control.

Participant research files are attached to their medical records, so they will be kept for 20 years after the last registration.

Statistical Methods {20}

Outcomes {20a}

The main analysis strategies will involve group comparison from the three timepoints and trajectory of symptoms and measures across time. Generalized Estimating Equations (GEE) analyses may be done with risk factors and potential moderators such social deprivation, parental styles, and oppositional symptoms to understand the relationship.

Additional analyses {20b}

The main additional analyses strategies will involve descriptive data to characterize the studied sample and to observe the correlations between features.

Analysis Population and Missing Data {20c}

Individuals that will not fill the information of the primary outcomes will have the other data analyzed: sociodemographic profile, BDNF dosages,

cytokines, and oxidative stress at baseline to understand potential populational bias.

Missing data will be handled as necessary for the chosen tests, for GEE for example we will include only individuals with the complete data set for primary outcomes. We will try to retrieve all information by WhatsApp contact.

Methods: Monitoring

Data Monitoring {21}

Formal Committee {21a}

Diagnosis data was done by thirteen years experienced child psychiatry and there is a team on UFMG to discuss any doubt prof. DMM and AASJ. They also have 15 years of experience in the field.

Interim Analyses {21b}

Individuals that will not fill the information of the primary outcomes will have the other data analyzed: sociodemographic profile, BDNF dosages, cytokines, and oxidative stress at baseline.

Socioeconomic data will be analyzed to evaluate any potential of sampling bias.

The clinical psychiatric assessment will be done by an experienced child and adolescent psychiatry, and any diagnosis divergence will be informed.

Adherence will be considered poor if the child takes less than 2/3 of prescribed doses of methylphenidate.

Harms {22}

Adverse events during the trial will be registered in the child's medical record and communicated to the relevant governmental agencies if necessary.

Assistance in case of adverse events will be guaranteed to the child via the public health system.

Auditing {23}

All data will be available to audit if necessary. The diagnosis divergence will be evaluated and informed as soon as it is verified.

Ethics and Dissemination

Research Ethics Approval {24}

Approved by the Research Ethics Committee of the Federal University of Minas Gerais. Number: 4.364.744. CAAE: 82870117.0.3001.5149. Written, informed consent to participate will be obtained from all parents, as well as informed assent from the children.

Protocol Amendments {25}

Any modifications to the protocol which may impact on the conduct of the study, potential benefit of the patient or may affect patient safety, will lead to a formal amendment to the protocol with REBEC, the ethics committee and clinical trial publication journal.

Consent or Assent {26}

Consent or Assent {26a}

All parents must sign the free and informed consent form, as well as the children the assent form. The terms will be delivered at the end of the child's screening if the child is considered eligible to be included in the study and they wish to participate voluntarily in the research.

Ancillary Studies {26b}

Not Applicable

Confidentiality {27}

All study-related information will be stored securely at the child’s medical record. All local databases will be secured with password-protected access systems.

Declaration of Interests {28}

The author(s) declare(s) that they have no competing interests.

Access to Data {29}

All researchers will have access to the final trial dataset without any contractual limitations.

Ancillary and Post-trial Care {30}

The children will continue clinical treatment at the child psychiatry outpatient clinic for as long as the family wishes, or they will be discharged or reach the age of 18, when they will be referred to adult services.

Dissemination Policy {31}

Trial Results {31a}

All research data and personal information will be under responsibility of the researchers to protect confidentiality before, during and after the trial. All parents or guardians' results will be communicated at the end and regarding the trial results.

Trial results will be published at REBEC, regardless of the magnitude or direction of effect. The results will also be reported in an original article and submitted for a relevant journal.

Authorship {31b}

ML is the Chief Investigator; she conceived the study, led the proposal and protocol development. L, B and J contributed to development of the proposal and methodology. SC e DMM contributed to study design and to development of the proposal and methodology. All authors will read and approve the final manuscript.

Reproducible Research {31c}

The anonymous data information might be available under request and reasonable demand.

Discussion

Inflammatory, neurotrophic, and oxidative parameters have been associated with the pathophysiology of ADHD. Limitations in the studies varies from small and heterogeneous samples of participants [12], lack of control for variables that may interfere with the results, such as diet, body mass index, short follow-up time, and level of physical activity, are some of these limitations. Moreover, the results are contradictory [98]. Here we propose a protocol trying to make clear clinical and biomarkers in response to medication in a cohort well characterized in a prospective follow up.

Many studies were cross-sectional and retrospective, which allows inferring only an association between inflammation and the disorder and not a causal relationship of pathogenesis [98]. Longitudinal studies are necessary to better establish the possible relationship between ADHD, inflammation,

neurotrophic, and oxidative stress [16]. Other important factors to be considered are comorbid mental disorders and potential confounding factors, in addition to being important to observe possible changes in the inflammatory profile after interventions [16].

This trial protocol methodology will follow the participants by 24 weeks, increasing the chance of evaluating the influence of the chronic use of methylphenidate on inflammatory, neurotrophic, and oxidative factors. In addition, there will be evaluation of the child's diet and physical activity through the food recall and the usual physical activity questionnaire, respectively. To reduce hormonal influences of puberty, we will exclude girls who had menarche and limited the age of both sexes to 14 years.

The results may provide information about the pathophysiology of ADHD, being able to collaborate in the identification of biomarkers of the disorder and response to treatment with methylphenidate.

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Appendices

Appendix 1. Informed consent materials {32}

Appendix 2. Consent and Assent forms {32}

Appendix 3. Biorepository constitution {32}

Biological specimens {33}

Collection and analysis of biological samples will follow standard protocols for these procedures in accordance with health surveillance recommendations.

Biorepository term was signed and contains the norms of storage and use in auxiliary and future studies. (Appendix 3)

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SPIRIT
STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

Supporting Information

S1_file. SPIRIT Checklist.

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	Item Nº	Description	Text location
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	Title
Trial registration	2ª	Trial identifier and registry name. If not yet registered, name of intended registry	Registry
	2b	All items from the World Health Organization Trial Registration Data Set	Data Set
Protocol version	3	Date and version identifier	Protocol version
Funding	4	Sources and types of financial, material, and other support	Funding
Roles and responsibilities	5ª	Names, affiliations, and roles of protocol contributors	Contributorship
	5b	Name and contact information for the trial sponsor	Sponsor contact information
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	Sponsor and Funder

	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	Committees
Introduction			
Background and rationale	6[a]	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	Background and rationale: §4, §5, §6, §7, §8, §9, §10, §11, §12
	6b	Explanation for choice of comparators	Choice of comparators: §1 and §2
Objectives	7	Specific objectives or hypotheses	Objectives
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	Trial Design
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	Study setting
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	Participants inclusion criteria and Participants exclusion criteria
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	Screening visits: §1, §2, §3 Assessment points: §1, §2, §3 Follow-up visits: §1 and §2 Blood sample

5. RESULTADOS E DISCUSSÃO

5.1. Artigo 2

Original Article

Methylphenidate impacts redox status in ADHD pediatric patients

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Abstract:

Background: Methylphenidate is highly effective in reducing ADHD symptoms, impacts mortality and morbidity, but its action mechanism remains partially unknown. There is some evidence that treatment may influence redox status, interfering in body homeostasis Methods: Sixty-two treatment-naïve ADHD children received methylphenidate for 24 weeks and redox parameters were measured at 3 follow-up time points (baseline, 12 and 24 weeks). Total antioxidant status, activity of superoxide dismutase, catalase and glutathione S transferase, lipid peroxidation and carbonyl protein were measured. Results: Changes in enzymatic antioxidants, lipid peroxidation and carbonyl protein occurred, suggesting increase in oxidative stress. Conclusion: There were signs of imbalance and worsening of oxidative stress in children using methylphenidate.

Keywords: ADHD; Antioxidative; Child; Methylphenidate; Oxidative

Introduction

Attention-deficit hyperactivity disorder (ADHD) is a neurodevelopmental disorder that affects 5% of children and 3% of adults (APA, 2013) and its etiology is attributed to a complex interaction of genetic, biological, and environmental factors, with twin studies attributing 70 to 80% of heritability. (Leffa et al.,2017)

In most cases, ADHD arises from several genetic and environmental risk factors that each have a small individual effect and act together to increase susceptibility. The multifactorial causation of ADHD is consistent with the heterogeneity of the disorder. (Faraone et al., 2015) Environmental factors such as low birth weight, prematurity, small size for gestational age, maternal substance use, and exposure to environmental toxins alongside polygenic risk may be involved with redox, inflammatory, immune system, neurotrophic factors, neurotransmitters, hormones changed status. (Tostes et al., 2012; Abdollahi et al., 2004; Binder and Scharfman, 2004; NG et al., 2008, Heyer and Meredith, 2017) ADHD's pathogenesis involves disruptions in dopaminergic and noradrenergic systems within prefrontal cortex circuits (Heyer and Meredith, 2017), so much so that the first-line treatment currently available, stimulants, acting by interfering with these neurotransmitters. (Advokat and Scheithauer, 2013; Cortese et al., 2018; Faraone SV et al. 2021)

Methylphenidate is highly effective in reducing ADHD symptoms mainly in children with the best benefit-to-risk ratios in this age group. (Cortese et al., 2018) Methylphenidate significantly impacts mortality and negative outcomes (school dropout, quality of life, criminality, injuries, other psychiatric diagnoses, transport accidents, sexually transmitted infections, suicide, substance abuse and teenage pregnancy. (Faraone et al., 2021) Although its action mechanism remains partially unknown, methylphenidate acts by blocking presynaptic dopamine (DA) and norepinephrine (NE) transporters, thus increasing catecholamine transmission. long-term use. There is some evidence that treatment could modify pro-inflammatory and oxidative profiles with dopamine neuron loss, microglia activation and increase in proinflammatory markers. (Quintero J et al., 2022) Catecholamine neurotransmitters (dopamine, epinephrine, and norepinephrine) can react with O_2 to produce superoxide and quinones/semiquinones that readily bind to sulfhydryl side chains and deplete the

already low cerebral GSH reserves. (Fraunberger EA, ET AL., 2015) Therefore, methylphenidate may influence redox status by its own hepatic metabolism, action mechanism and generation of highly reactive dopamine and DOPA quinones. (Miyazaki I, Asanuma M, 2008; Oakes HV et al., 2019)

Animal studies within murine model evaluated methylphenidate effects in redox imbalance. (Husson I et al., 2004; Martins MR et al., 2006; Fagundes AO et al., 2007; Andreazza AC et al., 2007; Gomes KM et al., 2007; Gomes KM et al., 2009; Schmitz F et al., 2011; Schmitz F et al., 2012; Comim CM et al., 2014; Loureiro-Vieira S et al., 2018; Foschiera LN et al., 2022) In generally, they showed relationship to oxidative stress altered parameters and damage, mainly in young animals, but the results are diverse and inconclusive. Foschiera LN et al (2022) made a review from animal studies and conclude that methylphenidate is capable of triggering oxidative stress even in an ADHD animal model. They suggested that monoamines metabolism and dopamine auto-oxidizes may contribute to redox imbalance and that young animals are more likely to present central nervous system damage, especially in long-term methylphenidate treatment. Adult rats could be more sensitive to the acute treatment effects.

Cross-sectional human studies suggest increased oxidative stress in unmedicated ADHD children when compared with health (Ceylan M et al., 2010; Oztop D et al., 2012; Ceylan MF et al, 2012; Kul M et al., 2015; Avcil S et al., 2019) and in medicated ADHD children (methylphenidate, atomoxetina) (Miniksar DY et al., 2023), but the results are preliminary and not conclusive. Guney (2015) suggested that MFD use for 12 weeks repaired oxidative imbalance primarily by increasing antioxidant defenses.

Redox status has been correlated with clinical and psychiatric pathophysiology in different age groups. Nevertheless, redox status has both physiological and pathophysiological roles in biology. (Sies and Jones, 2020; Ortis GG et al, 2017; Jîtcă G et al, 2022; Murphy MP et al., 2022) Changes in redox status levels are associated with imbalance in the activity of redox-sensitive cellular process and redox signaling, that plays a central role in several pathways to maintain body homeostasis. (Gagné François, 2014; Jîtcă G et al, 2022; Murphy MP et al., 2022)

Redox imbalance in psychiatry disorders has been correlated with depression and bipolar disorder (Kotzaeroglou A and Tsamesidis I., 2022)

schizophrenia (Jiao S, Cao T and Cai H., 2022; Rambaud V, Marzo A, Chaumette B., 2022), autism spectrum disorder (Usui N, Kobayashi H and Shimada S, 2023) and attention hyperactivity/impulsivity disorder (ADHD) (Ceylan M et al., 2010; Oztop D et al., 2012; Ceylan MF et al, 2012; Kul M et al., 2015; Avcil S et al., 2019; Miniksar DY et al., 2023) pathophysiology, but the results are still undefined.

The present study investigated biomarkers of redox status in treatment-naive ADHD children before and after 12 and 24 weeks of methylphenidate use to identify possible changes in antioxidant and oxidant parameters that may contribute to the ADHD' pathophysiology and treatment response.

Materials and Methods

Ethical Approval

This study was approved by the Research Ethics Committee of the Federal University of Minas Gerais. Number: 4.364.744. CAAE: 82870117.0.3001.5149, Minas Gerais, Brazil. The written informed consent and assent forms were obtained from at least one of the legal guardians and child respectively, after objective and procedures explanation.

Study design

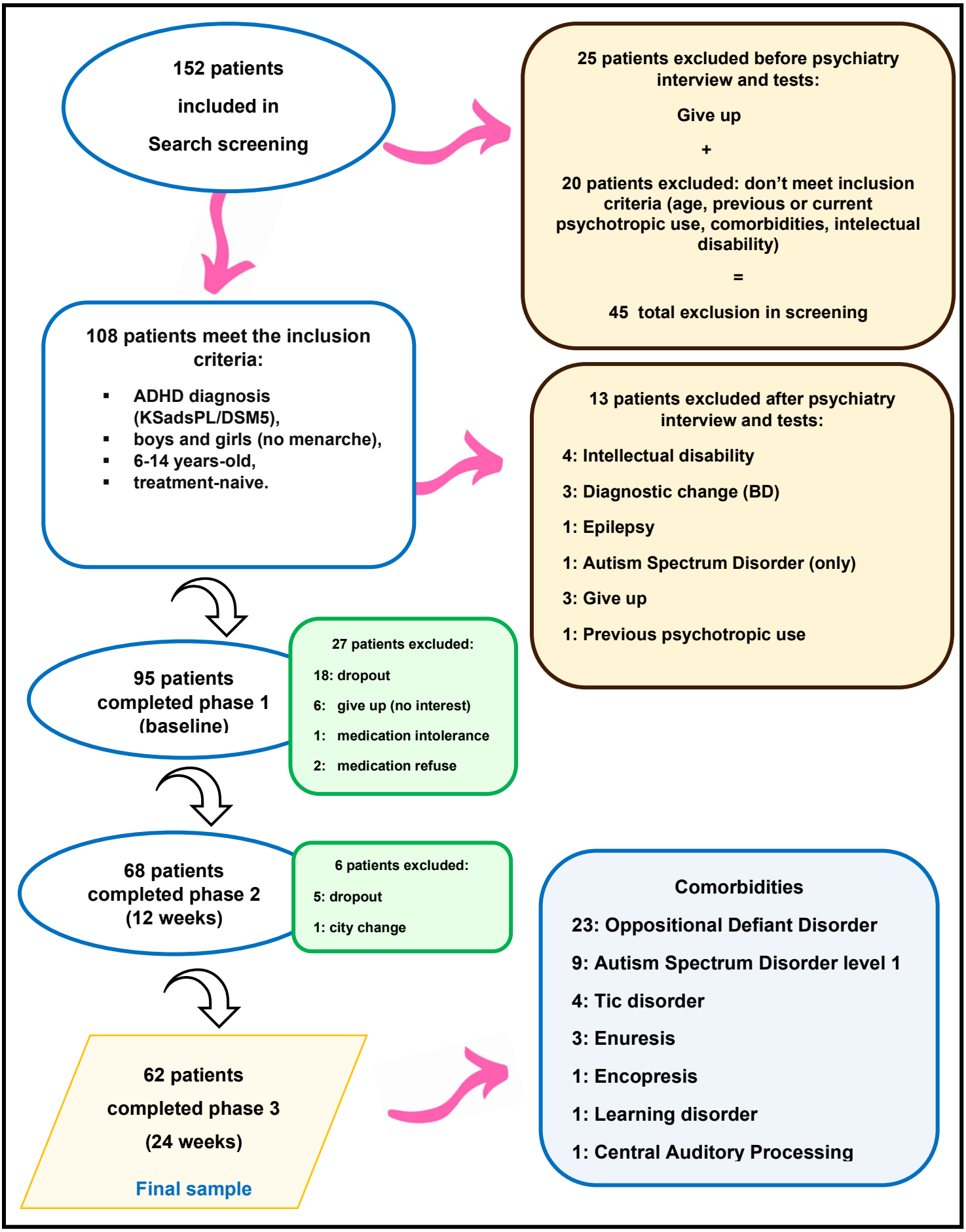
This trial used an open cohort single center design, with convenience sample. Study protocol were registered in ReBEC (Brazilian Registry of Clinical Trials) were is under review.

Subjects

All subjects were from the child psychiatry teaching outpatient clinic at the Medicine and Nursing Department in Federal University of Viçosa (UFV), state of Minas Gerais, Brazil. The study sample included 62 treatment-naïve ADHD children (44 boys, 18 girls), aged 6 to 14 years, that received a first-time diagnosis according to DSM-5 criteria. They were included between 2020, October to 2022,

august and were followed in this study for 24 weeks. (Figure 1) Children were included if were treatment-naïve ADHD children, of both sex in outpatient treatment; age between complete 6 and incomplete 15 years old. Children must have criteria for ADHD diagnosis according to the DSM-5 and must didn't have chronic diseases or use of any other medications. Children who need iron or vitamin replacement was included. The following comorbidities was included: oppositional defiant disorder (ODD), tic disorder, enuresis, encopresis, skin picking disorder. Autism spectrum disorder (ASD) level 1, Classification of Diseases and Related Health Problems (ICD) 10 F84.5, could be included if the diagnosis occurs during the study. Children were excluded if families and children refusal to participate in the research or refusal to use stimulant medications; presence of autoimmune, neurodegenerative diseases, and immunodeficiencies; intellectual disability, clinical or psychiatric comorbidities, except autism spectrum disorder level 1 (ICD 10 F84.5), ODD, tics, trichotillomania, skin picking disorder, enuresis, and encopresis; previous or current use of stimulants or other psychiatry medications; current use of medications for chronic diseases or any interfere in immune system or BDNF levels (antidepressants for example); girls who had menstruated; stimulant contraindication use were excluded.

Figure 1. Children recruitment process.



Investigations and instruments

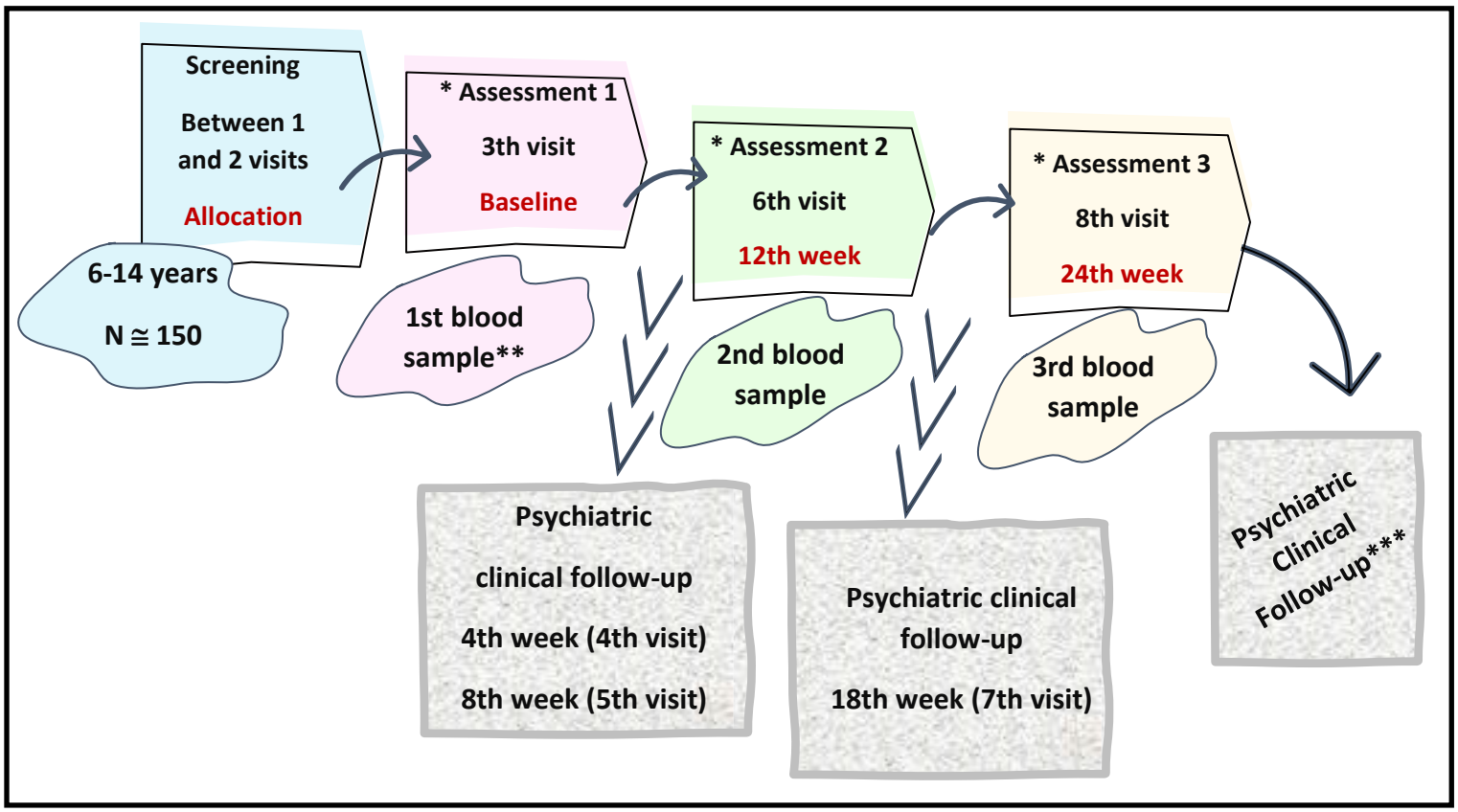
Each participant had about 8 visits during 24 weeks of the study (Figure 2). The initial assessment includes the semi-structured interview Kiddie-Schedule for Affective Disorders and Schizophrenia – Present and Lifetime Version (K-Sads-PL 2013), used to screen psychiatry disorders (Caye A et al., 2017). The frequency of ADHD and ODD symptoms were evaluated by the 26-itens SNAP-IV scale, Swanson, Nolan e Pelham version (Mattos P et al., 2006) and the Assessment Scale of Childhood and Adolescent Behaviors in ADHD in a Family Environment (ETDAH-parents) (Benczik E, 2018). The ETDAH scale parent' version investigates symptoms of inattention, hyperactivity/impulsivity, emotional regulation, and adaptive behavior (Benczik E, 2018). The diagnosis was confirmed by diagnostic interview with an experient Child and Adolescent Psychiatrist. Sociodemographic data was obtained from the Brazil Economic Ranking Criterion 2022 (Kamakura W and Mazzon JA, 2016; ABEP, 2022) and "Background information" part of the K-Sads-PL instrument (K-Sads-PL 2013) (Caye A et al., 2017). The children intelligence levels were determined Non-Verbal test of Children's Reasoning (TNVI) test was administered by experienced psychologist (Pasquali L, 2005) and through clinical interviews conducted by an experienced child psyquiatry specialist. All children who had the percentil classification in "intelectual disability" category was excluded. A standard clinical history was collected with data on Pregnancy, childbirth, neuropsychomotor development, and the child's previous and family history. Habitual physical activity questionnaire (Fernandes C, 2012), sleep questionnaire (Fernandes C, 2012 and Ferreira V, 2009) and instrument of measuring adherence to treatments (Delgado AB and Lima ML, 2011) were applied. Situations of violence and/or health issues identified during the research were duly forwarded to the necessary assistance network.

Timeline

There were three assessment timepoints: baseline (before methylphenidate use) and after 12 and 24 weeks of methylphenidate treatment. (Fig.1). In the first, blood sample is collected, family receives psychoeducation, and the methylphenidate is started. In the second -12 weeks- and third - 24 weeks - assessment, the symptoms were assessed. In these two timepoints,

standardized follow-up anamnesis was used. Treatment effects were measured using the Clinical Global Impressions (CGI) scale (Busner J et al., 2007), dose adjustment, presence of adverse effects, adherence to treatment. Participants were evaluated at 4, 8 and 18 weeks, dose adjustment, presence of adverse effects and adherence to treatment were evaluated. A day before, nurse confirmed the consultation by telephone (calls or WhatsApp messages). In case of absence, contact by phone was made to offer appointment rescheduling.

Figure 2. The participants visit (adapted from study protocol by these authors)



* Assessment points (Psychiatric clinical follow-up, Sample Blood, Questionnaires and Scales)

** Methylphenidate prescription after first blood sample.

***Children who finished the trial times were transferred to the general outpatient clinic of child psychiatry at the UFV medical school.

Methylphenidate treatment

Methylphenidate hydrochloride (immediate-release tablets) were used by all the children. The initial dose was prescribed as follow: 5mg in the morning and after lunch in the first week, with adjustment to 10mg twice a day in the next week until the evaluation after 4 weeks. From that moment on, the dose was titrated to optimize therapeutic effect and minimize adverse effects until 1mg/kg/day. The average dose was 0,65 mg/kg/day (0,65 ±0,19 and 0,65 ± 0,22 with 12 and 24 weeks respectively. The assessment methylphenidate adherence was by pill count and the Treatment Adherence Measure (MAT) questionnaire (Delgado AB and Lima ML, 2011) in all scheduled visits.

Biological samples

Three venous blood sample were collected from each participant: before medication treatment, 12 and 24 weeks after methylphenidate treatment. Following average 12 hours fast (range between 10 and 12 hours) in assessment 1, 2 and 3 respectively, blood samples were obtained in the morning, between 8 and 9 a.m. to adjust for any potential circadian rhythm effect in oxidative status. The average children corporal temperature (non-contact infrared forehead thermometer) was taken in three assessments. The samples were allowed to clot at room temperature for one hour and were not protected from light. The samples were then centrifuged for 10 minutes at 3000 rpm. The serum of the samples was separated and kept at - 20ºC in Eppendorf tubes until further analysis in the Immunochemistry and Glycobiology Laboratory of the General Biology Department of UFV.

Oxidative profile determination

The serum was used to all analyses. Serum TAS (total antioxidant status – FRAP - Ferric Reducing Antioxidant Power) was measured using fully automated colorimetric assay developed by Erel (2004) based on measurements of the OH radicals. FRAP measure the non-enzymatic antioxidant capacity. Catalase (CAT) activity was assayed by measuring the rate of decrease of H_2O_2 absorbance at 240 nm (Aebi H, 1984). Estimation of serum glutathione-S-

transferase (GST) was carried out as reported by Habig WH, Pabst MJ and Jakoby WB. (1974). Superoxide dismutase (SOD) activity was assayed by measuring the inhibition of pirogalol auto-oxidation, as described by Bannister JV and Calabrese L (1987). As a sensitive index of lipid peroxidation, we used the formation of thiobarbituric acid reactive substances (TBARS) during an acid heating reaction (Draper HH and Hadley M, 1990). The results are expressed as MDA (malondialdehyde) equivalents (nmol/mL protein). Briefly, the samples were mixed with 1 ml of TCA 10% and 1 ml of thiobarbituric acid 0.67%, then heated in a boiling water bath for 15 min. TBARS were determined by the absorbance at 535 nm. The oxidative damage to proteins was assessed by the determination of carbonyl groups based on the reaction with dinitrophenylhydrazine (DNPH), as previously described (Levine et al., 1990). Briefly, proteins were precipitated by the addition of 20% trichloroacetic acid (TCA) and redissolved in DNPH and the absorbance read at 370 nm. Introduction of carbonyl groups into amino acid residues of proteins is a hallmark for oxidative modification. Reaction of these groups with carbonyl specific reagents provides methods for detecting and quantitating metal-catalyzed oxidation. (Levine et al., 1990)

Statistical analyses

The Shapiro-Wilk and Kolmogorov-Smirnov test was used for normality analysis. Absolute and relative frequencies were used for numeric variables that were described by their means and standard deviations. Tukey's parametric test and variance analysis ANOVA (f) were performed for multiple comparisons in redox parameters and other variables respectively. Redox parameters analysis was performed using GraphPad Prism 7.0 program (GraphPad Software, Inc. San Diego, CA, USA) and other variables using SPSS software (version 23.0 for Windows). Statistical significance was considered at $p<0.05$.

Results

Participants characterization

The children average age was 8,58 ± 1,91, ranging from 6 to 14 years old with male predominance (71%) (Table 1).

Table 1. Children age and sex.

Variable	Male	Female	Total
Age (N years ± SD)	8,62 ± 2,04	8,64 ± 1,52	8,58 ± 1,91
Sex (N / %)	44 (71)	18 (29)	

* N = absolute number; SD = standard deviation; % = percentage number

Most children were from elementary school (82,3%) of state schools (80,6%), without failure years (96,8%) (Table 2).

Table 2. Children school data.

Child Schooling		
Kindergarten	3	4,8
Elementary school (first year)	**11**	**17,7**
Elementary school (second year)	**14**	**22,6**
Elementary school (third year)	**7**	**11,3**
Elementary school (fourth year)	**13**	**21**
Elementary school (fifth year)	**6**	**9,7**
Middle school (sixth year)	4	6,5
Middle school (seventh to ninth year)	3	4,8
No information	1	1,6
School Failure		
No	**60**	**96,8**
No information	2	3,2
Type of school		
Public	**50**	**80,6**
Private	11	17,7
No information	1	1,6

* N = absolute number; SD = standard deviation; % = percentage number

Biological mother and/or biological father being the householder. Regarding electronic devices, 79% of the families have at least two smartphones and 45,2% e 83,9% don’t have computer/notebook and tablets respectively (Table 3).

Table 3. Family sociodemographic and income data.

Variables	N	%
Socioeconomic data (Brazil Economic Ranking Criterion, 2022)		
Class A (average income of R$ 21.826,74)	2	3,2
Class B1 (average income of R$ 10.361,48)	4	6,5
Class B2 (average income of R$ 5.755,23)	**10**	**16,1**
Class C1 (average income of R$ 3.276,76)	**11**	**17,7**
Class C2 (average income of R$ 1.965,87)	**14**	**22,6**
Class D/E (average income of R$ 900,60)	6	9,7
No information	15	24,2
Smartphone (number of devices)		
0	5	8,1
1	6	9,7
2	**23**	**37,1**
3	**17**	**27,4**
4	**9**	**14,5**
No information	2	3,2
Notebook or Computer (number of devices)		
0	**28**	**45,2**
1	22	35,5
2	8	12,9
3	1	1,6
4	1	1,6
No information	2	3,2
Tablets (number of devices)		
0	**52**	**83,9**
1	9	14,5
No information	1	1,6
Householder		
Biological father	**22**	**35,5**
Biological mother	**22**	**35,5**
Both parents	9	14,5
Others	9	14,5
Father schooling		
Illiterate/incomplete elementary school	3	4,8
Complete elementary school - incomplete middle school	**7**	**11,3**
Complete middle school - incomplete high school	**8**	**12,9**
Complete high school - incomplete graduate	**8**	**12,9**
Complete higher education - incomplete university education	5	8,1
Complete university education - postgraduate	2	3,2
No information	29	46,8
Mother schooling		
Illiterate/incomplete elementary school	2	3,2
Complete elementary school - incomplete middle school	5	8,1
Complete middle school - incomplete high school	**8**	**12,9**
Complete high school - incomplete graduate	**23**	**37,1**
Complete higher education - incomplete university education	**8**	**12,9**
Complete university education - postgraduate	6	9,7
No information	10	16,1

* N = absolute number; % = percentage number

Clinical and laboratory data do not have a difference in three points: capillary blood glucose, diastolic blood pressure, physical activity level. There was significant difference in body mass index (BMI), systolic blood pressure, heart rate and body temperature in the three assessments (Table 4).

Table 4. Physical exam, physical activity, and screen time data

Mean ± SD or N / %	Baseline		Assessment 1		Assessment 2		Comparision
Capillary blood glucose (mg/dL)	94	± 11	93	± 11	93	± 7	*p = 0,687*
Body mass index (Kg/m²)	**17,92**	**± 3,74**	16,94	± 2,96	16,72	± 2,99	***p < 0,001***
Blood Pressure – Systolic (mmHg)	101	± 8	104	± 9	105	± 9	***p = 0,005***
Blood Pressure – Diastolic (mmHg)	59	± 9	61	± 9	61	± 8	*p = 0,131*
Heart Rate (bpm)	**79,23**	**±11,85**	86,50	±12,58	87,40	±13,75	***p < 0,001***
Temperature (ºC)	**36,3**	**±0,3**	**36,4**	**±0,3**	**36,5**	**±0,3**	***p< 0,009***
Physical activity (week/hours)	17,7	23,06	13,4	13,52	16,78	25,55	*p = 0,294*

* N = absolute number; SD = standard deviation; % = percentage number.

Twenty-three (37%), 12 (19,3%) and 13 (21%) of the children had BMI higher than expected for age in assessment 1, 2 and 3 respectively (Table 5). The underweight children were between 3,4 - 4,8%. Most children report sleep at least 8 hours per night (75,4% - 79%).

Table 5. Nutritional status and sleep data.

Mean ± SD or N / %		Baseline		Assessment 1		Assessment 2	
Nutritional Status	**Underweight**	3	4,8%	2	3,4%	3	4,8%
	Healthy Weight	36	58,1%	45	76,3%	46	74,2%
	Overweight	**11**	**17,7%**	**4**	**6,4%**	**5**	**8,1%**
	Obesity	**12**	**19,4%**	**8**	**12,9**	**8**	**12,9%**
Sleep time	**9-11 hours**	**34**	**54,8**	**38**	**61,3**	**35**	**56,4**
	8-9 hours	**14**	**22,6**	**11**	**17,7**	**12**	**19,4**
	7-8 hours	4	6,4	5	8	4	6,4
	5-7 hours	2	3,2	0	0	2	3,2
	Less than 5 hours	0	0	0	0	0	0
	No information	8	13	8	13	9	14,6

* N = absolute number; SD = standard deviation; % = percentage number.

Clinical ADHD characterization

ADHD-combined presentation was present at 58,1% (n = 36) of the children, while 32,3% (n = 20) were diagnosed with ADHD-inattentive presentation and 9,7% (n = 6) were diagnosed with ADHD-hyperactive/impulsive presentation. (Table 6) The ADHD severity was at least classified by moderately ill, most of which (74,2% of the children) with severely or the most extremely ill. Furthermore, 37,1% (23 children) and 14,5% (9 children) had the comorbidity oppositional defiant disorder and autism spectrum disorder level 1 respectively. The most of children showed very much or much disease clinical improvement (Table 6).

Table 6. ADHD presentation, comorbidities, disease severity and improvement.

Variables		**N**	**%**
ADHD presentation (SNAP IV)	Inattention	20	32,3
	Hyperactive/Impulsive	6	9,7
	Combined	**36**	**58,1**
Disease severity	Moderately ill	16	25,8
	Severely ill	**28**	**45,2**
	Among the most extremely ill	18	29
Comorbidities	Autism Spectrum Disorder	9	14,5
	Oppositional Defiant Disorder	**23**	**37,1**
	1 comorbidity	**33**	**53,2**
	2 comorbidities	3	4,8
	3 comorbidities	1	1,6
Variables		**Assessment 1 N (%)**	**Assessment 2 N (%)**
Disease improvement	**Very much improved**	**30 (48,4)**	**39 (62,9)**
	Much improved	**20 (32,3)**	**16 (25,8)**
	Minimally improved	10 (16,1)	5 (8,1)
	No change	2 (3,2)	2 (3,2)
	Total number of children	**62**	

* N = absolute number; % = percentage number

Methylphenidate treatment

The average methylphenidate dose (immediate release) was 0,65 mg/kg/day (0,65 ±0,19 and 0,65 ± 0,22 with 12 and 24 weeks respectively. The methylphenidate adherence level by MAT was 98,4% and 93,5%, with 12 and 24 weeks follow up respectively and by pill count was 53,2% and 54,8% respectively.

Oxidative profile

Patient inflammatory profile under methylphenidate treatment was evaluated in serum (Table 7).

Table 7. Redox biomarkers. FRAP, CAT, SOD, GST, MDA equivalents and proteins Carbonyl values (Mean ± SD).

Mean ± SD		Baseline	Assessment 1	Assessment 2	Comparision
ANTIOXIDANTS	**FRAP (nM/mL)**	624,8499 ± 159,2754[a]	615,0789 ± 234,6292[a]	619,0329 ± 192,4404[a]	*p = 0,954*
	CAT (KU/mL)	20,1239 ± 24,19588[a]	19,36193 ± 23,28952[a]	**↑ 61,50474 ± 47,49694[b]**	***p < 0,001***
	SOD (U/mL)	40,3749 ± 7,610411[a]	**↓ 28,32253 ± 12,46116[b]**	41,95306 ± 8,613972[a]	***p < 0,001***
	GST (U/mL)	ND	ND	ND	*not applicable*
OXIDANTS	**MDA (nmol/mL)**	9,046229 ± 4,155266[a]	8,001275 ± 3,300032[b]	**↑ 12,61539 ± 8,183838[a]**	***p < 0,001***
	PROTEINS CARBONYL (nmol/mL)	**↑ 89,58096 ± 37,6040[b]**	70,65574 ± 27,83834[a]	67,08874 ± 29,46145[a]	***p < 0,001***

* FRAP = Ferric Reducing Antioxidant Power (Total antioxidant status); CAT = catalase; SOD = superoxide dismutase; GST = glutathione – S – transferase; MDA = malondialdehyde equivalents (lipidic peroxidation – TBARS – thiobarbituric acid reactive substances); ND = not detectable

The total antioxidant status or total antioxidant capacity of non-enzymatic agents measured by FRAP remained unchanged over the 24 weeks of methylphenidate use (Figure 3).

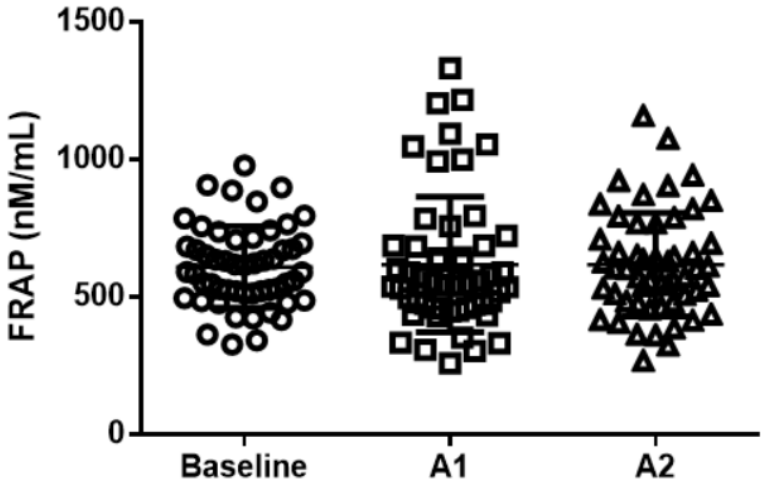


Figure 3. Total antioxidant capacity by ferric reduction (FRAP). Serum quantification of ADHD patients before the treatment (Baseline), after 12 or 24 weeks (A1, A2).

Serum enzymatic antioxidants, SOD and CAT, were evaluated. SOD activity, measured after 12 weeks of methylphenidate treatment, showed significant reduced serum values compared both at baseline and after 24 weeks of methylphenidate treatment. On the other hand, catalase showed significant higher serum values after 24 weeks of treatment with methylphenidate compared to baseline and 12 weeks of methylphenidate treatment. GST levels were undetectable in the three assessments (Figures 4).

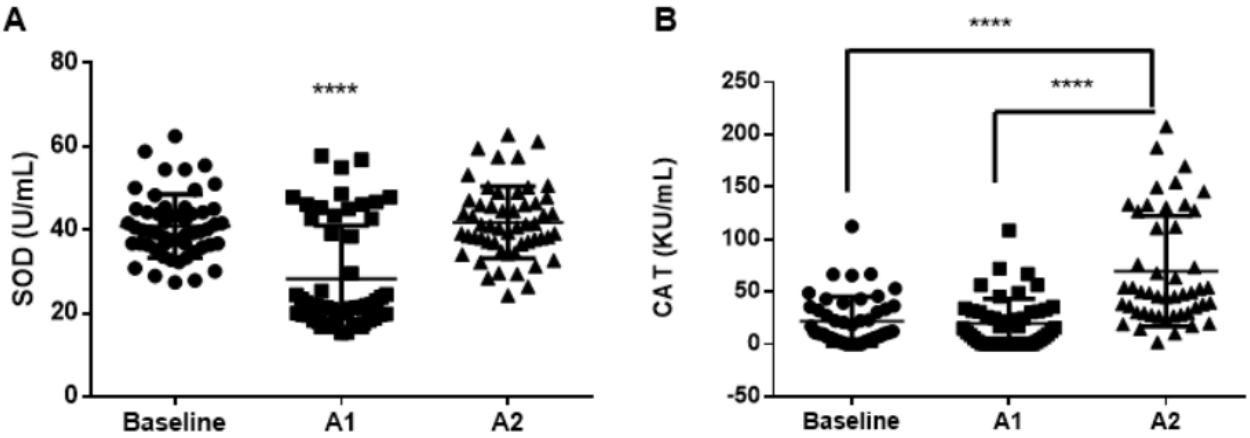


Figure 4. Antioxidant enzymatic activity**.** Serum quantification of SOD (A) and CAT (B). **** $p \leq 0{,}001$. Serum quantification of ADHD patients before the treatment (Baseline), after 12 or 24 weeks (A1, A2).

The lipidic peroxidation, evaluated by the formation of MDA, showed a significant increase in 24 weeks when compared to 12 weeks of methylphenidate use. Proteins carbonyl, another oxidative damage biomarker, had significant increased level in baseline when compared with 12th and 24th methylphenidate treatment weeks (Figure 5)

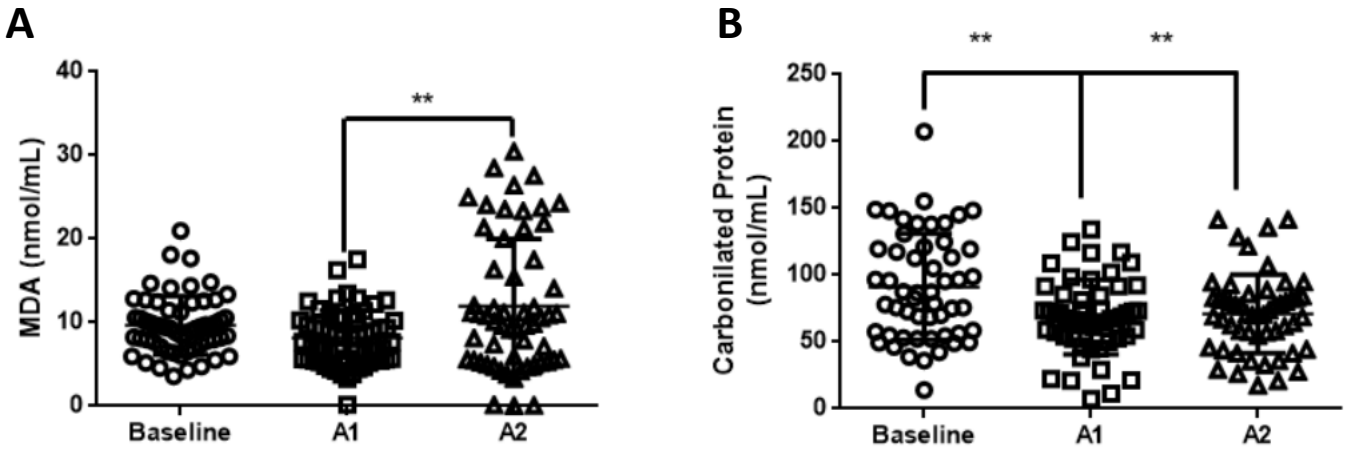


Figure 5. Quantification of damage markers related to oxidative stress. (A) MDA and (B) Protein carbonyl. Serum quantification of ADHD patients before the treatment (Baseline), after 12 or 24 weeks (A1, A2). ** $p \leq 0,01$.

Discussion

Human studies (Ceylan M et al., 2010; Oztop D et al., 2012; Ceylan MF et al, 2012; Guney et al., 2015; Joseph et al., 2015; Kul M et al., 2015; Avcil S et al., 2019; Miniksar DY et al., 2023) bring preliminary results of oxidative stress, ADHD e methylphenidate interplay. In generally, they are cross-sectional, with small samples and suggest increased oxidative and decreased antioxidants parameters in ADHD children with or without ADHD medication.

Our longitudinal clinic trial showed that oral immediate release methylphenidate (average dose 0,65mg/Kg/day) given to drug treatment naïve ADHD children for 24 weeks were associated with changes in redox profile suggesting redox imbalance and consequent oxidative damage risk.

Epidemiologic and clinical studies showed that ADHD is more common in males than females and often co-occurs with other psychiatric disorders. (Rodhe

LA et al., 2005; Ceylan MF et al., 2012; Avcil S et al., 2019; Faraone SV et al., 2021) In our study, we observed predominance of boys ADHD combined with oppositional defiant disorder comorbidity in almost 40% of children. Regarding nutritional status, our findings confirm previous studies that demonstrated children and adolescents with unmedicated ADHD were about 20 % most likely to be overweight or obese when compared with health children and the overweight/obese risk is greater in adults. (Nigg JT et al., 2016; Faraone et al., 2021) Weight loss may occur with methylphenidate (Cortese S et al., 2018; Faraone et al., 2021) and was significant with our children. Increased systolic blood pressure and heart rate are common adverse effects with stimulants (Cortese S et al., 2018; Faraone et al., 2021), but clinically they were not significant in our study. In literature, stimulants moderately reduced total sleep time (Faraone et al., 2021), but the most children in our study kept at least 8 hours per night during 24 follow-up weeks. Most children had a severe ill or most extremely ill level of symptoms (about 74%) and clinical improvement was classified as “much” or “very much” improvement in about 80% and 89% in second and third assessments respectively and there was no worsening of ADHD symptoms in any of the 62 children. Spencer T et al., (2005) found a marked therapeutic response for the methylphenidate treatment of ADHD symptoms that exceeded the placebo response (76% vs. 19%). Treatment was safe and well tolerated and the response to methylphenidate was independent of socioeconomic status, gender, and lifetime history of psychiatric comorbidity. (Spencer et al., 2005)

Regarding antioxidant status, we evaluated non-enzymatic (FRAP) and enzymatic parameters (superoxide dismutase, catalase, and glutathione S transferase activities). Antioxidants are substances that inhibit, delay, or slow the oxidation reactions rate and they act preventing the generation of free radicals, intercepting the generated radicals, repairing damage structures, and signaling redox pathways. (Desai, S. N., Farris, F. F., & Ray, S. D., 2014; Ighodaro OM, 2018)

Total antioxidant status (FRAP) showed no significant difference in the three assessments. Kul Mulsum et al., (2015) found lower total antioxidant status in ADHD children (without stimulants), when compared with health control group, this reduction was more important in ADHD children with oppositional defiant

disorder (ODD). Our study demonstrated also no different FRAP levels in relation to ADHD-associated comorbidities. Corroborating with our study, Miniskar DY et al., (2023) also found no changes in total antioxidant status when comparing children with ADHD to health controls and neither in children with ADHD methylphenidate/atomoxetine treated compared with children with ADHD without medication. In murine studies, chronic methylphenidate treatment in young rats showed decreased total non-enzymatic radical-trapping antioxidant (Schmitz F et al., 2011)

On the other hand, enzymatic antioxidant defenses showed changes. Enzymatic activity is the first redox defense line, working together to prevent superoxide radical accumulation and related damage. (Ighodaro OM and Akinloye OA, 2018) We found significant reduced superoxide dismutase activity level after 12 weeks of methylphenidate treatment, that could represent its consumption to organism protection against excessive superoxide levels, byproduct of oxygen metabolism. After 24 weeks of methylphenidate treatment, superoxide dismutase levels are comparable to baseline levels, suggesting that the organism used compensatory mechanisms to restore redox homeostasis, but they could not be sufficient to prevent oxidative damage.

Low SOD activity would be associated with superoxide accumulation and this radical could form very reactive oxygen species, including the hydroxyl radical for which we don’t have enzymatic detox systems. Consequently, these highly reactive forms could rapidly participate in toxic reactions in the body (lipid peroxidation, protein carbonylation, DNA damage and carbohydrates glycosylation), and may compromise homeostasis. (Mao GD, 1993; McCord JM, 2008; Forman HJ, Zhang H., 2021) Superoxide radical can act both as initiator and terminator of lipid peroxidation. Any concentration of SOD, other than the optimal may lead to increased lipid peroxidation and therefore to increased oxidative stress. In our results, the SOD activity in the 12ª week follow-up may have been inhibited by excess superoxide radical that was directed to damage reactions. Both the reduction in SOD activity at the 12th week, as well as its restoration at the 24th week may overlap with changes in the activity catalase enzyme showed. Superoxide radical impacts in the activity of SOD and oxygen peroxide generations which impacts in the catalase activity. (Mao GD, 1993; McCord JM, 2008)

Significant increase in catalase activity at 24th follow up week was found, when compared with baseline and 12th week of methylphenidate treatment. Reduced catalase activity in the 12th week may suggests that the reduced SOD produced less amount of catalase substrate (H_2O_2).

Ceylan M et al., (2010) found no significant difference in catalase and superoxide dismutase activity levels in ADHD children when compared with health children, but glutathione peroxidase activity in ADHD children was increased when compared with health children. Ceylan MF et al., (2012) in another study found glutathione S transferase in ADHD children significantly lower than control group. Miniksar D Y et al., (2023) found superoxide dismutase activity in ADHD children significantly lower than control group. In murine studies, chronic methylphenidate treatment in young rats was associated with increased SOD/CAT activity in cerebellum and prefrontal cortex (Schmitz F et al., 2012); acute and chronic methylphenidate in adults' rats showed decreased SOD and CAT activities (Comin et al., 2014). Gomes et al., (2008) found that acute methylphenidate treatment decreased SOD activity in pre-frontal cortex, increase SOD activity in brain cortex and decreased CAT activity in hippocampus. Chronic methylphenidate treatment increased SOD activity in the hippocampus and brain cortex and it decreased in the striatum. (Gomes et al., 2008) Chronic methylphenidate treatment in young rats increased catalase activities. (Schmitz F et al., 2011)

Our data demonstrate a treatment time dependent response in lipid peroxidation. Levels of lipid peroxidation, a biomarker of oxidative damage, showed significant increase in children with 24-weeks methylphenidate treatment when compared with twelve weeks of treatment. Therefore, this result suggested that at 24 weeks of methylphenidate treatment, there was an imbalance in the redox status in favor to oxidative parameters, leading to excessive amount of oxygen reactive species not counteracted by antioxidant defense, resulting in lipid peroxidation. (Desai, S. N., Farris, F. F., & Ray, S. D., 2014; Gagne F, 2014; Ortis GG, 2017; Ighodaro OM and Akinloye OA, 2018) Ceylan M et al., (2010) and Miniksar DY et al., (2023) also found increased MDA (malondialdehyde) levels in ADHD children when compared with health control group. Miniksar DY et al., 2023 found similar results to those found in our trial: high MDA level and low SOD activity and he suggested that this association may be predictor of

ADHD diagnosis in children and adolescents. Ostop D et al., (2012) found opposite result, with MDA (malondialdehyde) levels decreased in ADHD children when compared with health control group. These three studies are cross-sectional. In murine studies, the findings are also mixed. Greater methylphenidate dosage was associated with lipid peroxidation increase in the cerebellum, prefrontal cortex, hippocampus, and striatum in young animals chronically exposed to methylphenidate. (Husson I et al., 2004; Martins MR et al., 2006) Chronic methylphenidate treatment in young rats decreased thiobarbituric acid reactive substances. (Schmitz F et al., 2011), but acute and chronic methylphenidate in adults' rats increased TBARS. (Comin et al., 2014) Chronic methylphenidate treatment in young rats increase lipid peroxidation in prefrontal cortex. (Schmitz F et al., 2012)

The reactive oxygen species (ROS) can also direct damage lipids and alter fluidity and permeability of lipidic membrane, as well as promote ions transport alterations, inhibition of metabolic process, mitochondria injury and inefficient antioxidant defense systems. (Desai, S. N., Farris, F. F., & Ray, S. D., 2014) The lipidic membrane peroxidation may promote cell survival or induce cell death (apoptosis, necrosis), depending on subtoxic (low lipid peroxidation rates) or toxic (medium to high lipid peroxidation rates) conditions respectively. But both processes eventually lead to molecular cell damage, facilitating development of various pathological states and accelerated aging. Malondialdehyde (MDA) and 4-hydroxy-2-nonenal (4-HNE), products of peroxidation lipidic decomposition, can participate in secondary deleterious reactions by promoting intramolecular or intermolecular protein/DNA injury and more reactive species production. (Ayala A, Munõz MF, Argüelles S, 2014; Desai, S. N., Farris, F. F., & Ray, S. D., 2014)

Protein carbonyl level, permanent oxidative damage biomarkers, was increased in baseline when compared with 12^{th} and 24^{th} methylphenidate treatment weeks. In murine studies, different results were found: chronic methylphenidate treatment in young rats showed increase in protein damage in prefrontal cortex. (Schmitz F et al., 2012); acute and chronic methylphenidate in adults' rats increased carbonyl groups in the brain. (Comin et al., 2014) Small protein modifications may result in significant impact on protein function and are pivotal for cell signaling and survival. The proteins may be directly attacked by free radicals or from metabolites of lipid peroxidation process. This damage can

be reversible (nitrosylation) or irreversible (carbonilation). (Panis C, 2014) So, the protein oxidative injury, may affect potentially all protein dependent systemic functions such as antibodies, cell receptors, membrane structures, enzymes, and signal transduction proteins. In addition, oxidized proteins can trigger anomalous responses in the organism, being recognized as foreign structures. (Fedorova M, Bollineni RC, Hoffmann R., 2014; Panis C, 2014; Sies H, Berndt C and Jones DP, 2017) The oxidative damage proteins in the circulation may be repair or removal. A limited number of protein damages are repairable, and the most protein damages are removal by hepatic and phagocytic clearance mechanisms. Then, they are degraded intracellularly by the proteasome. (Griffiths HR et al., 2014) Lipid peroxidation and protein carbonyl changed in opposite directions, while the first had significant levels increase, protein carbonyl showed a level reduction. It can be hypothesized that the dysfunctional proteins may be removed by the hepatic and phagocytic clearance system looking for redox balance, when the antioxidant defense showed signs of SOD and CAT activities levels improvement. It highlights that ADHD children may have elevated protein carboxyl levels and may be associated with disorder pathophysiology. Future studies with control group, largest samples and longer follow-up time may help to clarify these gaps. Regarding changes in redox parameters in our study, we believe that there was no influence of blood collection variables. Blood temperature changed significantly between the three assessments, although this variation was clinically minimal. Therefore, our findings showing changes in redox status, signaling an increase in oxidative damage biomarkers, suggest that stimulants prescription need to remain careful. Nonetheless, methylphenidate treatment positively impacted the carbonyl proteins levels, showing reduced levels of these permanent oxidative damage biomarkers in 12^{th} and 24^{th} of stimulant use, when compared with baseline levels. Stimulants have important clinical role, high response power, impact on morbidity and mortality, but, in clinical practice, has been used for much longer periods than those used in studies, as well as in age groups with less safety evidence, such as children under 6 years and the elderly. (Cortese S et al., 2018; Sassi et al., 2020) Outside of clinic practice, people have used for off label purposes.

Conclusions

Our open cohort study evaluated the effect of methylphenidate treatment in redox parameters, showing that in ADHD children, methylphenidate may modify oxidative and antioxidative parameters, promoting a time dependent increase in lipidic peroxidation. Nonetheless, methylphenidate treatment positively impacted the carbonyl proteins levels, showing reduced levels of these permanent oxidative damage biomarkers in 12^{th} and 24^{th} of stimulant use, when compared with baseline levels. Therefore, it's not clear how and how much the brain buffering mechanisms act on these changes, nor how the results of these systems interaction can impact neurodevelopment throughout life, especially in young children.

Supplementary Materials: The following supporting information can be downloaded at: www.mdpi.com/xxx/s1, Figure S1: title; Table S1: title; Video S1: title.

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5.2. Artigo 3

Systematic Review and Metanalysis

Brain-Derived Neurotrophic Factor (BDNF) levels in children and adolescents before and after stimulant use a systematic review and metanalysis

O protocolo da revisão sistemática foi registrado no International Prospective Register of Systematic Reviews (PROSPERO; https://www.crd.york.ac.uk/prospero), intitulada “Brain-derived neurotrophic fator (BDNF) levels in children and adolescents before and after stimulant use: a systematic review”. Com número de identificação 261519.

PROSPERO
International prospective register of systematic reviews

National Institute for Health Research

University of York
Centre for Reviews and Dissemination

Systematic review

1. * Review title.

Give the title of the review in English

Brain-derived neurotrophic factor (BDNF) levels in children and adolescents before and after stimulant use: a systematic review.

2. Original language title.

For reviews in languages other than English, give the title in the original language. This will be displayed with the English language title.

Níveis de BDNF em crianças e adolescentes antes e após uso de estimulante: uma revisão sistemática.

3. * Anticipated or actual start date.

Give the date the systematic review started or is expected to start.

17/06/2021

4. * Anticipated completion date.

Give the date by which the review is expected to be completed.

17/01/2022

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Brain-derived neurotrophic factor (BDNF) levels in children and adolescents before and after stimulant use a systematic review and metanalysis

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ABSTRACT

Background: Attention deficit hyperactivity disorder (ADHD) is a neurodevelopmental disorder associated with cognitive, social, and academic impairment. Neurotrophins, particularly brain-derived neurotrophic factor (BDNF), have been implicated in the pathophysiology of ADHD and response to stimulant treatment. This review aims to investigate the relationship between BDNF levels in ADHD before and after treatment with stimulants in childhood.
Methods: This systematic review followed PRISMA-P guidelines and included 19 studies from PubMed, EMBASE, Cochrane, Capes Periodic, and Lilacs databases. The studies were evaluated for risk of bias and level of evidence.
Results: There was no significant difference in peripheral BDNF levels in ADHD children before or after methylphenidate treatment. Additionally, there was no statistically significant difference in BDNF levels between children with ADHD and controls.
Discussion: Understanding the role of BDNF in ADHD may provide insight into the disorder's pathophysiology and facilitate the development of biological markers for clinical use.
Conclusion: Our findings suggest that BDNF levels are not significantly affected by methylphenidate treatment in ADHD children and do not differ from controls.
Systematic review registration: "Brain-derived neurotrophic factor (BDNF) levels in children and adolescents before and after stimulant use: a systematic review". Number CRD42021261519.

1. Background

Attention deficit/hyperactivity disorder (ADHD) affects 5% of children and 3% of adults (APA, 2013), with twin studies attributing 70 to 80% of its etiology to heritability (Leffa et al., 2017). Diagnosis typically occurs in childhood and involves symptoms of inattention and hyperactivity/impulsivity before the age of 12, with intensity and duration higher and longer than expected for the age group evaluated, appearing in at least two environments and causing harm to the individual (APA, 2013).

Environmental factors such as low birth weight, prematurity, small size for gestational age, maternal substance use, and exposure to environmental toxins alongside genetic factors play a role in ADHD's etiology (Heyer and Meredith, 2017. An intricate interplay among genetic, environmental, oxidative, and inflammatory factors (NG et al., 2008) governs the mechanisms predisposing individuals to neuropsychiatric

Abbreviations: BDNF, Brain-Derived Neurotrophic Factor; ADHD, Attention-Deficit/Hyperactivity Disorder; PRISMA-P, Preferred Reporting Items for Systematic Reviews and Meta-Analyses Protocols.; PROSPERO, International Prospective Register of Systematic Reviews.; DECs, Descritores de Ciência e Saúde.; BDTD, Biblioteca Digital Brasileira de Teses e Dissertações.; CADTH, Canadian Agency for Drugs, Technologies in Health.; DSM, Diagnostic and Statistical Manual of Mental Disorders.; CID10, International Classification of Diseases.; GRADE, Grading of Recommendations, Assessment, Development and Evaluation.; NOS, Newcastle-Ottawa Scale..

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LEVELS IN CHILDREN AND ADOLESCENTS BEFORE AND AFTER STIMULANT USE A SYSTEMATIC REVIEW AND METANALYSIS

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ABSTRACT

BACKGROUND: Attention deficit hyperactivity disorder (ADHD) is a neurodevelopmental disorder associated with cognitive, social, and academic impairment. Neurotrophins, particularly brain-derived neurotrophic factor (BDNF), have been implicated in the pathophysiology of ADHD and response to stimulant treatment. This review aims to investigate the relationship between BDNF levels in ADHD before and after treatment with stimulants in childhood. METHODS: This systematic review followed PRISMA-P guidelines and included 19 studies from PubMed, EMBASE, Cochrane, Capes Periodic, and Lilacs databases. The studies were evaluated for risk of bias and level of evidence. RESULTS: There was no significant difference in peripheral BDNF levels in ADHD children before or after methylphenidate treatment. Additionally, there was no statistically significant difference in BDNF levels between children with ADHD and controls. DISCUSSION: Understanding the role of BDNF in ADHD may provide insight into the disorder's pathophysiology and facilitate the development of biological markers for clinical use. CONCLUSION: Our findings suggest that BDNF levels are not significantly affected by methylphenidate treatment in ADHD children and do not differ from controls.

SYSTEMATIC REVIEW REGISTRATION: “Brain-derived neurotrophic factor (BDNF) levels in children and adolescents before and after stimulant use: a systematic review”. Number CRD42021261519.

KEYWORDS: BDNF; ADHD; stimulants; children; adolescent; methylphenidate; meta-analysis, attention deficit/hyperactivity disorder, brain-derived neurotrophic factor.

BACKGROUND

Attention deficit/hyperactivity disorder (ADHD) affects 5% of children and 3% of adults (APA, 2013), with twin studies attributing 70 to 80% of its etiology to heritability (LEFFA et al., 2017). Diagnosis typically occurs in childhood and involves symptoms of inattention and hyperactivity/impulsivity before the age of 12, with intensity and duration higher and longer than expected for the age group evaluated, appearing in at least two environments, and causing harm to the individual (APA, 2013).

Environmental factors such as low birth weight, prematurity, small size for gestational age, maternal substance use, and exposure to environmental toxins alongside genetic factors play a role in ADHD's etiology (HEYER; MEREDITH, 2017). An intricate interplay among genetic, environmental, oxidative, and inflammatory factors (NG et al., 2008) governs the mechanisms predisposing individuals to neuropsychiatric disorders (TOSTES et al., 2012; ABDOLLAHI et al., 2004).

ADHD's pathogenesis involves disruptions in dopaminergic and noradrenergic systems within prefrontal cortex circuits (HEYER; MEREDITH, 2017). Among the genetic factors associated with ADHD, researchers have explored polymorphisms related to brain-derived neurotrophic factor (BDNF) activity (BINDER; SCHARFMAN, 2004).

Brain-derived neurotrophic factor (BDNF) is a critical component of neuroplasticity and development, with levels potentially affecting long-term brain activity (LIBMAN-SOKÓŁOWSKA, DROZDZOWICZ, & NASIEROWSKI, 2015). BDNF expression can be modulated by various physiological stimuli, such as physical activity, menstrual cycle, light exposure, osmotic and electrical stimuli (MITCHELMORE & GEDE, 2014), but acute and chronic stress, as well as epigenetic alterations like DNA methylation, can decrease its expression (BINDER & SCHARFMAN, 2004). Several studies have investigated BDNF levels in individuals with attention deficit hyperactivity disorder (ADHD) compared to typically developing children, with some examining changes in BDNF levels before and after treatment (AMIRI et al., 2013; SAHIN et al., 2014; CUBERO-MILLÁN et al., 2016; PEKCANLAR et al., 2017; GUMUS et al., 2022). These studies have produced mixed results, with some finding increases, decreases, or

no changes in BDNF levels relative to control groups or pre-treatment levels (SHIM et al., 2008; SAYYAH et al., 2009; SARGINI et al., 2012; SCASSELLATI et al., 2013; HAIMEI LI et al., 2014; SAHIN et al., 2014; SAADAT et al., 2015; SIMSEK et al., 2016; BILGIÇ et al., 2016; REDA et al., 2016; CUBERO-MILLÁN et al., 2016; PEKCANLAR et al., 2017; TAHA et al., 2017; WANG et al., 2019; YURTERI et al., 2019; CHANG et al., 2020; GHAMRY et al., 2021; GUMUS et al., 2022).

In this systematic review and meta-analysis, we aimed to consolidate and synthesize data from clinical studies involving children and adolescents diagnosed with ADHD (population), comparing them to typically developing children (control) while using stimulant interventions and measuring BDNF levels (outcome). Our objective was to determine whether differences in BDNF levels existed before and after stimulant treatment and whether these changes indicated up or downregulation. Additionally, we conducted subgroup analyses by sex, ADHD subtypes, and other factors.

MATERIALS AND METHODS

TYPE OF STUDY, PROTOCOL AND REGISTRY

This systematic review's protocol was registered with PROSPERO (International Prospective Register of Systematic Reviews; https://www.crd.york.ac.uk/prospero; 2021 CRD42021261519) and adhered to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses Protocols (PRISMA-P; http://www.prisma-statement.org/Extensions/Protocols) guidelines for source and database selection, search strategies, selection criteria, study quality assessment, and data extraction.

SEARCH STRATEGY

The search aimed to identify articles evaluating BDNF levels in children and adolescents before and after stimulant treatment, with or without control groups. The strategy employed Science and Health Descriptors (DECS) and combined the following terms using Boolean operators (Table 1): 1) Attention

deficit/hyperactivity disorder or its synonyms or acronym; 2) Brain Neurotrophic Derived Factor or its acronym; and 3) drugs used for ADHD treatment. The search was conducted in English, utilizing databases such as PubMed, Lilacs, Periódicos Capes, Embase, and Cochrane (Table 2). No time or language restrictions were imposed. In October 2022, the article search was updated, identifying an additional article published in 2022.

Grey literature was assessed using theses and dissertations platforms and health agency platforms, such as the CADTH (Canadian Agency for Drugs, Technologies in Health). Two researchers performed the platform search, article selection, and data extraction. Upon completing the platform search, articles were imported to EndNote, a reference manager, and duplicates were removed. The study "Serum brain-derived neurotrophic factor levels in treatment-naïve boys with attention deficit/hyperactivity disorder treated with methylphenidate: an 8-week, observational pretest–posttest study" served as a sentinel article to verify the search's accuracy.

Table 1. Terms used in the research.

Term (1) **AND**	**Term (2)** **AND**	**Term (3)**
1	*2*	*3*
ADHD/ *Attention-Deficit/Hyper activity Disorder/ Attention-Deficit-Hyperactivity Disorder/ attention deficitdisorder*	*BDNF/ Brain-derived neurotrophic factor*	*Methylphenidate*
		Ritalin
		Vyvanse
		Stimulant
		Lisdexamfetamine dimesylate
		Amphetamine
		Dextroamphetamine
		Adderall

Table 2. Research strategy according to the platform

Estratégia de Busca por Plataforma
PubMed
(BDNF OR *Brain-derived neurotrophic factor)* ***AND*** (ADHD OR *Attention deficit hyperactivity disorder* OR *Attention-Deficit/Hyperactivity Disorder OR attention deficit disorder)* **AND** stimulant
(BDNF OR *Brain-derived neurotrophic factor)* **AND** (ADHD OR *Attention deficit hyperactivity disorder* OR *Attention-Deficit/Hyperactivity Disorder OR attention deficit disorder)* **AND** *Ritalin*
(BDNF OR *Brain-derived neurotrophic factor)* **AND** (ADHD OR *Attention deficit hyperactivity disorder* OR *Attention-Deficit/Hyperactivity Disorder OR attention deficit disorder)* **AND** *Methylphenidate*

(BDNF OR *Brain-derived neurotrophic factor)* **AND** (ADHD OR *Attention deficit hyperactivity disorder* OR *Attention-Deficit/Hyperactivity Disorder OR attention deficit disorder)* **AND** (*Lisdexamfetamine dimesylate OR Vyvanse)*
(BDNF OR *Brain-derived neurotrophic factor)* **AND** (ADHD OR *Attention deficit hyperactivity disorder* OR *Attention-Deficit/Hyperactivity Disorder OR attention deficit disorder)* **AND** *DEXTROAMPHETAMINE*
(BDNF OR *Brain-derived neurotrophic factor)* **AND** (ADHD OR *Attention deficit hyperactivity disorder* OR *Attention-Deficit/Hyperactivity Disorder OR attention deficit disorder)* **AND** *Adderall*
(BDNF OR *Brain-derived neurotrophic factor)* **AND** (ADHD OR *Attention deficit hyperactivity disorder* OR *Attention-Deficit/Hyperactivity Disorder OR attention deficit disorder)* **AND** *AMPHETAMINE*
*BDNF **AND** Methylphenidate*
*BDNF **AND** Ritalin*
*BDNF **AND** Vyvanse*
*BDNF **AND** Adderall*
*BDNF **AND** Stimulant*
*BDNF **AND** Lisdexamfetamine dimesylate*
*BDNF **AND** Amphetamine*
*BDNF **AND** Dextroamphetamine*
Lilacs
ADHD **AND** BDNF **AND** methylphenidate
TDAH **AND** fator neurotrófico and metilfenidato
TDAH **AND** BDNF **AND** metilfenidato
Attention disorder **AND** BDNF **AND** methylphenidate
Attention disorder **AND** brain-derived neurotrophic factor **AND** methylphenidate
Capes periódicos
ADHD **AND** BDNF
Attention disorder **AND** BDNF
attention hyperactivity disorder **AND** BDNF
Cochrane
BDNF **AND** ADHD **AND** *AMPHETAMINE*
BDNF **AND** ADHD **AND** *Adderall*
BDNF **AND** ADHD **AND** *DEXTROAMPHETAMINE*
BDNF **AND** ADHD **AND** (*Lisdexamfetamine dimesylate OR Vyvanse)*
BDNF **AND** ADHD **AND** *Methylphenidate*
BDNF **AND** ADHD **AND** *Ritalin*
BDNF **AND** ADHD **AND** stimulant
Embase
'attention deficit disorder' **AND** 'brain derived neurotrophic factor' **AND** methylphenidate
'attention deficit disorder' **AND** 'brain derived neurotrophic factor' **AND** 'central stimulant agent'
'attention deficit disorder' **AND** 'brain derived neurotrophic factor' **AND** stimulant
'attention deficit disorder' **AND** 'brain derived neurotrophic factor' **AND** lisdexamfetamine
'attention deficit disorder' **AND** 'brain derived neurotrophic factor' **AND** 'amphetamine plus dexamphetamine' (adderall)
'attention deficit disorder' **AND** 'brain derived neurotrophic factor' **AND** 'amphetamine
'attention deficit disorder' **AND** 'brain derived neurotrophic factor' dexamphetamine
'attention disturbance' **AND** 'brain derived neurotrophic factor' **AND** lisdexamfetamine
'attention disturbance' **AND** 'brain derived neurotrophic factor' **AND** stimulant
'attention disturbance' **AND** 'brain derived neurotrophic factor' **AND** methylphenidate
‘attention disturbance’ **AND** 'brain derived neurotrophic factor' **AND** 'amphetamine
'attention disturbance' **AND** 'brain derived neurotrophic factor' **AND** 'dexamphetamine

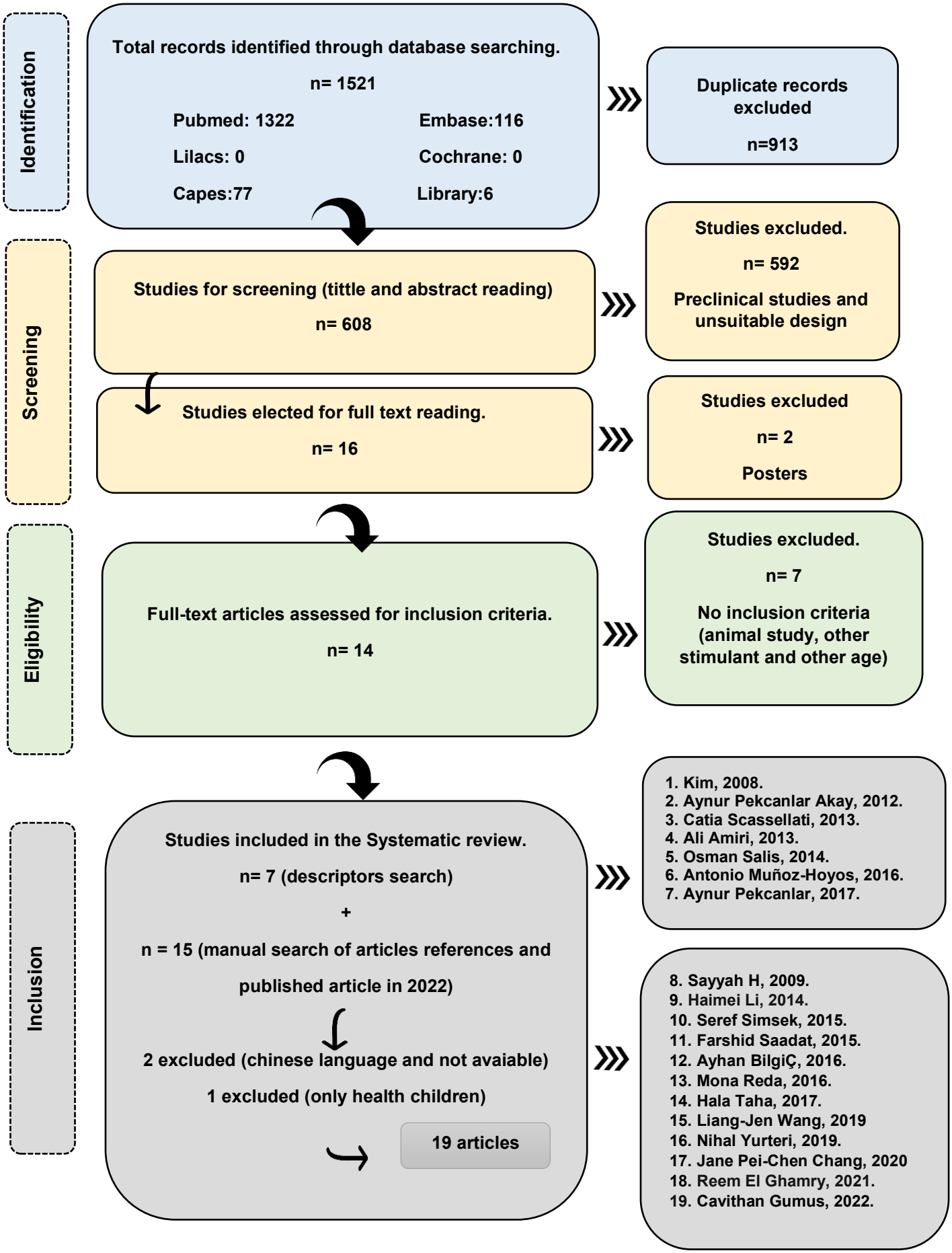
IDENTIFICATION AND SELECTION OF THE STUDIES

Following duplicate removal, articles were exported to Covidence, where two independent reviewers screened titles and abstracts, selecting articles for the next phase. Two researchers assessed the full text of these articles to determine their alignment with the inclusion criteria, with a third researcher resolving any discrepancies.

The inclusion criteria for this systematic review were based on the PICOT framework, which includes the Population (children and adolescents with ADHD), Intervention (therapeutic use of any psychostimulant drugs), Control (control group without medication, pre-treatment stage, or without mental disorders), Outcome (changes in peripheral blood BDNF levels), and Type of Studies (longitudinal studies, cross-sectional studies, quasi-experimental studies, cohort studies, and case-control studies). To be included in this review, studies had to have a diagnosis of ADHD for children and adolescents, in accordance with the DSM-III, DSM-III-R, DSM-IV, DSM-IV-R, DSM-5, ICD-10, direct evaluation by a qualified professional, or a valid instrument for ADHD. The control group was defined as individuals not receiving stimulants, those measured for BDNF levels before stimulant use, or children without mental disorders.

During the screening process, studies were excluded if they involved adults, animals, case reports, or stimulants not intended for ADHD treatment. Methamphetamine, for example, fell under this category. The inter-rater reliability status or level of agreement was evaluated using the Cohen's Kappa coefficient, which demonstrated a perfect agreement (K = 1). However, if a literature review was excluded based on the study type but met other inclusion criteria, the researchers still reviewed it and searched for additional relevant references. The detailed identification and selection process of articles included in this meta-analysis can be found in Figure 1.

Figure 1. PRISMA-based flow diagram for study selection.



EXTRACTED STUDY CHARACTERISTICS

Data extraction was performed by two authors using a standardized data sheet, with a third author resolving any discrepancies. The extracted information included the study's aim, design, year of publication, country/continent, inclusion, and exclusion criteria, total and included sample size, subject characteristics, randomization, group size, male-to-female ratio in each group, mean age of each group, prevalent ADHD subtype, presence of comorbidities, intellectual level, type of BDNF measured, other markers measured, main results, and medication status. The extraction details are listed in Tables 4, 5, and 6. In one study, the WebPlotDigitizer tool was used to extract data from the plot.

DATA SELECTION FOR SYSTEMATIC REVIEW

Data were synthesized based on two groups: (1) pre-intervention dosage or control group without medication, and (2) post-intervention BDNF dosage (following stimulant use).

BIAS ASSESSMENT (QUALITY ASSESSMENT)

Bias risk for case-control studies was assessed using the Newcastle-Ottawa Scale (NOS) and for quasi-experimental studies with the JBI Critical Appraisal Checklist. Two authors analyzed the data, resolving discrepancies through discussion and input from a third, experienced reviewer (Table 3).

Table 3. Quality assessment of the studies included in the meta-analysis.

Newcastle-Ottawa Scale (NOS) – case control				
Study	**Selection ★★★★ (Until 4)**	**Comparability ★★ (Until 2)**	**Outcome/ Exposure ★★★ (Until 3)**	**Total ★★★★ ★★★★ ★ (Until 9)**
1. Shim, 2008 (case control)	★★★	★★	★	6★
2. Sayyah H, 2009 (case control)	★	★	★★	4★
3. Sargini, 2012 (case control)	★★★	★★	★	6★
4. Catia Scassellati, 2013 (case control)	★★★	★	★	5★
5. Haimei Li, 2014 (case control)	★★★★	**----**	★★★	7★

6. Farshid Saadat, 2015 (case control)	★★★		★		★★★					7★
7. Seref Simsek, 2016 (case control)	★★★		★★		★					6★
8. Ayhan BilgiÇ, 2016 (case control)	★★		★		★★					5★
9. Mona Reda, 2016 (case control)	★★		----		★★★					5★
10. Hala Taha, 2017 (case control)	★★		★		★★★					6★
11. Liang-Jen Wang, 2019 (case control)	★★★		----		★★					5★
12. Nihal Yurteri, 2019 (case control)	★★★		★		★★					6★
13. Jane Pei-Chen Chang, 2020 (case control)	★★★		----		★★					5★
14. Reem El Ghamry, 2021 (case control)	★★★		★		★					5★
JBI Critical Appraisal Checklist - Quasi-Experimental Studies (non-randomized experimental studies										
15. Serkan Sahin, 2014 (quasi-experimental study with control group)	★	★	----	★	----	----	★	★	★	6★
16. Ali Amiri, 2013 (quasi-experimental without control group)	★	★	★	---	----	★	★	?	★	6★
17. Cubero-Millán, 2016 (quasi-experimental study with control group)	★	★	----	★	----	----	★	★	★	6★
18. Aynur Pekcanlar, 2017 (quasi-experimental study with control group)	★	★	----	★	----	----	★	★	★	6★
19. Cavithan Gumus, 2022 (quasi-experimental study with control group	★	★	----	★	----	----	★	★	★	6★

DATA ANALYSIS

Our primary outcome measures included differences in blood BDNF levels between individuals with ADHD before and after stimulant use, as well as BDNF level differences between treatment-naive ADHD children and typically developing children. We conducted both quantitative and qualitative data analyses. Due to expected differences in sample size, recruitment, procedures, and methods used to determine BDNF levels, we performed a random-effects meta-analysis. We either extracted the mean differences from the studies or calculated them from available data.

We conducted subgroup analyses to examine the effects of BDNF levels based on subtypes, male/female proportion, BDNF type (plasma vs. serum), biological sample storage temperature, BDNF unit (pg/mL vs. ng/mL), follow-up time, study quality, continent, and BDNF kit used for analysis. We performed univariate meta-regression using numerical variables: mean age and body mass index. We assessed heterogeneity with Cochran's Q test and the I^2 statistic by Higgins and Thompson, using a scale where 0% indicates no heterogeneity, 25% low, 50% moderate, and 75% high heterogeneity.

We represented the meta-analysis in a forest plot and evaluated publication bias through Begg's funnel plot and Egger's test. We considered meta-analysis results significant if the p-value was <0.05. We conducted the data analysis using STATA 11 software, metan command.

RESULTS

1) Qualitative Analysis

We included nineteen studies in this review: fourteen cross-sectional case-control studies and five longitudinal quasi-experimental studies. One longitudinal study did not have a control group. The ADHD group's total sample comprised 1,144 children (872 males and 268 females, with one study lacking sex information), with a mean age of 9.12 ± 2.10 years. This group included 241 inattentive type, 190 hyperactivity/impulsivity type, and 521 combined type participants (two studies without ADHD subtype information). The control group contained 859 children (only one study without a control group), with a mean age of 9.31 ± 2.13 years, including 524 males and 284 females (two studies without sex information) (Table 4).

Table 4. Characteristics of eligible studies.

19 studies	14 cross-sectional case-control	5 longitudinal quasi-experimental	1 longitudinal study didn´t have a control group	
ADHD group	1,144 children	872 boys and 268 girls 1 study without sex information	mean age 9,12 ± 2,10 years	241 inattentive type 190 hyperactivity/impulsivity type 521 combined type 2 studies without ADHD subtypes information
Control group	859 children only one study without control group	524 boys and 284 girls 2 studies without sex information	mean age 9,31 ± 2,13 years	

2) BDNF levels in each study

We analyzed BDNF levels in 19 studies. The control group and ADHD groups' mean BDNF levels did not differ significantly in eight studies (SAYYAH, 2009; SARGIN et al., 2012; SCASSELLATI et al., 2013; SAHIN et al., 2014;

ŞIMŞEK et al., 2016; BILGIÇ et al., 2016; Aynur Pekcanlar, 2017 and YURTERI et al., 2019). Conversely, six studies (SHIM et al., 2008; LI et al., 2014; REDA et al., 2016; TAHA et al., 2017; EL GHAMRY et al., 2021 and GUMUS et al., 2022) found that ADHD groups had significantly higher mean BDNF levels than controls, and four studies (SAADAT et al., 2015, CUBERO-MILLÁN et al., 2016 and CHANG et al., 2020) reported significantly lower mean BDNF levels in ADHD groups than controls.

Furthermore, we found divergent results in five longitudinal quasi-experimental studies. Ali Amiri (2013) and Aynur Pekcanlar (2017) observed significant increases in mean BDNF levels after 6 weeks of treatment with methylphenidate and 8 weeks of treatment with oros methylphenidate, respectively. Conversely, Serkan Sahin (2014), Cubero Millán (2016), and Cavithan Gumus (2022) observed significant reductions in mean BDNF levels post-treatment with long-acting methylphenidate for 8, 18.4 (± 9.16), 10 weeks, respectively.

Several studies investigated the relationship between BDNF levels and ADHD presentation types. Shim (2008) observed significant positive correlations between plasma BDNF levels and the severity of inattention symptoms. Sayyah (2009) identified a significant difference in BDNF serum levels between hyperactivity and inattentive types. In Ali Amiri's (2013) study, a negative correlation was observed between pretreatment plasma BDNF levels and reduction of hyperactivity symptoms after treatment. Serkan Sahin (2014), Farshid Saadat (2015), and Seref Simsek (2016) did not find any significant difference in BDNF levels between hyperactivity and combined ADHD groups. Cubero-Millán (2016) detected a decrease in baseline serum BDNF levels in children with ADHD, particularly those with hyperactive-impulsive/conduct disordered symptoms. Stimulant treatment led to further decreases in serum BDNF levels in predominantly inattentive children, without any changes in predominantly hyperactive children and without influencing depressive symptomatology. Aynur Pekcanlar's (2017) study revealed that the serum BDNF baseline was significantly lower in the inattentive group. Furthermore, the largest increase post-treatment with methylphenidate occurred in the same group. Reem El Ghamry's (2021) study showed significant differences between the

hyperactive and combined subtypes of ADHD and the control group, but no differences between the inattentive type and controls (Table 5).

Table 5: BDNF levels in each study

TYPE OF STUDY	MAIN RESULT	NUMBER OF STUDIES	STUDY	TYPE OF BDNF
ADHD GROUP X CONTROL GROUP	No significant differences in the BDNF levels between ADHD group and control group	8	Sayyah H, 2009	Plasma
			Enis Sargini, 2012	Serum
			Catia Scassellati, 2013	Serum
			Serkan Sahin, 2014	Serum
			Seref Sinsek, 2016	Serum
			Ayhan Bilgiç, 2016	Serum
			Aynur Pekcanlar, 2017	Serum
			Nihal Yurteri, 2019	Serum
	Mean BDNF levels significantly higher in ADHD group when compared with control group	6	Shim, 2008	Plasma
			Haimei Li, 2014	Plasma
			Mona Reda, 2016	Plasma
			Hala Taha, 2017	Serum
			Reem El Ghamry, 2021	Plasma
			Cavithan Gumus, 2022	Serum
	Mean BDNF levels significantly lower in ADHD groups when compared with control group	4	Farshid Saadat, 2015	Plasma
			Cubero-Millán, 2016	Serum
			Liang-Jen Wang, 2019	Plasma
			Jane Pei-Chen Chang, 2020	Plasma
ADHD BASELINE X ADHD ENDPOINT	Significantly increase in mean BDNF post-treatment	2	Ali Amiri, 2013	Plasma
			Aynur Pekcanlar 2017	Serum
	Significant reduction in mean BDNF levels post-treatment	3	Serkan Sahin, 2014	Serum
			Cubero Millán, 2016	Serum
			Cavithan Gumus, 2022	Serum

Some studies evaluated BDNF levels in relation to sex and comorbidities. Shim (2008) reported significant differences in plasma BDNF levels between ADHD patients and normal controls for both males and females. Li (2014), however, did not find any significant differences in BDNF levels between males and females in both the ADHD and control groups. In contrast, Wang (2019) found that mean BDNF levels in the male ADHD group were significantly increased when compared to control boys, while mean BDNF levels in the female ADHD group were significantly lower than control girls. Regarding

comorbidities, Simsek (2016) did not find any differences in BDNF levels between ADHD patients and those with comorbid oppositional defiant disorder and conduct disorder. In contrast, Wang (2019) found that children with greater oppositional defiant symptoms had higher BDNF levels.

Table 6. Descriptive data (general characteristics of studies included)

	AUTHOR YEAR	STUDY TYPE*	ADHD (N)	CONTROL (N)	ADHD MALE (%)	CONTROL MALE (%)	ADHD YEARS / MEAN	ADHD YEARS / SD	CONTROL YEARS / MEAN	CONTROL YEARS / SD	ADHD (N) I / HI / C	ADHD (%) I / HI / C	CONTINENT	BMI ADHD MEAN	BMI ADHD SD	BMI CONTROL MEAN	BMI CONTROL SD	BLOOD STORAGE T°
1	SHIM, 2008	1	41	107	78	38,3	8,8	2,3	9	1,3	Not avaiable	Not avaiable	ASIAN	17,7	2,3	17,3	2,1	Not avaiable
2	SAYYAH H, 2009	1	21	20	61,9	Not avaiable	7,26	2,07	Not avaiable	Not avaiable	6/4/11	28,6/19/52,4	AFICAN	Not avaiable	Not avaiable	Not avaiable	Not avaiable	- 80ºC
3	ENIS SARGINI, 2012	1	31	30	83,9	36,7	8,45	1,57	8,87	1,92	4/5/22	12,9/16,1/71	ASIAN/EUROPEAN	Not avaiable	Not avaiable	Not avaiable	Not avaiable	- 85ºC
4	CATIA SCASSELLATI, 2013	1	45	45	93	91	10,71	2,48	10,31	2,04	13/1/31	27/2/67	EUROPEAN	18,2	3,49	18,04	3,16	- 80ºC
5	ALI AMIRI, 2013	3	28	0	85,7	0	7,78	2	0	0	4/10/14	14,3/35,7/50	ASIAN	Not avaiable	Not avaiable	Not avaiable	Not avaiable	Not avaiable
6	HAIMEI LI, 2014	1	170	155	50,6	49	9,4	2,3	9,5	1,4	100/4/66	59,1/2,3/38,6	ASIAN	18,1	4,1	Not avaiable	Not avaiable	- 80ºC
7	SERKAN SAHIN, 2014	2	30	20	80	90	9,54	2,83	9,65	2,29	13/3/14	43,3/10/46,7	OCEANIA	17,73	2,67	18,06	3,38	- 80ºC
8	FARSHID SAADAT, 2015	1	29	29	82,8	79,3	7,59	2,01	7,43	1,95	4/10/15	13,8/34,5/51,7	ASIAN	Not avaiable	Not avaiable	Not avaiable	Not avaiable	- 20ºC
9	SEREF SIMSEK, 2016	1	49	40	86	80	8,6	2,4	8,7	2,2	17/0/32	34,7/0/65,3	ASIAN/EUROPEAN	16,4	1,7	17,6	2,5	- 80ºC
10	AYHAN BILGIÇ, 2016	1	110	44	80	70,4	10,3	2,1	10,9	2,8	0/0/110	0/0/100	ASIAN/EUROPEAN	Not avaiable	Not avaiable	Not avaiable	Not avaiable	- 80ºC
11	MONA REDA, 2016	1	29	30	Not avaiable	Not avaiable	Not avaiable	Not avaiable	Not avaiable	Not avaiable	10/12/7	34,5/41,4/24,1	AFICAN	Not avaiable	Not avaiable	Not avaiable	Not avaiable	- 20ºC
12	CUBERO-MILLÁN, 2016	2	82	41	79,4	73,2	9,59 / 9,33	2,77 / 2,41	10,22	2,58	29/78/0	27/73/0	EUROPEAN	18.76	4.16	19,8	4,14	- 30ºC
13	AYNUR PEKCANLAR, 2017	2	50	50	100	100	8,8	1,5	8,8	1,1	4/10/36	4,8/10,2/36,72	ASIAN/EUROPEAN	Not avaiable	Not avaiable	Not avaiable	Not avaiable	- 85ºC
14	HALA TAHA, 2017	1	35	30	82,9	86,7	8,1	2,5	8,8	3,3	7/19/9	20/54,3/25,7	AFICAN	Not avaiable	Not avaiable	Not avaiable	Not avaiable	- 20ºC
15	NIHAL YURTERI, 2019	1	49	36	69,4	66,7	Not avaiable	Not avaiable	Not avaiable	Not avaiable	6/12/31	12,2/24,5/63,3	ASIAN/EUROPEAN	Not avaiable	Not avaiable	Not avaiable	Not avaiable	- 80ºC
16	LIANG-JEN WANG, 2019	1	136	71	79,4	77,6	8,8	2.09	9,6	2.47	Not avaiable	Not available	ASIAN	18.36	4.1	17.74	2.91	Not avaiable
17	JANE PEI-CHEN CHANG, 2020	1	91	21	87,1	71,4	9,4	3,08	9,19	2,96	19/7/55	23,4/8,6/68	ASIAN	Not avaiable	Not avaiable	Not avaiable	Not avaiable	- 80ºC
18	REEM EL GHAMRY, 2021	1	60	30	75	66,7	8,18	1,61	8,37	1,79	5/15/40	8,3/25/66,7	AFICAN	16,4	1,06	16,22	1,03	- 70ºC
19	CAVITHAN GUMUS, 2022	2	58	60	67,2	68,3	10,1	1,6	10,07	1,31	30/0/28	51,7/0/48,3	ASIAN/EUROPEAN	18,17	4,05	18,54	2,63	- 80ºC

* Stydy type: 1 (case control cross-sectional); 2 (quasi-experimental longitudinal with control group); 3 (quasi-experimental longitudinal without control group).

I: inattentive type; HI: hyperactivity/impulsivity type; C: combined type.

BMI: body mass index (kg/m^2)

Table 7. BDNF levels and characteristics in the studies included in the meta-analysis.

	AUTHOR YEAR	EXCLUSION	BDNF ADHD BASELINE (MEAN)	BDNF ADHD BASELINE (SD)	BDNF ADHD ENDPOINT (MEAN)	BDNF ADHD ENDPOINT (SD)	BDNF CONTROL (MEAN)	BDNF CONTROL (SD)	BDNF TYPE	BDNF UNIT	COLLECTON TIME	FAST
1	SHIM, 2008	1: side effects 2: follow up lost	833.8	371.0	Not aplicable	Not aplicable	578.5	304.0	Plasma	pg/mL	8-12H AM	No information
2	SAYYAH H, 2009	No information	27171.4	25368,5	Not aplicable	Not aplicable	28689	12705	Plasma	pg/mL	8-12H AM	No information
3	ENIS SARGINI, 2012	4: excluded for Non-admission (ADHD)	2124.45	1044.31	Not aplicable	Not aplicable	2157.63	694.94	Serum	pg/mL	9-10H AM	12 H
4	CATIA SCASSELLATI, 2013	No information	39.33	10.41	Not aplicable	Not aplicable	38.82	8.29	Serum	ng/mL	8- 9H AM	Night fasting
5	ALI AMIRI, 2013	No information	193.06	95.38	271.06	11.35	0	0	Plasma	pg/mL	8-12H AM	No information
6	HAIMEI LI, 2014	No information	256.5	186.1	Not aplicable	Not aplicable	183.9	209.1	Plasma	pg/mL	10-12h am	No information
7	SERKAN SAHIN, 2014	No information	341.84	86.35	300.81	128.14	317.15	100.64	Serum	ng/mL	No information	12 H
8	FARSHID SAADAT, 2015	No information	190.70	9.06	Not aplicable	Not aplicable	374.91	175.60	Plasma	pg/mL	8-12H AM	No information
9	SEREF SIMSEK, 2016	No information	11.1	16.8	Not aplicable	Not aplicable	8.7	12.3	Serum	ng/mL	9-12 am	No information
10	AYHAN BILGIÇ, 2016	No information	14.7	8.4	Not aplicable	Not aplicable	16.4	7.6	Serum	ng/mL	8-10H AM	Overnight fast
11	MONA REDA, 2016	2: ADHD group with cardiac side effects during methylphenidate treatment	5476.9	5443	Not aplicable	Not aplicable	3138.5	2640.7	Plasma	pg/mL	8-12H AM	No information
12	CUBERO-MILLÁN, 2016	No information	30.64	11.36	28,05	12,2	36.36	11.62	Serum	ng/mL	9 AM 20 PM	No information
13	AYNUR PEKCANLAR, 2017	No information	2626.33	1528.05	3255	1908	2989.11	1420.08	Serum	pg/mL	9-10H AM	12 H
14	HALA TAHA, 2017	ADHD group - 141 26 excluded (exclusion criteria); 5: refused. Control group – 49 (5 excluded)	0.1596	0.0909	Not aplicable	Not aplicable	0.0744	0.111	Serum	ng/mL	No information	No information
15	NIHAL YURTERI, 2019	132 ADHD children. 67: refused (fear of injection hazards). 18: autistic features 2: pychotic features 5: epilepsy 11: type 1 diabetes mellitus.	13.41	9.36	Not aplicable	Not aplicable	14.87	7.22	Serum	ng/mL	8-10H AM	Overnight fasting
16	LIANG-JEN WANG, 2019	No information	4.25	4.13	Not aplicable	Not aplicable	3.73	4.03	Plasma	ng/mL	8H AM	Overnightfast
17	JANE PEI-CHEN CHANG, 2020	No information	778.81	380.58	Not aplicable	Not aplicable	1224.62	456.40	Plasma	pg/mL	8-10H AM	12 H
18	REEM EL GHAMRY, 2021	ADHD :2 refusals 14 exclusions regarding to exclusion criteria. Control group: 6 refusals	27.47	18.37	Not aplicable	Not aplicable	12.30	7.17	Plasma	ng/mL	No information	No information
19	CAVITHAN GUMUS, 2022	543 youth with ADHD, 105 fulfilled the recruitment criteria, and 98 participants participated in the study	2067.93	580.24	1857.66	316.07	1712.68	289.29	Serum	pg/ml	No information	12 H

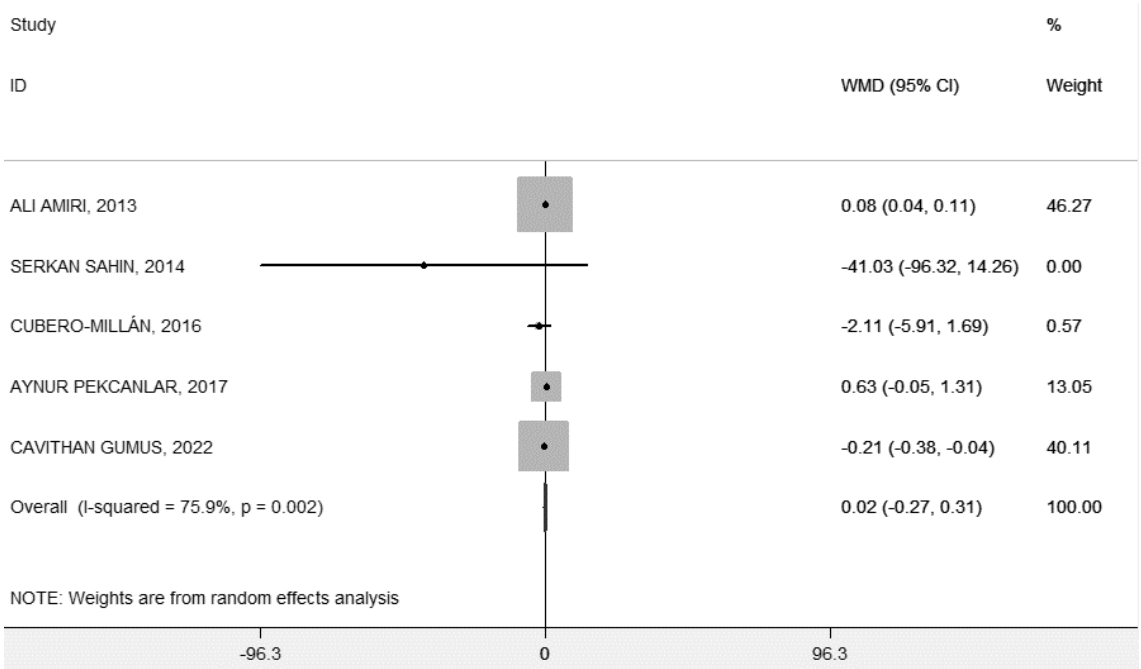
Table 8. Meta-analysis results.

	COMORBITIES	MAIN RESULTS
1	No information	Mean plasma BDNF levels were significantly higher in ADHD patients than in normal controls when adjusted for age and gender. Significant differences in the levels of BDNF in the plasma of ADHD patients and those of normal controls for males and females. Plasma BDNF levels have significant positive correlations with the severity of inattention symptoms.
2	No information	No significant difference between ADHD children and control group regarding the BDNF plasma level. BDNF level in the inattentive type of ADHD is much lower than that of the control group. Significant statistical difference regarding BDNF serum level between the hyperactive type of ADHD patients and inattentive type. No significant gender difference between ADHD children regarding all variables assessed in this study including BDNF plasma level.
3	67,7 % (ADHD children) had one or more comorbidities.	No statistically significant difference was found between children diagnosed with ADHD and control group for BDNF levels
4	17 (38 %) ADHD group with depressive symptoms.	Mean serum BNDF levels in patients with ADHD were not significantly different from those of controls.
5	Correlating baseline and 6-week BDNF level with symptoms of learning, behavior, anxiety, HI, psychosomatic, hyperactivity. (No detailed information).	Plasma BDNF levels increased after 6 weeks treatment with methylphenidate (main dose 17,85 (SD 3,95); Min 10MG; Max 20MG). There was a negative correlation between pretreatment plasma BDNF levels and reduction of hyperactivity symptoms after treatment. Follow-up 6 weeks.
6	No information	Mean plasma BDNF levels were significantly higher in ADHD patients than in unaffected controls after adjusted for age and sex. Similar results for both males and females when analysis which stratified for sex were done. No significant differences were observed in the BDNF levels Between males and females in the ADHD group and in control group.
7	ADHD patients: 17 had other disruptive behavior disorder, 5 anxiety disorder, 3 learning disability, 3 enuresis/encopresis, and 2 tic disorder; 22 (73.3%) patients had one or more comorbid psychiatric disease	Before the treatment, BDNF levels were found to be similar in the ADHD and control group. BDNF levels showed no difference between ADHD-C and ADHD-I In the ADHD group, the BDNF level was found to be significantly lower after the treatment.
8	Excluded.	Significant decrease in plasma levels of BDNF in children with all subtypes of ADHD compared with normal controls. Plasma BDNF levels between ADHD subtypes were not statistically significant.
9	Oppositional defiant disorder and conduct disorder. 28.6% (n=14) were diagnosed with ADHD plus ODD, and 20.4% (n=10) were diagnosed with ADHD plus CD.	No difference in BDNF levels between the ADHD and control groups. No relationship between ADHD symptom severity and BDNF levels. BDNF levels did not differ between the subtypes of ADHD. **N**o difference in BDNF levels between patients with ADHD and those with comorbid Oppositional defiant disorder and conduct disorder. In the ADHD group, there was no relationship between the Stroop test interference effect score and BDNF levels.
10	ADHD: oppositional defiant disorder (*n* = 68), conduct disorder (*n* = 38), specific phobias (*n* = 31), enuresis (*n* = 20), tic disorders other than Tourette's disorder (*n* = 7), encopresis (*n* = 6), generalized anxiety disorder (*n* = 5), social phobia (*n* = 4), separation anxiety disorder (*n* = 3), major depression (*n* = 2), and obsessive-compulsive disorder (*n* = 1).	No significant differences in serum BDNF levels between patients with ADHD and the controls.
11	No clear information. Some patients with ADHD had subsyndromal psychiatric conditions	Plasma BDNF was higher in children suffering from ADHD than in normal children. Moderate positive correlation of plasma BDNF with anxious/shy scores and a weak positive correlation with psychosomatic, socio-cognitive impairment, and inattentive scores. Moderate negative correlation with hyperactive/impulsive scores and a weak negative correlation with perfectionism, emotional liability, and oppositional scores.
12	Comorbid Depressive Symptoms.	At baseline, decreased serum BDNF in ADHD patients compared with healthy controls, these differences persisted after adjusting for age and gender. The values were higher in the morning. Decrease in the baseline serum BDNF levels of children with ADHD, due to a lower value in predominantly hyperactive–impulsive/conduct disordered children. Prolonged treatment with methylphenidate induced further decreases in serum BDNF due to decreases in predominantly inattentive children without any changes in predominantly hyperactive children and with no influence on depressive symptomatology. Before methylphenidate treatment, the BDNF profile of the predominantly inattentive children group was comparable to that of the control group. Follow-up: 4.61 ± 2.29 months.
13	Oppositional defiant disorder (ODD) as comorbidity with ADHD. More than half of the patients of the study group had a comorbid diagnosis. Some patients with ADHD had subsyndromal psychiatric conditions	There was no significant difference between baseline mean BDNF levels of boys with ADHD and the healthy control. There was a statistically significant increase of serum BDNF levels from pretreatment to post-treatment in boys with ADHD. Baseline serum BDNF was significantly lower in the inattentive group. The increase of serum BDNF levels with methylphenidate treatment after 8 weeks was significantly higher in the inattentive group. Follow-up: 8 weeks
14	No clear information.	The level of BDNF was higher in the patient group and was statistically significant different from control. BDNF was positively correlated with cognitive problems and negatively correlated with age and IQ.
15	Oppositional defiant disorder possible.	No significant differences in serum BDNF levels between treatment-naive patients with ADHD and controls.
16	No information.	BDNF levels in ADHD boys exceeded those in control boys, but BDNF levels in ADHD girls were lower than those in control girls. Children with greater oppositional defiant symptoms had higher BDNF levels.
17	54% Oppositional defiant disorder.	Youth with ADHD have lower levels of morning plasma TNFα and of BDNF.
18	No information.	Children with ADHD exhibit higher levels of plasma BDNF levels in comparison with age- and sex-matched healthy controls. No obvious correlation between the severity of symptoms in children with ADHD and their plasma BDNF level. Significant negative correlation between plasma BDNF levels and emotional lability. Significantly differences were found between the hyperactive and combined subtypes of ADHD and the control group but not between the inattentive type and controls.
19	Oppositional defiant disorder possible 25.9% (n = 15)	Serum BDNF levels in the ADHD group before treatment to be significantly higher compared to the control group. BDNF levels in the ADHD group following treatment to be significantly lower compared with before the treatment.

1.1) Meta-analysis of BDNF levels in ADHD children treated with methylphenidate

We conducted a meta-analysis on five studies that measured the levels of BDNF in the blood of children with ADHD before and after receiving methylphenidate treatment (N = 248). Our findings revealed no significant difference in the peripheral blood BDNF levels of ADHD children pre- and post-treatment [weighted mean difference (WMD) = 0.02, 95% CI = −0.27 to 0.31, $p > 0.05$] (Fig. 2). We also found significant statistical heterogeneity across the studies (I^2 = 75.9%, p = 0.002). We performed subgroup and meta-regression analyses to understand the factors correlated with heterogeneity, and we found no significant difference in BDNF levels pre- and post-treatment in the ADHD children group.

Figure 2: Forest plot of meta-analysis for peripheral BDNF levels in ADHD children pre- and post-treatment with random-effect analysis. WMD (95% CI) was used to present the data. Data sources are listed in tables 4 and 5.



1.2) Meta-analysis of peripheral blood BDNF level in ADHD children and control group

We analyzed 18 studies (N = 1,116) measuring the levels of BDNF in the blood of children with ADHD and a control group (N = 859). Our findings showed no significant difference in peripheral blood BDNF levels between children with ADHD and the control group [weighted mean difference (WMD) = 0.02, 95% CI = −0.12 to 0.17, p > 0.05] (Fig. 3). Significant statistical heterogeneity was found across the studies (I^2 = 88.4%, p = 0.000) (Fig. 4), but we found no publication bias based on Begg's test (p > 0.970) and Egger's test (p > 0.609). The funnel plot (Fig. 4) showed symmetrically scattered results on both sides of the null lines at the top of the plot. We performed subgroup and meta-regression analyses to identify factors correlated with heterogeneity, but no significant difference was found in BDNF levels between children with ADHD and the control group.

Figure 3: Forest plot of meta-analysis for peripheral BDNF levels in ADHD children and control group using random-effect analysis. Data presented as weighted mean difference (WMD, 95% CI). Source: Tables 4 and 5.

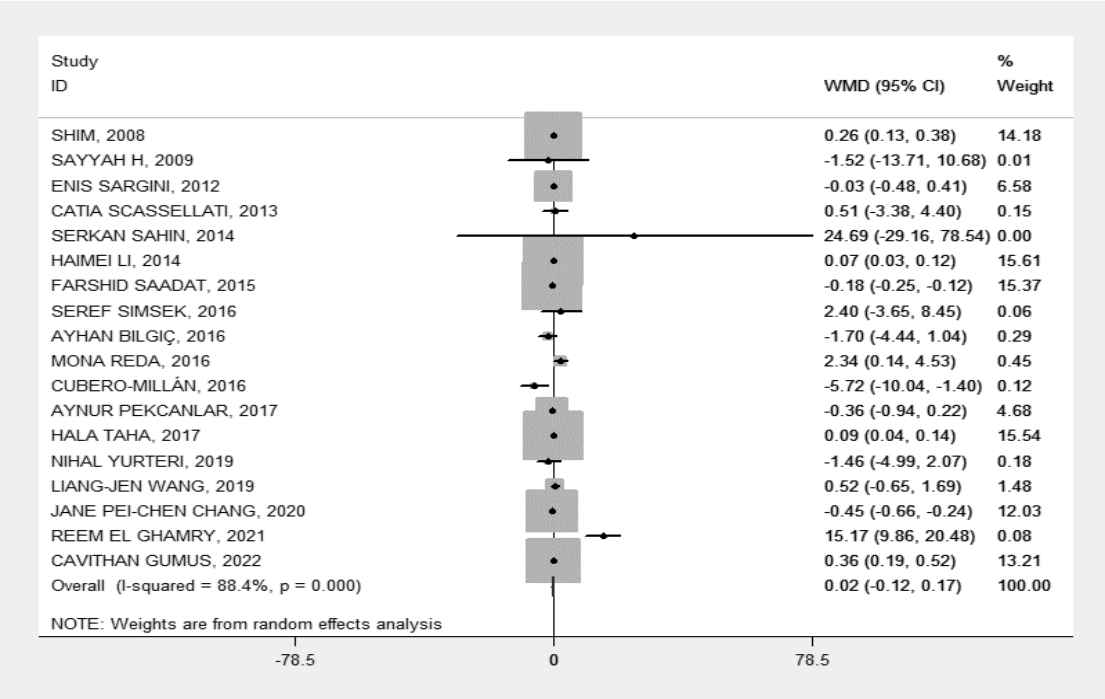
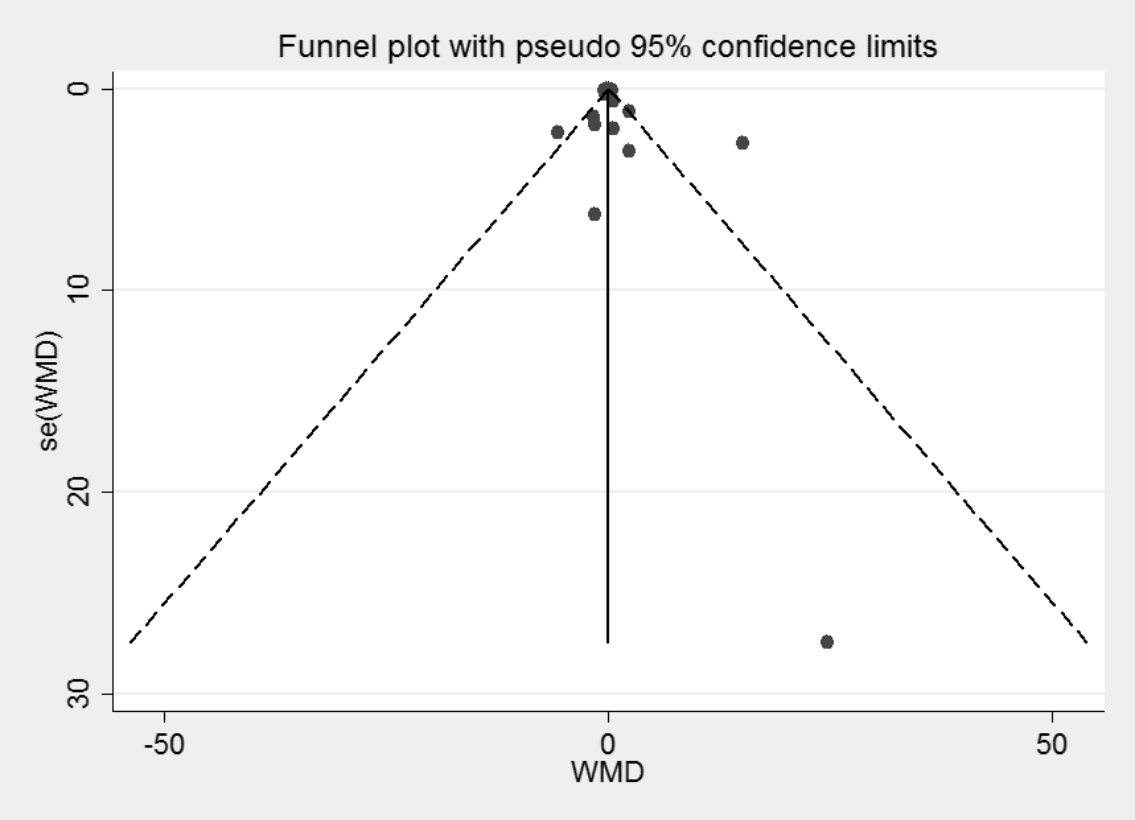


Figure 4: Funnel plot of meta-analysis for peripheral BDNF levels in ADHD children and control.

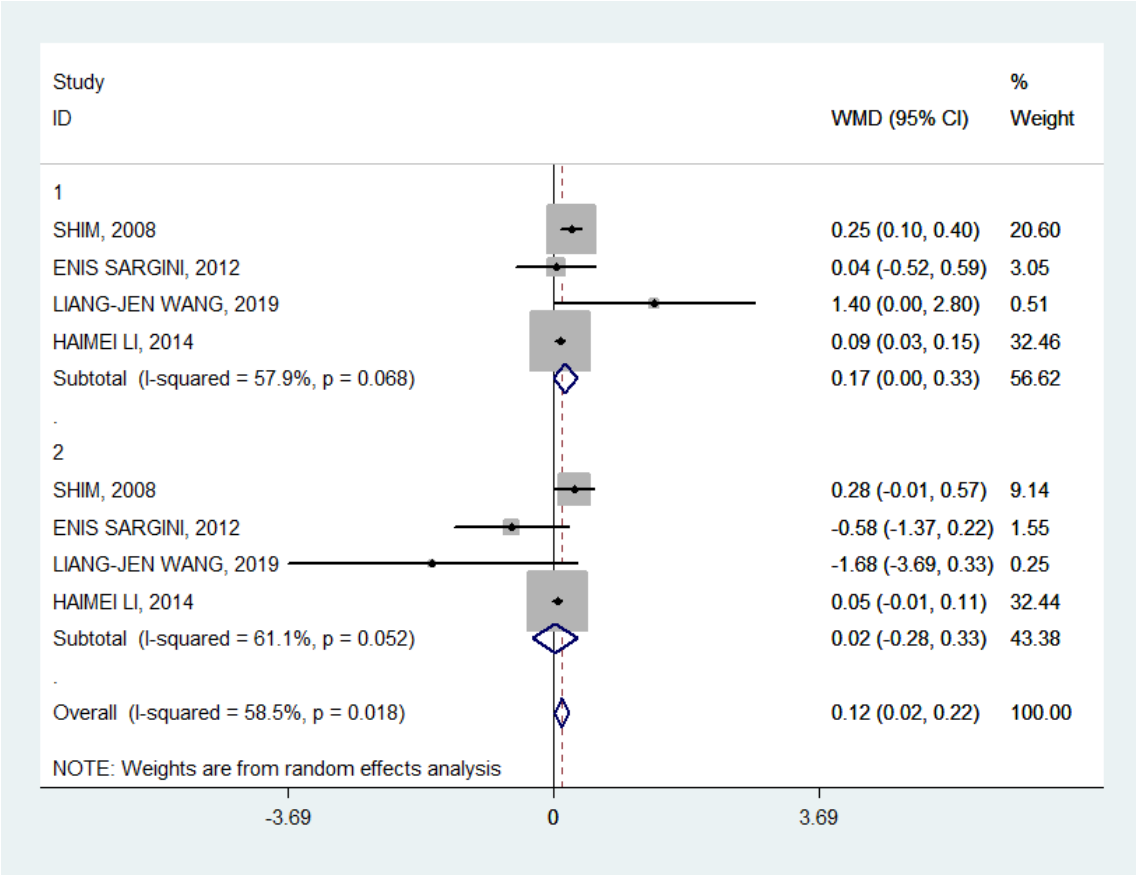


1.3) Meta-analysis of BDNF levels in subgroup male/female children with ADHD and male/female control subgroup.

We meta-analyzed four studies measuring BDNF levels in the blood (serum or plasma) of children with ADHD (N = 378; 252 male children and 126 female children) and control group (N = 363; 173 male children and 190 female children). We found no significant difference in peripheral blood BDNF levels between children with ADHD and control group [WMD = 0.12, 95% CI = −0.02 to 0.22, $p > 0.05$] (Fig. 5). Heterogeneity was moderate across the studies ($I^2 = 58.5\%$, P = 0.000) (Fig. 6), but publication bias was absent based on Begg's test ($p > 0.322$) and Egger's test ($p > 0.139$) and the symmetric scatter in the funnel plot. Subgroup and meta-regression analyses revealed no significant difference in BDNF levels between children with

ADHD and the control group.

Figure 5: Forest plot of meta-analysis for male and female peripheral BDNF levels in ADHD children and control group. Data presented as weighted mean difference [(WMD), (95% CI)]. Source: Tables 4 and 5.



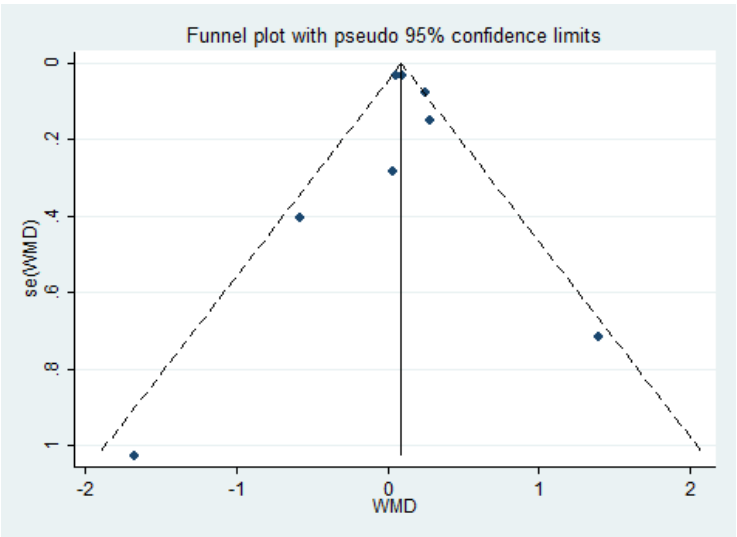


Figure 6: Funnel plot of meta-analysis for male and female peripheral BDNF levels in ADHD children and control.

1.4) Meta-analysis of ADHD subtypes peripheral blood BDNF level in ADHD children and control group.

We meta-analyzed ten studies that measured BDNF levels in children with ADHD (N = 550) and control groups (N = 377). Results revealed no significant difference in peripheral blood BDNF levels between children with ADHD and the control group [weighted mean difference (WMD) = -110.46, 95% CI = −230.04 to 10.01, p > 0.05] (Fig. 7). However, we noted significant statistical heterogeneity across the studies (I^2 = 88.9%, P = 0.000) (Fig. 4), but no publication bias was found (Begg's test: p > 0.343; Egger's test: p > 0.621) (Fig. 8). Our subgroup and meta-regression analyses revealed no significant difference in BDNF levels between children with ADHD and the control group.

Figure 7: Forest plot of meta-analysis for peripheral BDNF levels in ADHD subtypes using random-effect analysis. Data presented as weighted mean difference [(WMD), (95% CI)]. Source: Tables 4 and 5.

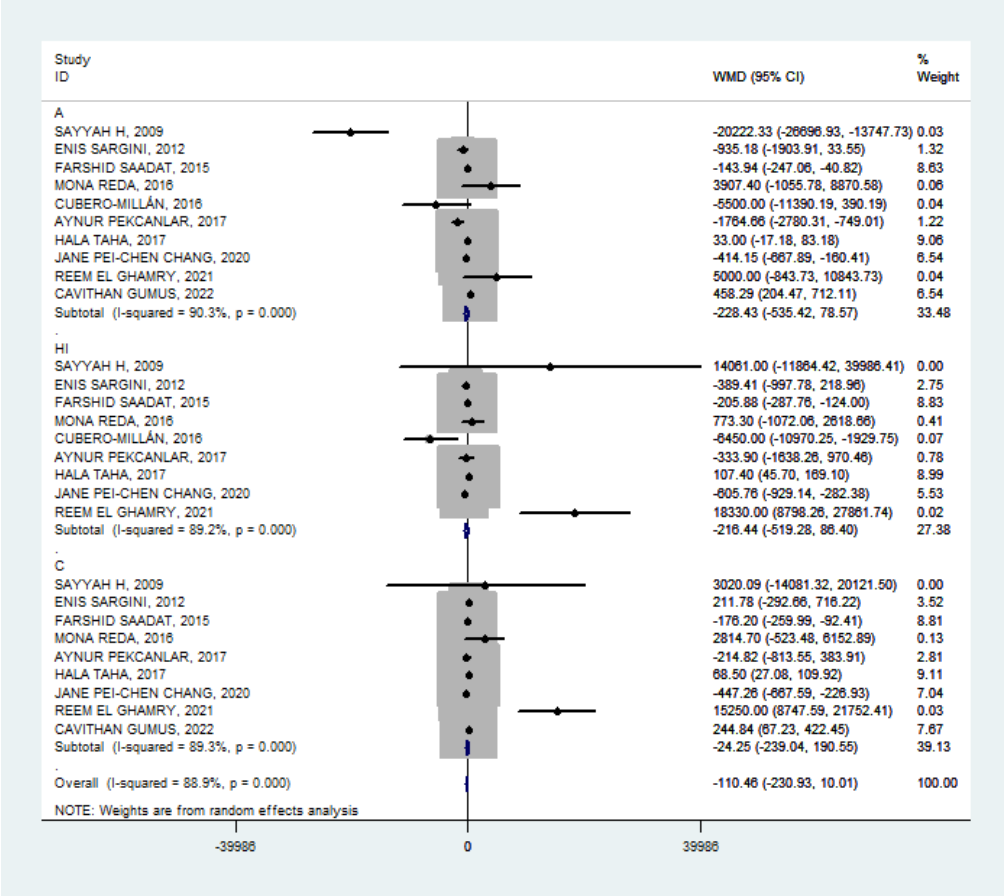
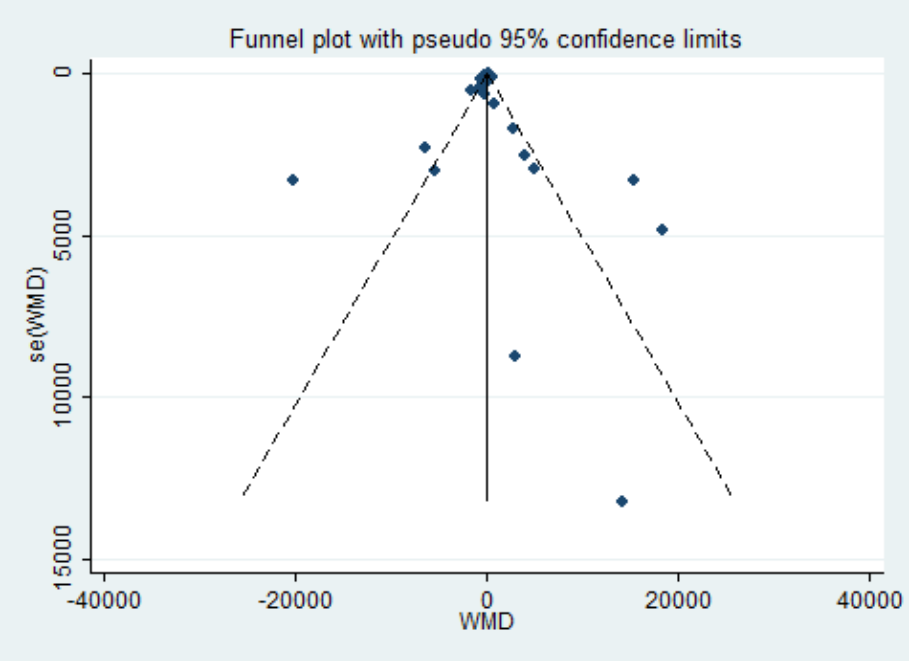


Figure 8: Funnel plot of meta-analysis for peripheral BDNF levels in ADHD subtypes.



1.1) Meta-analysis of ADHD subtypes peripheral blood BDNF level in pre- and post-treatment ADHD children.

We analyzed 11 studies measuring BDNF levels in the blood of 190 children with ADHD pre- and post-treatment. Peripheral blood BDNF levels were not statistically different between ADHD children and control group [weighted mean difference (WMD) = -53,23, 95% CI = −537,90 to 431,45, $p > 0.05$] (Fig. 9), with significant heterogeneity across studies (I^2 = 69,4%, P = 0.003) (Fig. 4). We found no evidence of publication bias (Begg's test P > 0,453 and Egger's test p > 0,350), and the funnel plot showed symmetrical scattering of study results (Fig 10). Subgroup and meta-regression analyses did not reveal any significant factors correlated with the heterogeneity.

Figure 9: Forest plot of meta-analysis for ADHD subtypes' peripheral BDNF levels in pre- and post-treatment using random-effect analysis. Data presented as weighted mean difference [(WMD), (95% CI)]. Data source listed in tables 4 and 5.

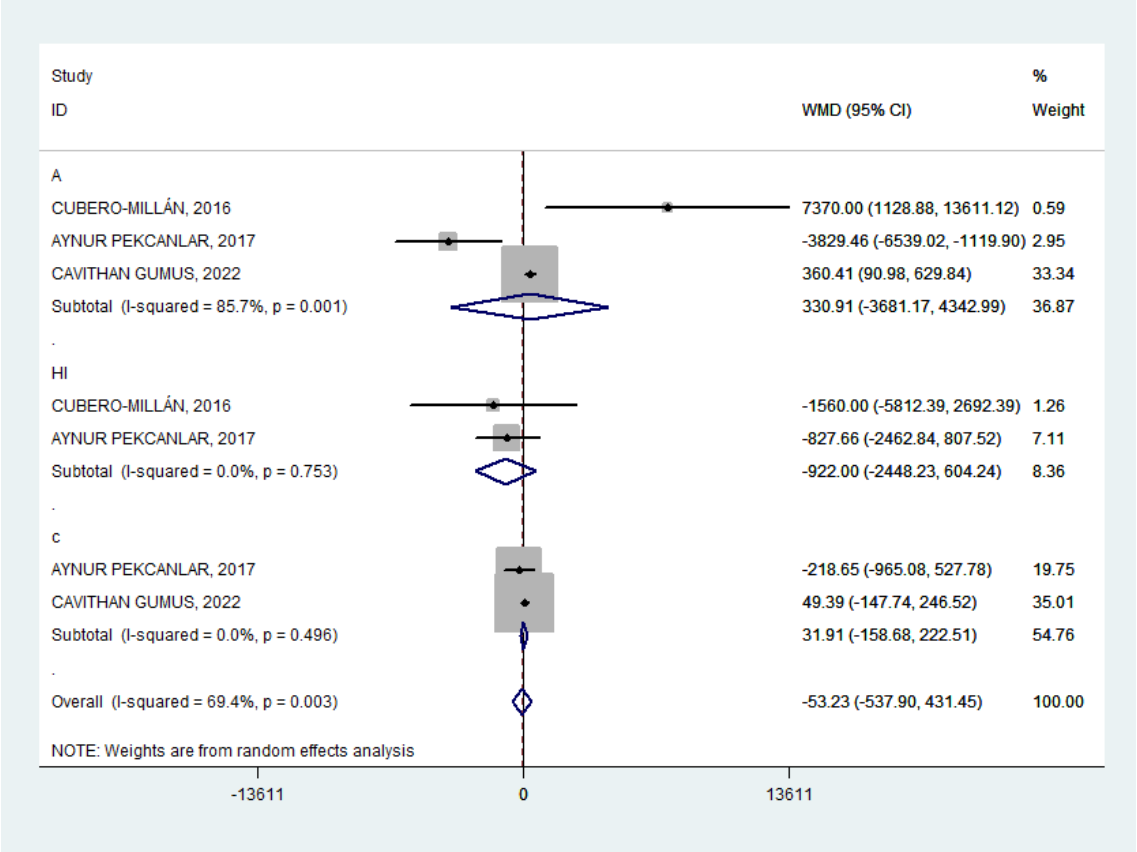
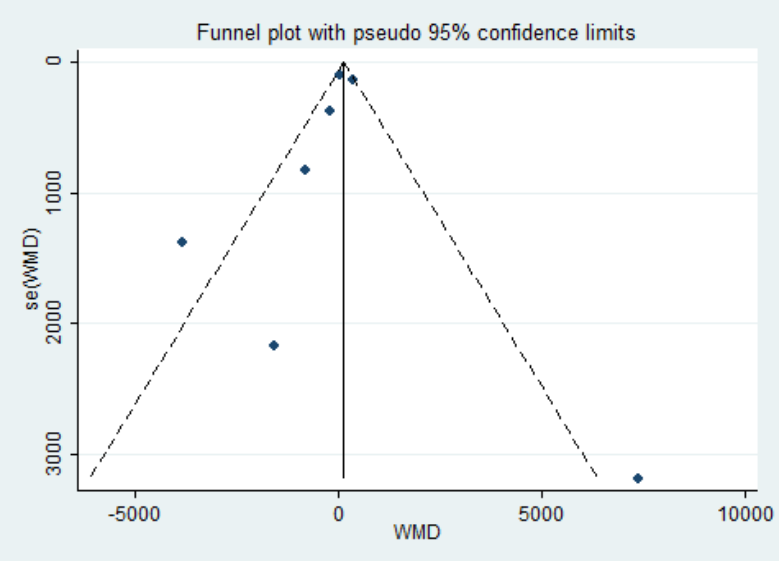


Figure 10: Funnel plot of meta-analysis for ADHD subtypes peripheral BDNF levels in pre- and post-treatment.



DISCUSSION

BDNF, a protein involved in neuroplasticity, may serve as a biomarker for ADHD (KERNIE, LIEBL, & PARADA, 2000). However, its expression and response to therapeutic intervention in individuals with ADHD remain uncertain. This systematic review and meta-analysis synthesized studies on BDNF levels in children and adolescents with ADHD. We analyzed data from five studies on therapeutic stimulant treatment, mostly cross-sectional control studies. Additionally, we extracted data from 18 studies comparing BDNF levels in children with ADHD not using methylphenidate and typically developing children. Our results suggest no significant difference in peripheral BDNF levels between pre- and post-treatment with methylphenidate in children with ADHD, or between children with ADHD and control groups.

Zhang *et al.* (2018) conducted a meta-analysis of ten articles and reported no disparity in peripheral blood levels between control subjects and individuals with ADHD. Nevertheless, they noticed that males with ADHD had higher BDNF levels than the control group, indicating a gender difference. Our study, which had a larger sample, revealed no difference between male or female ADHD and control groups. Moreover, our analysis of ten studies on ADHD subtypes found no significant differences.

Unaddressed features, such as the genetic profile of the population and physiological and pathological factors (e.g., physical exercise, menstrual cycle, light level, osmotic and electrical stimuli, obesity, stress, medications, inflammation status, and endothelial lesions) may have contributed to the inconsistent findings among the studies and influenced our meta-analysis results (GLUD et al., 2019; AMIRI et al., 2013; MITCHELMORE; GEDE, 2014; LIBMAN-SOKOŁOWSKA; DROZDOWICZ; NASIEROWSKI, 2015). Limitations identified in the included studies, such as the lack of diet analysis, assessment of physical activity level, data collection time, and comorbidity assessment (AMIRI, 2013; CUBERO-MILLÁN, 2016; PEKCANLAR, 2017; SHIM, 2008; SARGINI, 2012; SCASSELLATI, 2013; SIMSEK, 2016; GHAMRY, 2021; SAYYAH, 2009; SAADAT, 2015; BILGIÇ, 2016; REDA, 2016; TAHA, 2017; WANG, 2019; YURTERI, 2019), may also have influenced the results. In this meta-analysis, most studies collected blood samples in the morning.

MOLTENI *et al*. (2002) observed that highly saturated fats and refined sugar diets reduced BDNF levels in animals, which could impact neuroplasticity and memory. Glud (2019) found that women had higher baseline serum BDNF concentrations than men, and diet reduced circulating BDNF levels in women only, whereas exercise reduced circulating BDNF levels in men. Rodrigo de Poli (2021) noted that high-intensity intermittent exercise increased circulating BDNF levels. Previous studies (MOLTENI et al., 2012; GLUD, 2019; Rodrigo de Poli, 2021) suggest that diet, exercise, and sex may influence circulating BDNF levels.

Our meta-analysis included children of varying ages (3.5 to 18 years old), with an average age of 9 years, and identified age as a possible factor affecting BDNF expression. BDNF transcriptional expression in the human prefrontal cortex peaks in early childhood, indicating a role in early development (MITCHELMORE; GEDE, 2014). Corominas-Roso (2013) found that adults with ADHD had significantly lower serum BDNF levels than those with typical development, indicating a potential role for BDNF in ADHD throughout life.

Sex differences impact circulating BDNF levels, with women exhibiting significantly higher platelet BDNF levels than men (WEI; WANG; XU, 2017; LOMMATZSCH et al., 2005). However, none of the reviewed studies reported data on pubertal development. Female subjects exhibit higher BDNF levels in the prefrontal context, but hippocampal BDNF content does not significantly differ between men and women (HAYLEY et al., 2015). Women exhibit higher circulating BDNF levels during the last phase of their menstrual cycle compared to the first phase (LOMMATZSCH et al., 2005), suggesting that gonadal hormones may impact sex differences in BDNF levels (CUBEDDU et al., 2011; PLUCHINO et al., 2009).

Methodological differences, such as sample size, proportion of ADHD subtypes, boy/girl ratio, BDNF type, fasting status prior to collection, storage temperature of biological material, longitudinal or cross-sectional study design, presence of psychiatric or subsyndromal psychiatric symptoms, intellectual level, and sample collection time, can impact results. Additionally, variations in ELISA methods or sampling tubes can lead to differences in measured BDNF levels (SAYYAH, 2009).

BDNF exists as an abundant neurotrophin in the central nervous system. It can cross the blood-brain barrier in both directions, and its peripheral levels

associate with central levels. BDNF is initially synthesized as pro-BDNF, which cleaves to generate the mature form. Both pro-BDNF and mature BDNF actively participate biologically. Platelets store most of the peripheral BDNF, with only a small amount of free BDNF present in plasma. Consequently, platelet associated BDNF may not reflect the levels of BDNF in the brain. However, GEJL et al. (2019) suggest that serum BDNF and plasma BDNF do not reflect the same BDNF pool. The collection-to-centrifugation time affects serum BDNF levels, while the centrifugation strategy influences plasma BDNF levels. Strong correlations exist between BDNF levels in normal plasma and platelet-poor plasma, allowing for comparison of different protocols for analyzing relative levels of plasma BDNF.

Essentially, to investigate potential differences in BDNF levels between children and adolescents with ADHD and to determine if stimulant treatment has an impact on these levels, it is crucial to employ rigorous collection and analysis protocols and utilize large sample sizes. This review underscores the significance of refining future studies to enhance the probability of identifying the correlation between BDNF and ADHD and validating BDNF's utility as a biological marker for the condition.

CONCLUSION

Our meta-analysis showed no significant difference in peripheral BDNF levels before and after methylphenidate treatment in ADHD children, nor between ADHD children and control group, even in subgroups analyses (male/female; ADHD subtypes, plasma/serum). These findings call into question the validity of BDNF as a reliable biological marker for ADHD and suggest that alternative methodologies may be necessary to accurately assess this relationship.

LIST OF ABBREVIATIONS

1. BDNF: BRAIN-DERIVED NEUROTROPHIC FACTOR
2. ADHD: ATTENTION-DEFICIT/HYPERACTIVITY DISORDER
3. PRISMA-P: PREFERRED REPORTING ITEMS FOR SYSTEMATIC REVIEWSAND META-ANALYSES PROTOCOLS.
4. PROSPERO: INTERNATIONAL PROSPECTIVE REGISTER OF SYSTEMATICREVIEWS.
5. DECs: DESCRITORES DE CIÊNCIA E SAÚDE.
6. BDTD: BIBLIOTECA DIGITAL BRASILEIRA DE TESES E DISSERTAÇÕES.
7. CADTH: CANADIAN AGENCY FOR DRUGS, TECHNOLOGIES IN HEALTH.
8. DSM: DIAGNOSTIC AND STATISTICAL MANUAL OF MENTAL DISORDERS.
9. CID10: INTERNATIONAL CLASSIFICATION OF DISEASES.
10. GRADE: GRADING OF RECOMMENDATIONS, ASSESSMENT,DEVELOPMENT AND EVALUATION.
11. NOS: NEWCASTLE-OTTAWA SCALE.

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- **AUTHORS' INFORMATION (OPTIONAL)**

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DECLARATION OF COMPETING INTEREST

None.

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Table 9. PRISMA 2020 Checklist

Section and Topic	Item #	Checklist item	Location where item is reported
TITLE			
Title	1	Identify the report as a systematic review.	TITLE
ABSTRACT			
Abstract	2	See the PRISMA 2020 for Abstracts checklist.	ABSTRACT
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of existing knowledge.	BACKROUND ¶ 4 and ¶ 5
Objectives	4	Provide an explicit statement of the objective(s) or question(s) the review addresses.	BACKROUND ¶ 5
METHODS			
Eligibility criteria	5	Specify the inclusion and exclusion criteria for the review and how studies were grouped for the syntheses.	IDENTIFICATION AND SELECTION OF THE STUDIES ¶ 2, ¶ 3 and ¶ 4
Information sources	6	Specify all databases, registers, websites, organisations, reference lists and other sources searched or consulted to identify studies. Specify the date when each source was last searched or consulted.	SEARCH STRATEGY ¶ 1 and ¶ 2
Search strategy	7	Present the full search strategies for all databases, registers and websites, including any filters and limits used.	SEARCH STRATEGY ¶ 1 and ¶ 2, TABLES 1 and 2
Selection process	8	Specify the methods used to decide whether a study met the inclusion criteria of the review, including how many reviewers screened each record and each report retrieved, whether they worked independently, and if applicable, details of automation tools used in the process.	IDENTIFICATION AND SELECTION OF THE STUDIES ¶ 1
Data collection process	9	Specify the methods used to collect data from reports, including how many reviewers collected data from each report, whether they worked independently, any processes for obtaining or confirming data from study investigators, and if applicable, details of automation tools used in the process.	STUDY CHARACTERISTICS TO BE EXTRACTED ¶ 1, ¶ 2 and ¶ 3
Data items	10a	List and define all outcomes for which data were sought. Specify whether all results that were compatible with each outcome domain in each study were sought (e.g. for all measures, time points, analyses), and if not, the methods used to decide which results to collect.	STUDY CHARACTERISTICS TO BE EXTRACTED ¶ 2 and TABLES 6, 7 and 8
	10b	List and define all other variables for which data were sought (e.g. participant and intervention characteristics, funding sources). Describe any assumptions made about any missing or unclear information.	TABLES 6, 7 and 8
Study risk of bias assessment	11	Specify the methods used to assess risk of bias in the included studies, including details of the tool(s) used, how many reviewers assessed each study and whether they worked independently, and if applicable, details of automation tools used in the process.	BIAS ASSESSMENT ¶ 1 and TABLE 3

Section and Topic	Item #	Checklist item	Location where item is reported
Effect measures	12	Specify for each outcome the effect measure(s) (e.g. risk ratio, mean difference) used in the synthesis or presentation of results.	DATA ANALYSIS ¶ 1 to ¶ 4
Synthesis methods	13a	Describe the processes used to decide which studies were eligible for each synthesis (e.g. tabulating the study intervention characteristics and comparing against the planned groups for each synthesis (item #5)).	DATA ANALYSIS ¶ 1 and ¶ 2. RESULTS ¶ 1 QUALITATIVE DESCRIPTION OF DATA
	13b	Describe any methods required to prepare the data for presentation or synthesis, such as handling of missing summary statistics, or data conversions.	STUDY CHARACTERISTICS TO BE EXTRACTED ¶ 3 DATA ANALYSIS ¶ 3
	13c	Describe any methods used to tabulate or visually display results of individual studies and syntheses.	TABLES 6, 7 and 8
	13d	Describe any methods used to synthesize results and provide a rationale for the choice(s). If meta-analysis was performed, describe the model(s), method(s) to identify the presence and extent of statistical heterogeneity, and software package(s) used.	DATA ANALYSIS ¶ 3 and ¶ 4.
	13e	Describe any methods used to explore possible causes of heterogeneity among study results (e.g. subgroup analysis, meta-regression).	DATA ANALYSIS ¶ 3
	13f	Describe any sensitivity analyses conducted to assess robustness of the synthesized results.	DATA ANALYSIS ¶ 4
Reporting bias assessment	14	Describe any methods used to assess risk of bias due to missing results in a synthesis (arising from reporting biases).	DATA ANALYSIS ¶ 4
Certainty assessment	15	Describe any methods used to assess certainty (or confidence) in the body of evidence for an outcome.	DATA ANALYSIS ¶ 4
RESULTS			
Study selection	16a	Describe the results of the search and selection process, from the number of records identified in the search to the number of studies included in the review, ideally using a flow diagram.	FIGURE 1
	16b	Cite studies that might appear to meet the inclusion criteria, but which were excluded, and explain why they were excluded.	FIGURE 1
Study characteristics	17	Cite each included study and present its characteristics.	RESULTS TABLE 6, 7 AND 8
Risk of bias in studies	18	Present assessments of risk of bias for each included study.	METHODS TABLE 3
Results of individual studies	19	For all outcomes, present, for each study: (a) summary statistics for each group (where appropriate) and (b) an effect estimate and its precision (e.g. confidence/credible interval), ideally using structured tables or plots.	QUALITATIVE DESCRIPTION OF DATA ¶1 TO 8, TABLES 4 AND 5. RESULTS TABLE 8

Section and Topic	Item #	Checklist item	Location where item is reported
Results of syntheses	20a	For each synthesis, briefly summarise the characteristics and risk of bias among contributing studies.	RESULTS ITEMS 2.1, 2.2,2.3,2.4
	20b	Present results of all statistical syntheses conducted. If meta-analysis was done, present for each the summary estimate and its precision (e.g. confidence/credible interval) and measures of statistical heterogeneity. If comparing groups, describe the direction of the effect.	RESULTS ITEMS 2.1, 2.2,2.3,2.4
	20c	Present results of all investigations of possible causes of heterogeneity among study results.	RESULTS ITEMS 2.1, 2.2,2.3,2.4
	20d	Present results of all sensitivity analyses conducted to assess the robustness of the synthesized results.	RESULTS ITEMS 2.1, 2.2,2.3,2.4
Reporting biases	21	Present assessments of risk of bias due to missing results (arising from reporting biases) for each synthesis assessed.	RESULTS ITEMS 2.1, 2.2,2.3,2.4
Certainty of evidence	22	Present assessments of certainty (or confidence) in the body of evidence for each outcome assessed.	RESULTS ITEMS 2.1, 2.2,2.3,2.4
DISCUSSION			
Discussion	23a	Provide a general interpretation of the results in the context of other evidence.	DISCUSSION ¶ 1, ¶ 2 and ¶ 3
	23b	Discuss any limitations of the evidence included in the review.	DISCUSSION ¶ 4, ¶ 5, ¶ 7, ¶ 8, ¶ 9 and ¶ 10
	23c	Discuss any limitations of the review processes used.	DISCUSSION ¶ 1
	23d	Discuss implications of the results for practice, policy, and future research.	DISCUSSION ¶ 11 and CONCLUSION
OTHER INFORMATION			
Registration and protocol	24a	Provide registration information for the review, including register name and registration number, or state that the review was not registered.	TYPE OF STUDY, PROTOCOL AND REGISTRY
	24b	Indicate where the review protocol can be accessed, or state that a protocol was not prepared.	TYPE OF STUDY, PROTOCOL AND REGISTRY
	24c	Describe and explain any amendments to information provided at registration or in the protocol.	NOT APPLICABLE
Support	25	Describe sources of financial or non-financial support for the review, and the role of the funders or sponsors in the review.	FUNDING
Competing interests	26	Declare any competing interests of review authors.	DECLARATION OF COMPETING INTEREST
Availability of data, code and other materials	27	Report which of the following are publicly available and where they can be found: template data collection forms; data extracted from included studies; data used for all analyses; analytic code; any other materials used in the review.	CORRESPONDING AUTHOR AND SUPPLEMENTARY MATERIAL

5.3. Artigo 4: Can we really modify neuroplasticity using psychostimulants?

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Can we really modify neuroplasticity using psychostimulants?

Human neurodevelopment occurs as a program with biologically pre-established, sequential, and automatic phases, with more favorable moments to the acquisition of complex functions. Besides an innate component, adequate stimuli and environment are needed throughout this process start, run, and develop satisfactorily (Reh et al., 2020). In other words, it is necessary to insert proper environmental components along life to have fully biological expression. Best timing is necessary to synchronize stimuli presentation, development predisposition, and readiness to response. Beyond timing, age- and brain region- are also important (Kolb et al., 2010; Sta Maria et al., 2019).

The number of ongoing neurobiological processes that occur during brain maturation confer both considerable vulnerability, adaptability, and potential for recovery. The structural and functional maturation walk together, and connectivity still going on throughout adolescence (Sta Maria et al., 2019). Brain maturation differs among mammalian species, demanding careful assumptions in trying each species findings generalizations to humans. A complete mapping of human brain maturation may help to better understand the neuroplasticity (Luca Bonfanti, 2021). Human brain passes for axis grown, myelinization waves, synaptic pruning in frequencies which still being described. Human prolonged neuroplasticity programed may confer more risk for developmental psychopathologies (Sydnor VJ et., al, 2021; Diniz and Crestani, et al., 2023)

Neuroplasticity consists in the brain capacity to alter structure and function in response to some stimuli, so called “experience-dependent plasticity” (Kolb et al., 2010). Besides the simplicity of the concept of neuroplasticity, the mechanism still not fully clarified (Diniz and Crestani, 2023). Plastic changes in brain may improve motor, cognitive functions and interfere with behavior (Kolb et al., 2010).

Altering the balance of local circuit inhibition to excitation or the opposite through drugs, sensory experience, stress, nutrition, genetic, aging, or epigenetic factors can modulate crucial periods of neurodevelopmental (Reh et al., 2020). This would be useful if we think about the possibility of a drug contributing to the restoration and/or reduction of brain damage or stimulate brains with underdeveloped areas. Therefore, we must be careful with the possibility of inadvertently stimulate pathological plasticity, including trough the pharmacological treatments given. For example, psychomotor stimulants, such as amphetamine, may stimulate plastic changes in the dorsal striatum, nucleus accumbens, and prefrontal cortex (Robinson and Kolb, 2004).

ADHD is a neurodevelopmental disorder, whose maturational delay hypothesis has continued to be a predominant theory in the field (Dutta et al., 2022). ADHD has been increasingly considered a disorder of brain-wide network dysconnectivity (Kaiser et al., 2022; Ohnishi T et al., 2023).

ADHD has been associated with structural and functional changes in brain areas responsible to amphetamine and methylphenidate modulations of dopamine and norepinephrine activity (Faraone, 2018). Psychostimulants primarily increase catecholamine availability in striatal and cortical regions, as evidenced in preclinical and human studies (Faraone, 2018; Cortese et al., 2018).

Dopamine system undergoes significant alterations throughout development, so the methylphenidate-induced effects on functional connectivity may be modulated by age (Kaiser et al., 2022). These drugs class are the first line in ADHD treatment, having 70 to 80% of pharmacological response (Advokat and Scheithauer, 2013), and evidence of reducing morbimortality of affected subjects (Boland et al., 2020). Although there is an unsolved doubt, when treating disorders as ADHD, can we really modify neuroplasticity using psychostimulants? Is it beneficial in long term to the ADHD subjects?

Regarding effects of psychostimulant in neuroplasticity, studies have yielded mixed results about brain structure and function. It is assumed the neuroplasticity is changed by treatment, but what evidence is available suggesting the neuroplasticity is really changed by these drugs?

Exposure to ADHD medication influenced striatal dopamine transporter (DAT) density (Fusar-Poli P et al., 2012). Long-term effects of stimulant treatment may result from several alterations in the dynamic expression and quantity of transporters at the plasmatic membrane, and the induction of downstream gene expression, but there is still a limited counter regulation and understanding of their long-term effects (Vles JS et al., 2003; Dutta et al., 2022, Aster HC et al., 2022). The higher striatal DAT density in previous medicated ADHD individuals and down-regulation of dopamine turn over when compared with unmedicated subjects seem to be an adaptive response to stimulant exposure. This suggests that a high dopamine transporter level is not part of the key ADHD pathophysiology, but it is secondary to years of psychostimulant use (Fusar-Poli P et al., 2012; Faraone, 2018). Decrease in DAT after methylphenidate

administration in children (9-16 years) was observed with methylphenidate treatment for at least 6 months (Aster HC et al., 2022).

In imaging studies of ADHD, Rubia et al., (2021) summarized results of structural volumetric studies. Reduced gray matter in subcortical regions were observed prominently in the basal ganglia and, but also limbic areas such as the amygdala and hippocampus and reduced gray matter, surface area and cortical thickness in frontal, temporal and parietal regions. There is an apparent delay in the peak of cortical thickness and surface area maturation in frontal, temporal and parietal regions and for impaired white matter tract, most prominently in frontal-striatal, frontal-cerebellar, interhemispheric tracts and long-distance tracts such as frontal-occipital tracts (Rubia et al., 2021).

Overall imaging studies have yielded mixed results about the effects of stimulant medication on brain structure. The effects may be too local to be picked up by volumetric analysis (DUTTA ET AL, 2022). In longitudinal studies, the age of medication onset and treatment duration seem to play a role in predicting the magnitude of medication effects on brain structure and function (DUTTA ET AL, 2022). In randomized, double-blind, methylphenidate and placebo-controlled trial of effects on right medial cortical thickness, there was difference between children and adults. In children, less cortical thinning was observed, but in adults or the placebo groups, there is no cortical thinning, suggesting methylphenidate action on developing gray matter in specific brain region (Walhovd KB et al.,2020). Regarding myelination, Bouziane et al., (2019) found after four months of methylphenidate treatment, changes in specific tracts in brain white matter in boys with attention deficit/hyperactivity disorder, but not in adults

methylphenidate treated, suggesting age-dependent methylphenidate effect again.

ADHD subjects and stimulant use have been linked to changes in brain connectivity (Yoo JH et al., 2018; Pape L et al., 2021; Kaiser et al., 2022; Henry TR et al., 2022; Mizuno et al., 2023). Kaiser et al (2022) ponder that the efficacy of stimulant therapy may depend on combinations of factors, including age, brain maturation that return network organization to typical topology for some systems while reorganizing others. They found that pre-methylphenidate participants with ADHD showed aberrant connectivity and centrality in frontal regions. After a single dose of methylphenidate, measures of connectivity and centrality in the striatum and thalamus decreased in children with ADHD but increased in adults with ADHD. No effects were observed in the dorsal anterior cingulate cortex and pre-frontal cortex in children or adults.

In molecular level, the brain-derived neurotrophic factor (BDNF) is the most abundant expressed neurotrophin in the central nervous system. The BDNF plays a vital role in neuroplasticity and alterations in its levels could have effects on long terms brain activity (Libman-Sokolowska, Drozdowicz and Nasierowski, 2015). Despite its intimate relationship with neurodevelopment and neuroplasticity, the studies carried out so far have yielded mixed results. A meta-analysis including 19 studies evaluated the BDNF, and psychostimulant relationship in ADHD. No difference in peripheral BDNF levels in ADHD children was observed before and after methylphenidate treatment or between ADHD children and control group, even considering gender and ADHD subtypes (de Lucca, MS et al., 2023).

Despite the evidence of the clinical benefits of stimulants, as well as morbimortality reduction, the available evidence shows only preliminary findings that stimulants may modify neuroplasticity, but don't ensure risk-free neurodevelopment, especially in young children. We await further studies that can clarify if psychostimulants really can influence neuroplasticity in the short and long terms, as the optimum time point and duration of treatment to reach best results and benefits, without causing damage.

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6. CONSIDERAÇÕES FINAIS

O objetivo deste trabalho de doutorado foi a avaliação do impacto do tratamento com cloridrato de metilfenidato em crianças com Transtorno de Déficit de Atenção e Hiperatividade. O estudo incluiu metodologia variada, contendo estudo de revisão sobre o efeito de metilfenidato sobre o fator neurotrófico derivado do cérebro (BDNF) e estudo de coorte experimental.

Os resultados contribuem com o conhecimento sobre as ações farmacológicas e o mecanismo de ação do metilfenidato, principal tratamento do TDAH nessa faixa etária e amplamente usado.

7. APÊNDICES

7.1. Apêndice I: Termo de Consentimento Livre e Esclarecido (TCLE) – Pais - Frente

ANEXO 2 - TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO - RESPONSÁVEL -

Via do Responsável

Você e seu (sua) filho (a) estão sendo convidados (as) como voluntários(as) a participar da pesquisa "**NÍVEIS DE BDNF, PERFIL INFLAMATÓRIO E OXIDATIVO DE CRIANÇAS COM TRANSTORNO DE DÉFICIT DE ATENÇÃO E HIPERATIVIDADE (TDAH) ANTES E APÓS TRATAMENTO COM METILFENIDATO**". Neste estudo pretendemos **comparar os níveis de BDNF (fator neurotrófico de crescimento cerebral) e o perfil inflamatório/oxidativo de crianças com transtorno de déficit de atenção e hiperatividade antes e após tratamento com metilfenidato, medicação que seu filho usará para o tratamento.** O motivo que nos leva a estudar esse assunto é tentar aprofundar no conhecimento de fatores que podem estar associados "a causa" do TDAH, possibilitando assim pensar em estratégias preventivas e terapêuticas coadjuvantes diferentes das já estabelecidas. Para este estudo adotaremos o(s) seguinte(s) procedimento(s): **Aplicação de questionários e escalas, consultas psiquiátricas pela médica pesquisadora, assim como três coletas de amostras de sangue venoso, no início do tratamento, com 12 e 24 semanas de tratamento com metilfenidato. Essas avaliações serão realizadas concomitantemente aos atendimentos de assistência, os quais seus filhos realizariam independente da pesquisa. O tempo médio das consultas será 2 horas no ambulatório da UFV ou da UFMG.** Para participar deste estudo, você sendo o(a) responsável pela criança, deverá autorizar e assinar esse termo de consentimento, assim como o termo de assentimento da criança.

Você não terá nenhum custo além dos custos que já teria com o tratamento do TDAH de seu filho, nem receberá qualquer vantagem financeira. Você será esclarecido(a) em qualquer aspecto que desejar e estará livre para participar ou recusar-se. O Sr. (a) poderá retirar o consentimento ou interromper a participação de seu (sua) filho (a) a qualquer momento. A participação é voluntária e a recusa em participar após iniciado o acompanhamento não acarretará qualquer penalidade ou modificação na forma em que seu (sua) filho (a) é tratado (a) no ambulatório desta instituição, que irá tratar a identidade dele (a) com padrões profissionais de sigilo e não será identificado(a) em nenhuma publicação. Portanto, caso ocorra a desistência, a continuidade do atendimento será realizada no mesmo ambulatório sob supervisão dos preceptores responsáveis. Ao participar dessa pesquisa, o risco e desconforto serão mínimos. O desconforto poderá ser sentido durante a punção venosa para coleta do sangue. Os riscos, se ocorrerem serão mínimos por se tratar de uma punção venosa no antebraço por profissional experiente. Reações locais poderão ocorrer, tais como: hematomas e muito raramente inflamação na veia puncionada. Nestes casos, os (as) voluntários(as) da pesquisa serão orientados e encaminhados adequadamente para atendimento. Caso sejam identificados outros problemas de saúde e/ou situações de violência sofridas pela criança, o encaminhamento à rede de assistência pública também será feito. Os resultados estarão à sua disposição quando finalizada. A devolutiva dos resultados da pesquisa, dos exames, consultas e questionários coletados será dada a você e seu responsável durante os atendimentos neste ambulatório.

Rubrica pesquisadora: MARINA SILVA DE LUCCA:01186121661 Assinado de forma digital por MARINA SILVA DE LUCCA:01186121661 Dados: 2021.06.26 21:23:12 -03'00'

Rubrica responsável:

Apêndice I: Termo de Consentimento Livre e Esclarecido (TCLE) – Pais – Verso

Durante a realização desses procedimentos, você pode sentir um pouco de desconforto e ansiedade, sentir-se cansado, mas respeitaremos seu ritmo e daremos apoio psicológico durante todo o processo da pesquisa.

O nome do seu (sua) filho (a) ou o material que indique a participação não será liberado sem a permissão do Sr. (a). Os dados e instrumentos utilizados na pesquisa ficarão arquivados com o pesquisador responsável por um período de 10 anos, e após esse tempo serão destruídos. Este termo de consentimento encontra-se impresso em duas vias, sendo que uma via será arquivada pelo pesquisador responsável, e a outra será fornecida ao (à) Sr. (a).Eu, ..., portador(a) do documento de Identidade ....., fui informado(a) dos objetivos do presente estudo de maneira clara e detalhada e esclareci minhas dúvidas. Sei que a qualquer momento poderei solicitar novas informações, e poderei modificar a decisão de participar se assim o desejar. Declaro que concordo em participar desse estudo. Recebi uma via deste termo de consentimento e me foi dada a oportunidade de ler e esclarecer as minhas dúvidas.

Viçosa, ... de ..... de 20.....

Assinatura do (a) responsável

MARINA SILVA DE LUCCA:01186121661 Assinado de forma digital por MARINA SILVA DE LUCCA:01186121661 Dados: 2021.06.26 21:23:52 -03'00'

Assinatura da pesquisadora

Em caso de dúvidas com respeito aos aspectos éticos deste estudo, você poderá consultar:

COEP –Comitê de Ética em Pesquisa da Universidade Federal de Minas Gerais
AV. Presidente Antônio Carlos, 6627, Pampulha - Belo Horizonte - MG - CEP 31270-901Unidade Administrativa II - 2° Andar - Sala: 2005 Telefone: (031) 3409-4592 - E-mail: coep@prpq.ufmg.br Horário de atendimento: 09:00 às 11:00 / 14:00 às 16:00
Pesquisadora responsável: Marina Silva de Lucca marinadelucca@ufv.br
Departamento de Medicina e Enfermagem Av. Peter Henry Rolfs, s/n Campus Universitário – Viçosa, MG CEP: 36570-900. +55 (31) 36125580 dem@ufv.br

7.2. Apêndice II: Termo de Assentimento (TA) – Crianças e Adolescentes

ANEXO 1 - TERMO DE ASSENTIMENTO
CRIANÇAS DE 6 A 12 ANOS IMCOMPLETOS

Via do responsável e Criança

Você está sendo convidado(a) como voluntário(a) a participar da pesquisa **"NÍVEIS DE BDNF, PERFIL INFLAMATÓRIO E OXIDATIVO DE CRIANÇAS COM TRANSTORNO DE DÉFICIT DE ATENÇÃO E HIPERATIVIDADE (TDAH) ANTES E APÓS TRATAMENTO COM METILFENIDATO"**. O motivo que nos leva a estudar esse assunto é tentar aprofundar no conhecimento de fatores que podem estar associados "a causa" do TDAH, possibilitando assim, pensar em estratégias de prevenção e tratamento da doença. Para este estudo adotaremos o(s) seguinte(s) procedimento(s): **Realização de perguntas, consultas médicas, assim como três exames de sangue durante os 6 meses de estudo. Após o término do estudo, você continuará sendo atendido no ambulatório da UFV ou UFMG sob supervisão dos preceptores deste serviço.** Para participar dessa pesquisa, seu responsável também precisará autorizar. Você terá risco e desconforto mínimos. O desconforto poderá ser sentido durante a coleta do sangue no antebraço. Reações locais poderão ocorrer, tais como: hematomas e muito raramente inflamação na veia puncionada. Nestes casos, você será orientado (a) e encaminhado (a) adequadamente para atendimento. Este termo de assentimento encontra-se impresso em duas vias, sendo que uma via será arquivada pelo pesquisador responsável, e a outra será fornecida ao seu responsável. Eu, ..., portador(a) do documento de Identidade e/ou CPF ....., fui informado(a) dos objetivos do presente estudo de maneira clara e detalhada e esclareci minhas dúvidas. A devolutiva dos resultados da pesquisa, dos exames, consultas e questionários coletados será dada a você e seu responsável durante os atendimentos neste ambulatório. Durante a realização desses procedimentos, você pode sentir um pouco de desconforto e ansiedade, sentir-se cansado, mas respeitaremos seu ritmo e daremos apoio psicológico durante todo o processo da pesquisa.

Viçosa, ... de ..... de 20.....

Assinatura do (a) responsável: .....

Assinatura da criança: .....

Assinatura da pesquisadora: MARINA SILVA DE LUCCA:01186121661

Em caso de dúvidas com respeito aos aspectos éticos deste estudo, você poderá consultar:

COEP –Comitê de Ética em Pesquisa da Universidade Federal de Minas Gerais
AV. Presidente Antonio Carlos, 6627, Pampulha - Belo Horizonte - MG - CEP 31270-901 Unidade Administrativa II - 2° Andar - Sala: 2005 Telefone: (031) 3409-4592 - E-mail: coep@prpq.ufmg.br Horário de atendimento: 09:00 às 11:00 / 14:00 às 16:00
Pesquisadora responsável: Marina Silva de Lucca. CRMMG 39.542 lucca_marina@hotmail.com
Departamento de Medicina e Enfermagem Av. Peter Henry Rolfs, s/n Campus Universitário – Viçosa, MG CEP: 36570-900. 55 (31) 36125580 dem@ufv.br

Procedimentos:







Consulta medica | Exame Fisico | Coletas de sangue

8. ANEXOS

8.1. Anexo I: Parecer consubstanciado do Comitê de Ética em Pesquisa da Universidade Federal de Minas Gerais – Pág. 1

UNIVERSIDADE FEDERAL DE MINAS GERAIS

PARECER CONSUBSTANCIADO DO CEP

Elaborado pela Instituição Coparticipante

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: AVALIAÇÃO DO PERFIL INFLAMATÓRIO E OXIDATIVO DE PACIENTES ATENDIDOS EM SERVIÇO DE NEUROPSIQUIATRIA.

Pesquisador: Silvia Almeida Cardoso

Área Temática:

Versão: 4

CAAE: 82870117.0.3001.5149

Instituição Proponente: UNIVERSIDADE FEDERAL DE MINAS GERAIS

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 4.364.744

Apresentação do Projeto:

Resposta a diligência de parecer de número 3.799.146 do projeto de pesquisa de número de CAAE: 82870117.0.3001.5149.

Lista de pendências apresentadas em parecer anterior:

- Anexar a Declaração da Gerência de Ensino e Pesquisa, autorizando a realização da pesquisa no HC pela coordenação do GEPE. A declaração do GEP de submissão do projeto para análise e aprovação também é aceito pelo CEP
- Anexar o termo de constituição de biorrepositório assinados (modelo podem ser encontrado na página do COEP-UFMG).
- Há a necessidade de trocar o termo "cópia" por "via", nos TCLE's e TALE.
- Inserir a aprovação do parecer da Câmara Departamental, no qual, a pesquisadora responsável está alocada na UFMG.
- Informar nos TCLE's que haverá a devolutiva dos resultados aos participantes da pesquisa e seus responsáveis, quantos aos resultados dos exames, consultas e questionários coletados.
- Considerar o risco de desconforto,ansiedade e cansaço durante as atividades propostas ao participante, e incluir na descrição dos riscos nos TCLE's e TALE,e informar como serão minimizados.

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Bairro: Unidade Administrativa II **CEP:** 31.270-901
UF: MG **Município:** BELO HORIZONTE
Telefone: (31)3409-4592 **E-mail:** coep@prpq.ufmg.br

Anexo I: Parecer consubstanciado do Comitê de Ética em Pesquisa da Universidade Federal de Minas Gerais – Pág. 2

UNIVERSIDADE FEDERAL DE MINAS GERAIS

Plataforma Brasil

Continuação do Parecer: 4.364.744

Objetivo da Pesquisa:

O presente projeto tem como objetivo central determinar o perfil inflamatório e oxidativo associados com desordens neuropsiquiátricas.

Objetivos secundários: - Categorização dos pacientes atendidos no serviço de referência; - Levantamento de dados socioeconômicos/ambientais e clínicos dos pacientes, voluntários; - Avaliação metabólica (glicemia, colesterol total, frações e triglicéride); - Avaliação bioquímica (hemograma, ureia, creatinina, AST, ALT, Vitamina B12 e D); - Avaliação sorológica de marcadores inflamatórios (albumina, PCR e VHS); - Dosagem de enzimas relacionadas com estresse oxidativo; - Dosagem de marcadores de dano celular pelo estresse oxidativo (óxido nítrico, proteína carbonilada e peroxidação lipídica); - Quantificação de citocinas; - Definição de perfil de proteínas séricas

Avaliação dos Riscos e Benefícios:

Como não há mudança na metodologia e nos critérios de inclusão e alteração, os riscos estão inalterados em relação à versão atual do projeto aprovado pelo centro coordenador em parecer de número 3.581.990

Comentários e Considerações sobre a Pesquisa:

Inalterados em relação ao projeto mais recente aprovado pelo centro coordenador em parecer de número 3.581.990.

Considerações sobre os Termos de apresentação obrigatória:

- Os pesquisadores anexaram a Declaração da Gerência de Ensino e Pesquisa
- Os pesquisadores anexaram o termo de constituição de biorrepositório assinado.
- Há a necessidade de trocar o termo “cópia” por “via”, nos TCLE's e TALE.
- Os pesquisadores anexaram o parecer da Câmara Departamental da UFMG.
- Os pesquisadores incluíram nos TCLE's que haverá a devolutiva dos resultados aos participantes da pesquisa e seus responsáveis, quantos aos resultados dos exames, consultas e questionários coletados.
- Os pesquisadores incluíram nos TCLE´s risco de desconforto,ansiedade e cansaço durante as atividades propostas ao participante, e incluir na descrição dos riscos nos TCLE's e TALE,e informar como serão minimizados.

Endereço: Av. Presidente Antônio Carlos,6627 2º Ad SI 2005
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Anexo I: Parecer consubstanciado do Comitê de Ética em Pesquisa da Universidade Federal de Minas Gerais – Pág. 3

UNIVERSIDADE FEDERAL DE MINAS GERAIS

Plataforma Brasil

Continuação do Parecer: 4.364.744

Recomendações:

É importante que caso nos TCLEs e no TALE conste a o local em que foi realizada a pesquisa. Portanto, sugerimos para os pacientes que forem recrutados no âmbito dos ambulatórios do Hospital das Clínicas o TCLE e o TALE seja incluída a cidade de Belo Horizonte (no modelo atual encontra-se descrita a cidade de Viçosa)

Conclusões ou Pendências e Lista de Inadequações:

Aprova-se a emenda da pesquisa.

Considerações Finais a critério do CEP:

Tendo em vista a legislação vigente (Resolução CNS 466/12), o CEP-UFMG recomenda aos Pesquisadores: comunicar toda e qualquer alteração do projeto e do termo de consentimento via emenda na Plataforma Brasil, informar imediatamente qualquer evento adverso ocorrido durante o desenvolvimento da pesquisa (via documental encaminhada em papel), apresentar na forma de notificação relatórios parciais do andamento do mesmo a cada 06 (seis) meses e ao término da pesquisa encaminhar a este Comitê um sumário dos resultados do projeto (relatório final).

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_1437619.pdf	10/10/2020 20:28:10		Aceito
Declaração de Instituição e Infraestrutura	COMISSAO_CIENTIFICA_UFMG.pdf	10/10/2020 20:26:45	Silvia Almeida Cardoso	Aceito
Outros	GEP_UFMG_OUTUBRO.pdf	10/10/2020 20:25:49	Silvia Almeida Cardoso	Aceito
Outros	GEP_UFMG.pdf	10/10/2020 20:25:27	Silvia Almeida Cardoso	Aceito
Declaração de Instituição e Infraestrutura	VIABILIDADE_ECONOMICA_FINANCEIRA_UFMG.PDF	10/10/2020 20:24:16	Silvia Almeida Cardoso	Aceito
Solicitação registrada pelo CEP	CENTRO_PESQUISA_UFMG.pdf	10/10/2020 20:23:09	Silvia Almeida Cardoso	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	ASSENTIMENTO.pdf	10/10/2020 20:18:28	Silvia Almeida Cardoso	Aceito
TCLE / Termos de Assentimento / Justificativa de	TCLEprofessores.pdf	10/10/2020 20:17:46	Silvia Almeida Cardoso	Aceito

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Telefone: (31)3409-4592 **E-mail:** coep@prpq.ufmg.br

Anexo I: Parecer consubstanciado do Comitê de Ética em Pesquisa da Universidade Federal de Minas Gerais – Pág. 4

UNIVERSIDADE FEDERAL DE MINAS GERAIS

Plataforma Brasil

Continuação do Parecer: 4.364.744

Ausência	TCLEprofessores.pdf	10/10/2020 20:17:46	Silvia Almeida Cardoso	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLEpais.pdf	10/10/2020 20:17:28	Silvia Almeida Cardoso	Aceito
Outros	CARTA.pdf	10/10/2020 20:16:50	Silvia Almeida Cardoso	Aceito
Declaração de Manuseio Material Biológico / Biorepositório / Biobanco	BIORREPOSITORIO.pdf	10/10/2020 20:15:09	Silvia Almeida Cardoso	Aceito
Declaração de Pesquisadores	cartareposta.pdf	04/12/2019 14:42:38	Silvia Almeida Cardoso	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLEresponsavelmodificado.pdf	04/12/2019 14:42:23	Silvia Almeida Cardoso	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLEprofessormodificado.pdf	04/12/2019 14:42:14	Silvia Almeida Cardoso	Aceito
Declaração de Instituição e Infraestrutura	NITIDAmodificada.pdf	04/12/2019 14:42:05	Silvia Almeida Cardoso	Aceito
Declaração de Instituição e Infraestrutura	autorizacaomodificada.pdf	04/12/2019 14:41:52	Silvia Almeida Cardoso	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TAmodificado.pdf	04/12/2019 14:41:39	Silvia Almeida Cardoso	Aceito
Outros	FORMULARIO.pdf	21/10/2019 17:20:50	Silvia Almeida Cardoso	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	NOVO.pdf	21/10/2019 17:20:31	Silvia Almeida Cardoso	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TERMO.pdf	21/10/2019 17:20:02	Silvia Almeida Cardoso	Aceito
Outros	questionarioFREQUENCIAALIMENTAR.pdf	09/08/2019 14:55:41	Silvia Almeida Cardoso	Aceito
Outros	questionarioSISVAN.pdf	09/08/2019 14:54:54	Silvia Almeida Cardoso	Aceito

Endereço: Av. Presidente Antônio Carlos,6627 2º Ad SI 2005
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Anexo I: Parecer consubstanciado do Comitê de Ética em Pesquisa da Universidade Federal de Minas Gerais – Pág. 5

UNIVERSIDADE FEDERAL DE MINAS GERAIS

Continuação do Parecer: 4.364.744

Outros	questionarioCTSPC.pdf	09/08/2019 14:54:35	Silvia Almeida Cardoso	Aceito
Outros	questionarioCGI.pdf	09/08/2019 14:54:20	Silvia Almeida Cardoso	Aceito
Outros	questionarioBULLYING.pdf	09/08/2019 14:53:19	Silvia Almeida Cardoso	Aceito
Outros	QUESTIONARIOATIVIDADEFISICA.pdf	09/08/2019 14:53:04	Silvia Almeida Cardoso	Aceito
Outros	questionarioadesaotratamento.pdf	09/08/2019 14:52:16	Silvia Almeida Cardoso	Aceito
Outros	qiestionarioETDAH.pdf	09/08/2019 14:50:50	Silvia Almeida Cardoso	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	termodeacentimeto.pdf	25/01/2018 09:57:40	Silvia Almeida Cardoso	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE.pdf	25/01/2018 09:57:16	Silvia Almeida Cardoso	Aceito
Projeto Detalhado / Brochura Investigador	projeto.pdf	25/01/2018 09:56:55	Silvia Almeida Cardoso	Aceito

Situação do Parecer:
Aprovado

Necessita Apreciação da CONEP:
Não

BELO HORIZONTE, 27 de Outubro de 2020

Assinado por:
Críssia Carem Paiva Fontainha
(Coordenador(a))

Endereço: Av. Presidente Antônio Carlos,6627 2º Ad SI 2005
Bairro: Unidade Administrativa II **CEP:** 31.270-901
UF: MG **Município:** BELO HORIZONTE
Telefone: (31)3409-4592 **E-mail:** coep@prpq.ufmg.br

8.2. Anexo II: Termo de Autorização da Unidade de Atenção Especializada em Saúde

UFV Universidade Federal de Viçosa

D
EPARTAMENTO DE MEDICINA E ENFERMAGEMUNIDADE DE ATENÇÃO ESPECIALIZADA EM SAÚDE

DEM Departamento de Medicina e Enfermagem Universidade Federal de Viçosa - MG

Praça w s/n – Viçosa, MG – 36570-133 – Telefone: (31) 3612-5580 - E-mail: uaesufv@gmail.com

AUTORIZAÇÃO

Eu, Dalila Teixeira Leal, na qualidade de responsável pela Comissão de Pesquisa e Extensão da Unidade Atenção Especializada em Saúde (UAES) autorizo a realização da pesquisa intitulada "NÍVEIS DE BNDF E PERFIL OXIDATIVO/INFLAMATÓRIO DE CRIANÇAS COM TRANSTORNO DE DÉFICIT DE ATENÇÃO E HIPERATIVIDADE (TDAH) ANTES E APÓS TRATAMENTO COM METILFENIDATO", a ser conduzida sob a responsabilidade da pesquisadora Marina Silva de Lucca. Declaro, que esta Instituição apresenta infraestrutura necessária à realização da referida pesquisa. Esta autorização só é válida no caso de haver parecer favorável do Comitê de Ética em Pesquisa com Seres Humanos da Universidade Federal de Viçosa para a referida pesquisa. A pesquisa será custeada pelos pesquisadores, sem custos para a UAES.

Dalila Teixeira Leal
ENFERMEIRA
COREN - MG 180[illegible]

Dalila Teixeira Leal
Enfermeira
Comissão de Pesquisa e Extensão
Unidade de Atenção Especializada em Saúde

8.3. Anexo III: Termo de Constituição de Biorrepositório – Página 1

UFMG **Termo de Constituição de Biorrepositório** UFV

O presente acordo estabelece as normas para operacionalização, compartilhamento e utilização do material biológico humano coletado e armazenado em Biorrepositório, vinculado ao Projeto de Pesquisa: NÍVEIS DE BDNF, PERFIL INFLAMATÓRIO E OXIDATIVO DE CRIANÇAS COM TRANSTORNO DE DÉFICIT DE ATENÇÃO E HIPERATIVIDADE (TDAH) ANTES E APÓS TRATAMENTO COM METILFENIDATO, a ser gerenciado pela pesquisadora Marina Silva de Lucca, com participação da Universidade Federal de Minas Gerais, localizada à Av. Antônio Carlos, 6627, Pampulha - Belo Horizonte - MG - CEP 31270-90, CNPJ nº 17.217.985/ 0001-04, da Universidade Federal de Viçosa, localizada à Av. Peter Henry Rolfs, s/n Campus Universitário – Viçosa, MG, CEP: 36570-900, CNPJ 25.944.455/001-96 e das professoras DÉBORA MARQUES DE MIRANDA E SÍLVIA ALMEIDA CARDOSO das Faculdades de Medicina da Universidade Federal de Minas Gerais e da Universidade Federal de Viçosa, respectivamente , localizadas nos endereços e com os CNPJs citados anteriormente, conforme definido na legislação competente, atendendo, em especial, ao disposto nas Resoluções nº 441/11 e nº 466/12, ambas do CNS.

1

1- O Biorrepositório, constituído por amostras de soro, plasma e DNA, atenderá às normas do Regimento Institucional de Biorrepositório da instituição depositária e será sediado e armazenado na Faculdade de Medicina da Universidade Federal de Minas Gerais, localizada à Av. Prof. Alfredo Balena, 190 Belo Horizonte - MG - Brasil - Cep 30130-100, CNPJ 17.217.985/0001-04 no laboratório de medicina molecular, inscrito no CNPJ sob o nº 17.217.985/0001-04 , situado no mesmo endereço da Faculdade de Medicina (Primeiro andar – sala 114).

2- O material biológico constituinte do Biorrepositório será mantido em geladeira (plasma a 4° C) e freezer (plasma a -80°C e soro a -4°C e -20°C) até sua utilização;

3- O prazo de armazenamento do Biorrepositório será o mesmo definido no cronograma do projeto de pesquisa aprovado pelo Comitê de Ética em Pesquisa da UFMG (COEP-UFMG);

4- As instituições acordantes, devidamente representadas, poderão ter acesso aos dados e materiais obtidos em decorrência da execução do projeto, durante sua vigência, mediante solicitação aos membros da equipe do projeto;

RUBRICAS: Lucca

Anexo III: Termo de Constituição de Biorrepositório – Página 2

5- A solicitação de acesso a dados e materiais do Biorrepositório somente poderá ser feita por meio dos membros da equipe do projeto de pesquisa, devidamente cadastrados na Plataforma Brasil, dentro dos parâmetros estabelecidos pelo Projeto de Pesquisa e mediante aprovação da análise ética;

6- O Biorrepositório estará sob a responsabilidade do pesquisador, competindo aos acordantes o cumprimento das disposições aqui constantes e observância das normas contidas no regulamento de Biorrepositório;

7- A requisição de amostras durante a vigência da pesquisa deverá ser feita por escrito e não poderá causar prejuízo ao regular desenvolvimento do Projeto de Pesquisa;

8- Havendo a retirada ou desistência por parte do participante da pesquisa, referente à amostra coletada e armazenada, deverá o pesquisador e a instituição que mantém a guarda disponibilizarem a amostra, nos termos da regulamentação vigente. Nesse caso, será facultado ao participante da pesquisa requerer a amostra ou solicitar que ela seja destruída pelo pesquisador;

9- Em caso de dissolução da parceria entre as instituições durante a vigência do projeto, a partilha e destinação dos dados e materiais que compõem o Biorrepositório serão objeto de novo acordo que deverá ser submetido à análise de ética dos Comitês de Ética Institucionais;

10- Em caso de encerramento do projeto de pesquisa, havendo interesse de uso futuro das amostras do Biorrepositório e quando autorizado pelo participante da pesquisa em Termo de Consentimento Livre e Esclarecido, o pesquisador responsável pelo projeto deverá manifestar seu interesse por escrito e assinado pelos pesquisadores e instituições parceiras. A partilha e destinação dos dados e materiais que compõem o Biorrepositório serão objeto de novo acordo entre as instituições, que deverá ser submetido à análise ética dos Comitês de Ética em Pesquisa envolvidos;

11- Para uso futuro das amostras em nova pesquisa, em atendimento ao disposto na Resolução nº 441/2011 do CNS, deverá haver submissão de novo Projeto de Pesquisa ao Sistema CEP/CONEP;

12- Todos os materiais armazenados no Biorrepositório serão destruídos ao final do projeto de pesquisa, caso não haja manifestação nos termos da Cláusula 10;

13- Os casos não contemplados pelo presente Termo de Constituição de Biorrepositório serão submetidos à análise conjunta dos acordantes e resolvidos de comum acordo pelas partes envolvidas.

RUBRICAS: _____; _____; _____; _____; _____.

2

Anexo III: Termo de Constituição de Biorrepositório – Página 3

UFMG

Assinaturas (com a inclusão de carimbos):

UFV
Universidade Federal de Viçosa

Marina Silva de Lucca
CRM-MG 39.542
Professora/Técnica UFV
Matrícula 11067-1 / 10997-5

Pesquisadora do Projeto – Doutoranda do Serviço de Pós-Graduação em Ciências da Saúde - Saúde da Criança e do Adolescente da UFMG

(Marina Silva de Lucca)

Débora Marques de Miranda
Coordenadora Geral
Centro Detecnologia em Medicina Molecular

Débora Marques de Miranda
Coordenadora Geral
Centro Detecnologia em Medicina

Pesquisadora Principal do Projeto – UFMG (Orientadora: Débora Marques de Miranda)

Pesquisadora responsável do Projeto – UFV (Coorientadora: Sílvia Almeida Cardoso)

Prof. Humberto José Alves
Vice-Diretor da Faculdade de Medicina da UFMG
Inscrição UFMG: 109037
Inscrição SIAPE: 323221

Representante Legal da Instituição

Prof. Humberto José Alves
Vice-Diretor da Faculdade de Medicina da UFMG
Inscrição UFMG: 109037
Inscrição SIAPE: 323221

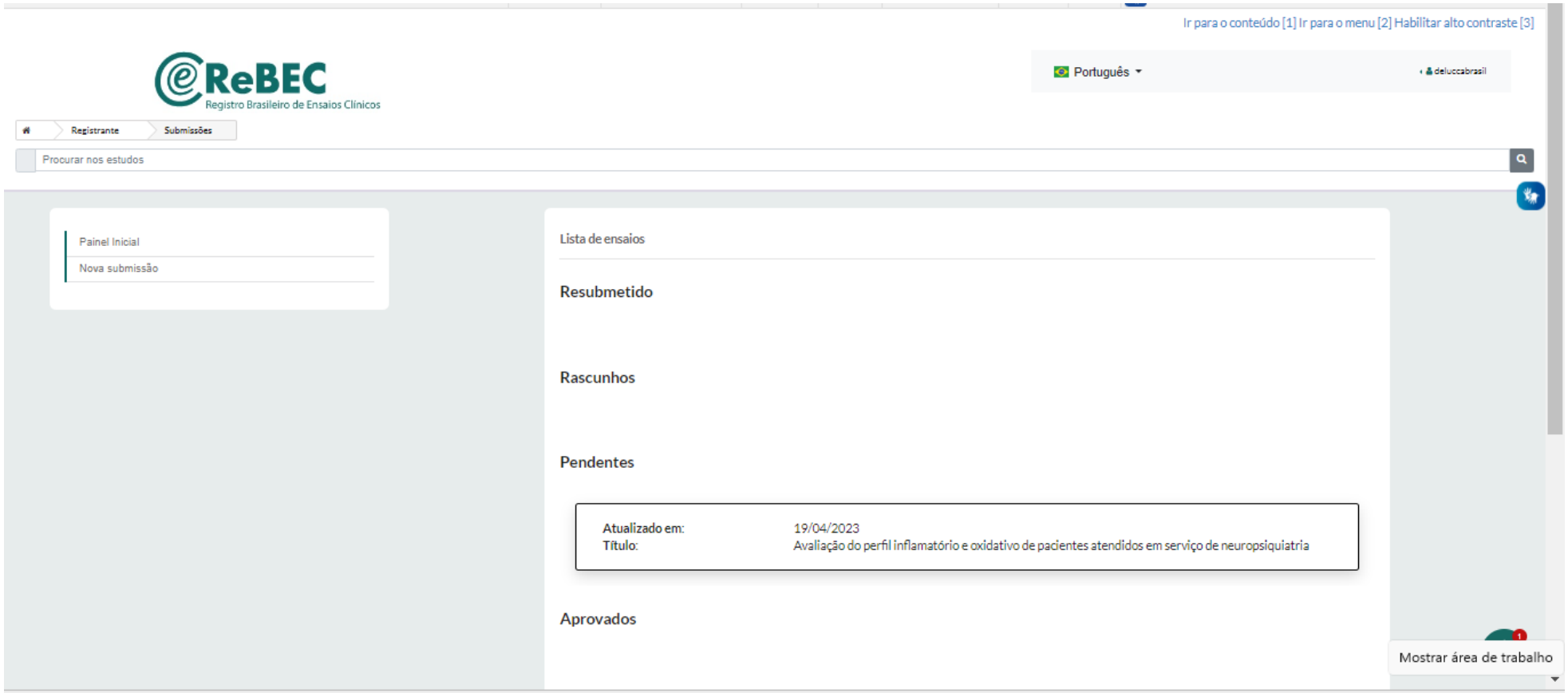
Chefia do Serviço de Pesquisa

Profª Cristiane Chaves de Souza
Chefe do Deptº. de Medicina
e Enfermagem/UFV
Matrícula: 12036-7

Responsável Legal pela Instituição Acordante – Chefe do Departamento de Medicina e Enfermagem da UFV (Cristiane Chaves de Souza)

3

8.4. Anexo IV: Sumissão ao ReBEC (Registro Brasileiro de Ensaios Clínicos).



8.5. Anexo V: Autorização de uso do instrumento Medida de Adesão aos Tratamentos (MAT)

De: Maria Luísa Lima
Enviado:segunda-feira, 12 de agosto de 2019 12:31
Para: Marina de Lucca
Assunto: RE: Autorização para uso MAT

Cara Marina,
Muito obrigada pelo seu contacto e pelo seu interesse no nosso trabalho.
Autorizo a utilização da MAT, desde que a referencie correctamente em publicações futuras desta investigação:
Delgado, A.B., & Lima, M.L. (2001). Contributo para a validação concorrente de uma medida de adesão aos tratamentos. *Psicologia: Saúde e Doenças, 1,* 81-100.
Com os melhores cumprimentos, desejo-lhe os melhores sucessos.

Luisa Lima

De: Marina de Lucca <lucca_marina@hotmail.com>
Enviado: 9 de agosto de 2019 16:39
Para: Maria Luísa Lima <luisa.lima@iscte-iul.pt>
Assunto: Autorização para uso MAT

Excelentíssima Profª Drª Maria Luisa Lima, gostaria de solicitar, por gentileza, autorização para uso do instrumento Medida de Adesão aos Tratamentos (MAT). Ele será usado em minha pesquisa de doutorado intitulada Níveis de BNDF e perfil oxidativo/inflamatório de crianças com Transtorno de Déficit de Atenção e Hiperatividade (TDAH) antes e após tratamento com Metilfenidato. O instrumento será usado com os pais das crianças para verificar nível de adesão ao uso do metilfenidato. A pesquisa será realizada no Programa de Pós-Graduação em Ciências da Saúde – Saúde da criança e do Adolescente da UFMG sob orientação da Profª Débora Marques de Miranda da UFMG e coorientação da Profª Sílvia Cardoso da UFV/MG. Estou à disposição para maiores esclarecimentos e agradeço sua atenção desde já.
Atenciosamente,
Marina Silva de Lucca
Professora do Curso de Medicina (Psiquiatria) da Universidade Federal de Viçosa.

Enviado do Email para Windows 10

8.6. Anexo VI: Anamnese padronizada de primeira consulta – Página 1

ID:	DATA:

Dra. Marina de Lucca
CRMMG 39.542
Psiquiatra da Infância e Adolescência

Nome:	
Data de nascimento:	CPF: (obrigatório)
Cartão SUS:	
Mãe:	DN:
Pai:	DN:
Responsável:	Parentesco:
Endereço atual (colocar CEP):	
Telefones de contato: Whatsapp:	

Anexar cópia da certidão de nascimento da criança, carteira de identidade da criança e carteira de identidade da mãe.

Data do primeiro atendimento:	
Motivo do encaminhamento:	
Profissional que encaminhou:	Data do encaminhamento:
UBS de referência:	Tel da UBS: ( )

TERMO DE ANUÊNCIA PARA CONSULTA

Aceito e estou ciente que a consulta de minha criança e/ou adolescente será realizada por ACADÊMICOS E/OU RESIDENTES DE MEDICINA, ENFERMAGEM, NUTRIÇÃO, PSICOLOGIA, EDUCAÇÃO FÍSICA, EDUCAÇÃO INFANTIL, sob supervisão do (a) médico (a), enfermeiro (a), nutricionista, psicólogo, educador físico e/ou educador infantil responsável do Departamento de Medicina e Enfermagem, demais departamentos da UFV e profissionais vinculados aos projetos de pesquisa sob coordenação da Profª Marina Silva de Lucca.

Nome: .....
CNS: .....
Data: ..... / ..... / 202.....
Assinatura do (a) responsável: .....

Anexo VI: Anamnese padronizada de primeira consulta – Página 2

TERMO DE AUTORIZAÇÃO E USO DE IMAGEM

Eu, ...,

Portador do CPF . . - , AUTORIZO o uso de minha imagem ou do (a) meu (minha) filho (a), ... assim como dos responsáveis e irmãos da criança se for o caso, para fins acadêmicos e científicos, ou seja, discussão científica de casos clínicos, aulas, congressos e eventos científicos. A presente autorização é concedida a título gratuito, abrangendo o uso da imagem (foto, vídeo, som) acima mencionada em todo território nacional e/ou no exterior.

Por meio desta autorização ora concedida, autorizo a Dra. Marina Silva de Lucca, professora do Departamento de Medicina e Enfermagem (DEM) da Universidade Federal de Viçosa(UFV/MG) realizar nas imagens e sons captados, cortes, reduções e edições para o uso acadêmico e científico como mencionado acima.

Esta autorização não gera e não gerará no futuro e, também não ensejará interpretação de existir quaisquer vínculos ou obrigações trabalhistas, securitárias, previdenciárias, indenizatória ou mesmo empregatícia, entre o (a) cedente e a Dra. Marina Silva de Lucca e o DEM/UFV.

Declaro, portanto, que estou de acordo com essas imagens, que não violam os direitos de imagem e de privacidade do cedente, e que tenho ciência que este material constituído por imagens, vídeos e sons pertence exclusivamente à Dra. Marina Silva de Lucca – DEM/UFV/MG, que poderá usá-lo a seu exclusivo critério.

Viçosa, ... / ..... / ..... .

_____

Assinatura do Cedente **(paciente se adulto ou o responsável se menor de idade ou interditado/incapaz)**

Anexo VI: Anamnese padronizada de primeira consulta – Página 3

RESULTADOS DE EXAMES

Exames	*Data:*	*Data:*	*Data:*	*Data:*
Hemácias				
Hemoglobina				
Hematócrito				
VCM				
HCM				
CHCM				
RDW				
Leucócitos Totais				
Neut/Linf/Eos				
Plaquetas				
Ferritina/Fe sérico				
Glicemia jejum				
HA1C				
CT/HDL/TG				
TGO/TGP/FA/GGT				
Creatinina/Uréia				
TSH/T4 livre				
Prolactina				
Vitamina D				
Viamina B12				
Ácido Fólico				
Ácido Úrico				
Ácido Lático				
Bicarbonato				
DHL				
Gasometria venosa				
CPK				
Aldolase				
Dosagem quantitativa de aminoácidos no sangue				
Estudo completo de ácidos orgânicos na urina				
Na/K/Mg/P/Ca/Cl				
Litemia sérica				
Ácido Valpróico				
Carbamazepina				

Anexo VI: Anamnese padronizada de primeira consulta – Página 4

Exames	*Data:*	*Data:*	*Data:*	*Data:*
Hemácias				
Hemoglobina				
Hematócrito				
VCM				
HCM				
CHCM				
RDW				
Leucócitos Totais				
Neut/Linf/Eos				
Plaquetas				
Glicemia jejum				
HA1C				
CT/HDL/TG				
TGO/TGP				
Creatinina/Uréia				
TSH/T4 livre				
Prolactina				
Litemia sérica				
Ácido Valpróico				
Carbamazepina				
EEG				
ECG				
Polissonografia				
Processamento Auditivo Central				
Audiometria				
Cariótipo		Pesquisa X- frágil		
RNM Encéfalo				
Avaliação Visual				

Anexo VI: Anamnese padronizada de primeira consulta – Página 5

Responsável pelo preenchimento do questionário	
	Ambos os pais biológicos
	Mãe biológica
	Pai biológico
	Avó materna
	Avó paterna
	Avô materno
	Avô paterno
	Tia materna
	Tia paterna
	Conselho tutelar
	Cuidador de Casa de Acolhimento
	Babá
	Mãe adotiva
	Pai adotivo
	Padrasto
	Madrasta
	Outro

Raça declarada	
	Branca
	Negra
	Parda
	Amarela
	Indígena
	Não quis informar

Provedor de Saúde	
	Sus
	Convênio de Saúde
	Particular

Religião	
	Católico
	Protestante/Evangélico
	Espírita
	Sem religião
	Ateu
	Outra

Ano Escolar	
	Educação infantil segundo ano
	Primeiro ano ensino fundamental I
	Segundo ano ensino fundamental I
	Terceiro ano ensino fundamental I
	Quarto ano ensino fundamental I
	Quinto ano ensino fundamental I
	Sexto ano ensino fundamental I
	Ensino fundamental II
	Fora da escola
Anos reprovados na escola	
	1 ano
	2 anos
	3 anos
	4 anos
	5 anos
	nenhum
Tipo de Escola	
	Pública
	Particular
Nome da escola	

Chefe da família é:			
	Pai		Tia
	Mãe		Outro
	Avó materna		Pai e mãe ganham igualmente
	Avó paterna	Chefe de família = pessoa que contribui com a maior parte da renda do domicílio.	
	Avô materno		
	Avô paterno		

Grau de instrução do chefe da família é	
A	Analfabeto – fundamental I Incompleto
B	Fundamental I Completo – Fundamental II Incompleto
C	Fundamental Completo – Médio Incompleto
D	Médio Completo – Superior Incompleto
E	Superior Completo
F	Pós-graduação Completa

Grau de instrução da mãe, caso ela não seja a chefe da família
Colocar letra acima descriminadas:
Grau de instrução do pai, caso ele não seja a chefe da família
Colocar letra acima descriminadas:

Anexo VI: Anamnese padronizada de primeira consulta – Página 6

	Mora atualmente com:		Cuidador Primário		Cuidador Secundário
	Ambos os pais biológicos		Ambos os pais biológicos		Ambos os pais biológicos
	Ambos os pais biológicos, mas em guarda compartilhada		Mãe biológica		Mãe biológica
	Mãe biológica e padrasto		Pai biológico		Pai biológico
	Pai biológico e madrasta		Avó materna		Avó materna
	Mãe biológica e namorado/a		Avó paterna		Avó paterna
	Pai biológico e namorada/o		Avô materno		Avô materno
	Apenas mãe biológica		Avô paterno		Avô paterno
	Apenas pai biológico		Tia/tio maternos		Tia/tio maternos
	Apenas madrasta		Tia/tio paternos		Tia/tio paternos
	Apenas padrasto		Conselho tutelar		Conselho tutelar
	Avô/Avó		Cuidador de Casa de Acolhimento		Cuidador de Casa de Acolhimento
	Pais adotivos		Babá		Babá
	Outro familiar/amigo		Mãe adotiva		Mãe adotiva
	Lar adotivo/instituição		Pai adotivo		Pai adotivo
	Fuga		Madrasta		Madrasta
	Mora independente		Padrasto		Padrasto
	Outro		Outro		Outro
	Escolaridade:		Escolaridade: Nome:		Escolaridade: Nome:

A criança vive com a mãe biológica?	sim	Se sim, a qualidade dessa relação é: o Excelente o Boa o Regular o ruim	não	Se não: o Mãe falecida o Mãe viva, visita regularmente o Mãe viva, contato esporádico o Mãe viva, mas não mantém contato
A criança vive com o pai biológica?	sim	Se sim, a qualidade dessa relação é: o Excelente o Boa o Regular o ruim	não	Se não: o Pai falecida o Pai viva, visita regularmente o Pai viva, contato esporádico o Pai viva, mas não mantém contato
A criança vive com		Grau de parentesco: Nome: Idade	sim	Se sim, a qualidade dessa relação é: o Excelente o Boa o Regular o ruim
A criança vive com		Grau de parentesco: Nome: Idade:	sim	Se sim, a qualidade dessa relação é: o Excelente o Boa o Regular o ruim
A criança vive com		Grau de parentesco: Nome: Idade:	sim	Se sim, a qualidade dessa relação é: o Excelente o Boa o Regular o ruim
A criança vive com		Grau de parentesco: Nome: Idade:	sim	Se sim, a qualidade dessa relação é: o Excelente o Boa o Regular o ruim

Anexo VI: Anamnese padronizada de primeira consulta – Página 7

Escolha a opção que corresponde à situação de sua família					
Automóveis de passeio exclusivamente para uso particular (excluir van, taxi usados para fretes, uso misto excluir)	0	I	2	3	≥4
Empregados mensalistas, que considerando apenas os que trabalham pelo menos cinco dias por semana.	0	I	2	3	≥4
Máquinas de lavar roupa, excluindo tanquinho	0	I	2	3	≥4
Banheiros (ter vaso sanitário), excluir banheiro coletivo (mais de uma casa)	0	I	2	3	≥4
DVD, incluindo qualquer dispositivo que leia DVD (notebook, videogame, computadores) e desconsiderando DVD de automóvel	0	I	2	3	≥4
Geladeiras	0	I	2	3	≥4
Freezers independentes ou parte da geladeira duplex	0	I	2	3	≥4
Microcomputadores (computadores de mesa, laptops, notebooks e netbooks e desconsiderando tablets, palms ou smartphones)	0	I	2	3	≥4
Tablets	0	I	2	3	≥4
Smartphones	0	I	2	3	≥4
Lavadora de louças	0	I	2	3	≥4
Microondas (considerar forno elétrico que tem função microondas)	0	I	2	3	≥4
Motocicletas, desconsiderando as usadas exclusivamente para uso profissional. Considerar as de uso misto (pessoal + profissional)	0	I	2	3	≥4
Secadoras de roupas, considerando lava e seca	0	I	2	3	≥4
Número de pessoas que mora em casa					
A água utilizada em sua casa é proveniente de:		Rede geral de distribuição			
		Poço ou nascente			
		Outro			
Considerando o trecho de rua do seu domicílio, você diria que a rua é		Asfaltada/Pavimentada			
		Terra/Cascalho			

Anexo VI: Anamnese padronizada de primeira consulta – Página 8

Equipe multidisciplinar que acompanha o paciente:			
Psicologia	Nome:		
tel:	Início:	Interrupção:	Término:
Terapia ocupacional	Nome:		
tel:	Início:	Interrupção:	Término:
Fonoaudiologia	Nome:		
tel:	Início:	Interrupção:	Término:
Psicopedagogia	Nome:		
tel:	Início:	Interrupção:	Término:
Fisioterapia	Nome:		
tel:	Início:	Interrupção:	Término:
Prof. de apoio	Nome:		
tel:	Início:	Interrupção:	Término:
APAE	Nome:		
tel:	Início:	Interrupção:	Término:
Nutricionista	Nome:		
tel:	Início:	Interrupção:	Término:
Médico	Nome:		
tel:	Início:	Interrupção:	Término:
Médico	Nome:		
tel:	Início:	Interrupção:	Término:
Esporte	Nome:		
tel:	Início:	Interrupção:	Término:

Anexo VI: Anamnese padronizada de primeira consulta – Página 9

ID: **DATA:** **RESPONSÁVEL:**

História gestacional:

∞ Planejada ∞ Não planejada ∞ Desejada ∞ Não desejada

Reação da mãe: 0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95 100
Muito triste — Muito feliz

Reação do pai: 0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95 100
Muito triste — Muito feliz

Reação da família: 0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95 100
Muito triste — Muito feliz

Descobriu a gravidez com **semanas aproximadamente.**

Tentativa de aborto nesta gestação? O sim O não O duvidoso
Se sim, descreva:

Fez pré-natal: O Não O Sim O Informação desconhecida

Início no: O 1° Trimestre (≤ 13 sem) O 2° trimestre (≥14 sem ≤ 27 sem) O 3° trimestre (≥28 sem ≤ 41 sem) O Informação desconhecida

N° consultas de PN: O Menos de 6 consultas O Entre 6 e 8 consultas O 9 consultas ou mais O Informação desconhecida

N° de gestações: O 1 O 2 O 3 O 4 O 5 O 6 O Outro O Informação desconhecida
Se respondeu **OUTRO**, especifique:

N° de partos: O 1 O 2 O 3 O 4 O 5 O 6 O Outro O Informação desconhecida
Se respondeu **OUTRO**, especifique:

N° de abortos: O 0 O 1 O 2 O 3 O 4 O 5 O 6 O Outro O Informação desconhecida
Se respondeu **OUTRO**, especifique: O espontâneo O Provocado
Com quantas semanas? Como?

Ordem do paciente dentre os demais filhos: O Primeiro filho (a) O Segundo filho (a) O Terceiro filho (a) O Quarto filho (a) O Outro O Informação desconhecida
Se respondeu **OUTRO**, especifique:

Há história de natimorto? O Não O Sim O Informação desconhecida
Se respondeu SIM, descreva a causa:

Há consanguinidade entre os pais? O Não O Sim O Informação desconhecida
Se respondeu SIM, defina o grau de parentesco:

Idade da mãe na gravidez: **Idade do pai na gravidez:**

Uso de substâncias na gravidez. **Quantidade? Frequência? Quais trimestres? Cessou uso?**	**álcool**	O Não	O Sim. O 1° Trimestre O 2° trimestre O 3° trimestre O Lactação materna O Info desconhecida Quantidade e frequência?
	tabaco	O Não	O Sim. O 1° Trimestre O 2° trimestre O 3° trimestre O Lactação materna O Info desconhecida Quantidade e frequência?
	cocaína/crack	O Não	O Sim. O 1° Trimestre O 2° trimestre O 3° trimestre O Lactação materna O Info desconhecida Quantidade e frequência?
	maconha	O Não	O Sim. O 1° Trimestre O 2° trimestre O 3° trimestre O Lactação materna O Info desconhecida Quantidade e frequência?
	outras*	O Não	O Sim. O 1° Trimestre O 2° trimestre O 3° trimestre O Lactação materna O Info desconhecida Quantidade e frequência?

*Se faz uso de outras substâncias na gravidez, descreva qual (is):

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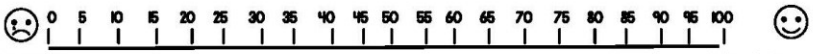
Uso de medicamentos na gravidez Quantidade? Frequência? Quais trimestres? Cessou uso?	O Sulfato Ferroso. O Pré- gestacional O1° Trimestre O 2° trimestre O 3° trimestre O Lactação materna O Informação desconhecida
	O Ácido Fólico. O Pré- gestacional O1° Trimestre O 2° trimestre O 3° trimestre O Lactação materna O Informação desconhecida
	O O Pré- gestacional O1° Trimestre O 2° trimestre O 3° trimestre O Lactação materna O Informação desconhecida
	O O Pré- gestacional O1° Trimestre O 2° trimestre O 3° trimestre O Lactação materna O Informação desconhecida
	O O Pré- gestacional O1° Trimestre O 2° trimestre O 3° trimestre O Lactação materna O Informação desconhecida
Bem-estar materno durante a gravidez	 Muito triste Muito feliz
Estado emocional da mãe e intercorrências psiquiátricas no período perinatal	O Violência Doméstica O Sim O Não O Informação desconhecida O Estresse no ambiente de trabalho O Sim O Não O Informação desconhecida
	Se respondeu SIM no item VIOLÊNCIA DOMÉSTICA, especifique qual e descreva: O Física O Psicológica ou moral O Sexual O Patrimonial O Informação desconhecida
Tipos de violência doméstica, em material anexo.	O Psicose durante a gravidez O Sim O Não O Informação desconhecida O Psicose pós-parto O Sim O Não O Informação desconhecida
	O Síndrome Ansiosa na gravidez O Sim O Não O Informação desconhecida O Síndrome Ansiosa no pós-parto O Sim O Não O Informação desconhecida
	O Depressão durante a gravidez O Sim O Não O Informação desconhecida O Depressão pós-parto O Sim O Não O Informação desconhecida
Intercorrências clínicas ou obstétricas na gravidez ISTs = infecções sexualmente transmissíveis.	O HAS O Não O Sim O 1° Trim. O 2° trim. O 3° trim. O Info. desconhecida Manejo: O DM O Não O Sim O 1° Trim. O 2° trim. O 3° trim. O Info. desconhecida Manejo:
	O Sangramento O Não O Sim O 1° Trim. O 2° trim. O 3° trim. O Info. desconhecida Manejo: O Perda de líquido O Não O Sim O 1° Trim. O 2° trim. O 3° trim. O Info. desconhecida Manejo:
	O ITU O Não O Sim O 1° Trim. O 2° trim. O 3° trim. O Info. desconhecida Manejo: O ISTs O Não O Sim O 1° Trim. O 2° trim. O 3° trim. O Info. desconhecida Manejo:
	O Anemia O Não O Sim O 1° Trim. O 2° trim. O 3° trim. O Info. desconhecida Manejo: O Baixo Peso O Não O Sim O 1° Trim. O 2° trim. O 3° trim. O Info. desconhecida Quantos kg ganhou na gestação: Manejo:
	Outras: O Não O Sim O 1° Trim. O 2° trim. O 3° trim. O Info. desconhecida Se SIM, especifique:

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História do Parto	Tipo: O Vaginal O Cesárea O Fórceps O Informação desconhecida Se vaginal, pergunte se houve uso de fórceps.
	Se respondeu CESÁREA, especifique o tipo: O Eletiva O De urgência O Info. desconhecida Se DE URGÊNCIA, especifique o motivo:
	Duração do trabalho de parto: horas e minutos. O Não se aplica
	IG: semanas e dias. O Pré-termo. O A termo O Pós- termo O Informação desconhecida
	Intercorrências: O Não O Sim - Qual (is)? O Informação desconhecida
	Estado do bebê: Chorou ao nascer? O Não O Sim O Informação desconhecida Cianótico? O Não O Sim O Informação desconhecida Líquido meconial? O Não O Sim O Informação desconhecida Icterícia? O Não O Sim O Informação desconhecida Alojamento conjunto O Não O Sim O Informação desconhecida UTI* O Não O Sim O Informação desconhecida PCR O Não O Sim O Informação desconhecida *Se SIM, quantos dias: Sofrimento fetal: O Não O Sim O Informação desconhecida
	Se SIM em ICTERÍCIA, selecione quando ocorreu: O Nas primeiras 24h após o nascimento O Entre 24h e 15 dias de nascimento O Após 15 dias de nascimento O Informação desconhecida
	Tratamento para a ICTERÍCIA: O Nenhum (chá de picão) O Fototerapia (banho de luz no hospital) O Exanguineotransfusão O Informação desconhecida
	Tempo de permanência no hospital: O Até 24h O Entre 25 e 48h O Entre 49 e 72h O Acima de 72h O Informação desconhecida Se respondeu ACIMA DE 72H, especifique o tempo e o motivo:
Triagens /avaliações neonatais e na infância e adolescência Bera, audiometria e processamento auditivo são exames diferentes do teste de orelhinha, feitos em outros momentos da vida da criança	Teste do pezinho: O Não O Sim O Informação desconhecida O Teste visto O teste relatado pelo responsável Resultado:
	Teste da orelhinha: O Não O Sim O Informação desconhecida O Teste visto O teste relatado pelo responsável Resultado:
	Bera/Audiometria: O Não O Sim O Informação desconhecida O Teste visto O teste relatado pelo responsável Resultado:
	Processamento auditivo: O Não O Sim O Informação desconhecida O Teste visto O teste relatado pelo responsável Resultado:
	Exame oftalmológico em idade pré-escolar ou escolar: não se trata do teste reflexo vermelho O Não O Sim O Informação desconhecida O Teste visto O teste relatado pelo responsável Resultado:

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História do Desenvolvimento	
Motor	Sentou Engatinhou Andou Desfralde diurno Desfralde noturno Coordenação motora fina (escrever, desenhar, pegar objetos pequenos em pinça) Brincadeiras motoras (bola, correr, pular, subir e descer escadas) Classificação MOTOR: O Adequado O Atrasado O Informação desconhecida
Linguagem Consegue uma comunicação verbal e gestual adequada com os pares?	Virar o rosto em direção a barulhos Balbucio Primeiras palavras, exceto mamãe e papai Frases de 2 palavras. Diálogo Jogar conversa fora Entender ironias, girias, piadas, mensagens subentendidas Classificação LINGUAGEM: O Adequado O Atrasado O Informação desconhecida
Social Consegue uma interação adequada com os pares? Com adultos? Faz e mantém amigos?	Sorriso social Beijo Tchau Faz-de-conta Interação com pares Olhar compartilhado Empatia Brincadeiras com crianças da mesma idade Classificação SOCIAL: O Adequado O Atrasado O Informação desconhecida
Cognitivo Entender o contexto, correlacionar com o que já sabe e resolver o problema.	Entendimento de comandos Curiosidade em aprender coisas novas Escola (alfabetização) Capacidade de abstração para a idade: conseguir aprender algo, passar a imaginar como se resolve aquilo, criar situações de criatividade e inventar modelos novos a partir do inicial ou apenas repete o que viu ou aprendeu e não coloca elementos dela, capacidade de generalização. Ex. O lápis serve para escrever, mas posso usar para prender o cabelo. Classificação COGNITIVO: O Adequado O Atrasado O Informação desconhecida
Adaptativo (resolver problemas de forma autônoma e adequada para a idade)	Atividades de vida diária para a idade (ex. vestir-se, despir-se, tomar banho, escovar dente, comer, dar recado, saber troco) – avaliar autonomia esperada para a idade. Aprender adequadamente as tarefas, os recursos e instrumentos do ambiente. Classificação ADAPTATIVO: O Adequado O Atrasado O Informação desconhecida
Processamento Sensorial	Audição: O Sem alterações O Hiperresponsivo O Hiporresponsivo O Informação desconhecida Visão: O Sem alterações O Hiperresponsivo O Hiporresponsivo O Informação desconhecida Olfato: O Sem alterações O Hiperresponsivo O Hiporresponsivo O Informação desconhecida Tato: O Sem alterações O Hiperresponsivo O Hiporresponsivo O Informação desconhecida (abraço, roupas etc) Gustação: O Sem alterações O Hiperresponsivo O Hiporresponsivo O Informação desconhecida (restrição alimentar, textura de alimentos) Dor: O Sem alterações O Hiperresponsivo O Hiporresponsivo O Informação desconhecida Interocepção: O Sem alterações O Hiperresponsivo O Hiporresponsivo O Informação desconhecida (sensações interiores de fome, sede, sono, bexiga cheia, batimentos cardiacos, cansaço) Vestibular: O Sem alterações O Hiperresponsivo O Hiporresponsivo O Informação desconhecida Termocepção: O Sem alterações O Hiperresponsivo O Hiporresponsivo O Informação desconhecida Se respondeu HIPO/HIPERRESPONSIVO em algum item, explique:
Desenvolvimento puberal	
Menarca: O SIM O NÃO O NÃO SE APLICA Se SIM, diga quando ocorreu:	DUM

Anexo VI: Anamnese padronizada de primeira consulta – Página 13

Descrever! **História Patológica Pregressa do paciente** Qual? Que idade começou? Qual tratamento? Complicações? Sequelas?	Transtornos mentais. O Informação desconhecida	O Não	O Sim
	Tratamento com psicólogo O Informação desconhecida	O Não	O Sim
	Tentativas de suicídio O Informação desconhecida	O Não	O Sim
	Internações? TCE? O Informação desconhecida	O Não	O Sim
	Cirurgias O Informação desconhecida	O Não	O Sim
	Alergias O Informação desconhecida	O Não	O Sim
	Epilepsia (Crise de ausência) O Informação desconhecida	O Não	O Sim
	Desnutrição O Informação desconhecida	O Não	O Sim
	Anemia O Informação desconhecida	O Não	O Sim
	Queixas cardíacas? Problema cardíaco? (arritmia, taquicardia, dor precordial, dispneia? etc) O Informação desconhecida	O Não	O Sim
	Outras doenças O Informação desconhecida	O Não	O Sim
História de doenças na família Descrever qual transtorno e quem o possui. Qualquer nível de parentesco, mesmo muito distante como bisavós, etc Perguntar por sintomas dos transtornos, muitas pessoas não têm o diagnóstico.	TDAH O Informação desconhecida	O Não	O Sim
	TEA O Informação desconhecida	O Não	O Sim
	Outros transt. mentais (Depressão, Ansiedade, Esquizofrenia, TOC, etc) O Informação desconhecida	O Não	O Sim
	Alcoolismo O Informação desconhecida	O Não	O Sim
	Drogas ilícitas O Informação desconhecida	O Não	O Sim
	Internações psiquiátricas O Informação desconhecida	O Não	O Sim
	Tentativas de suicídio O Informação desconhecida	O Não	O Sim
	Epilepsia O Informação desconhecida	O Não	O Sim
	Doenças cardíacas em familiares jovens? Arritmias? Morte Súbita? IAM? O Informação desconhecida	O Não	O Sim
	Outras doenças (DM, HAS, Câncer etc) O Informação desconhecida	O Não	O Sim

Se respondeu SIM no item TDAH, especifique: O Mãe O Pai O Irmão (s) O Primos paternos O Primos maternos O Tios paternos O Tios maternos O Avós paternos O Avós maternos O Outro* O Informação desconhecida *Se OUTRO, especifique:

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Guarda do paciente ao longo da vida:			
	Mãe		Avós
	Pai		Outro

Estrutura familiar:			
	Estável – composição mantida ao longo da vida da criança		Instável. Mudança na composição familiar ao longo da vida da criança. Motivo:

14

Cuidadores ao longo do dia	Segunda	
	Terça	
	Quarta	
	Quinta	
	Sexta	
	Sábado	
	Domingo	

		Manhã	Tarde	Noite
Rotina da criança	Segunda			
	Terça			
	Quarta			
	Quinta			
	Sexta			
	Sábado			
	Domingo			

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	Nome	Quando?	Suspenso por quê?
Medicamentos já usados O Não O Sim O Informação desconhecida			

15

Medicamentos atuais (escrever a lápis) posologia, data de início, horários

O Não O Sim O Informação desconhecida

Medicamento	Apresentação	Posologia	Data de início
Está fazendo uso de Ferro: O Não O Sim O Não realizado O Informação desconhecida			
Está fazendo uso de Vitaminas: O Não O Sim O Não realizado O Informação desconhecida			

Lista de Problemas	

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Escola (a criança se sente segura na escola e no trajeto casa / escola / casa; disciplinas favoritas, disciplinas de que menos gosta, relacionamento com pares e professores)

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Entrevista inicial com responsável (entendendo o problema):

16

Nome do responsável: *Data:*

Importante: identificar o motivo da procura. Quando começou, como evoluiu, como está agora esta queixa? Verificar funcionamento prévio e atual do adoecimento. Lembrar de questionar sobre sono, apetite (distorção de imagem corporal), uso de telas, funções fisiológicas, atividades lazer, sociais e domésticas. Hábitos (sucção de polegar, tiques, onicofagia, tricotilomania, etc). Sociabilidade (timidez, inibição, isolamento, agressividade, etc). Questionar comportamentos autolesivos, suicidas. Autoestima. Autonomia em atividades de vida diária básicas e instrumentais de acordo com faixa etária. **Se houver risco de suicídio, realizar avaliação de risco (folha específica)**

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Anexo VI: Anamnese padronizada de primeira consulta – Página 17

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Evidência de algum trauma psicológico (Vivenciado, Presenciado e/ou Relatado):

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Em relação ao cuidador, você observou:

	Fisionomia extremamente expressiva, inquietação e agitação;
	Pessimismo em relação à criança;
	Conduta em relação à atividade constante da criança, com ordens inúteis e sucessivas;
	Tolerância para a constante intervenção de terceiros;
	Perguntar a outrem sobre o próprio filho (devemos considerar que nestes tempos pós-modernos, muitas vezes há a necessidade de se perguntar à babá informações que a mãe simplesmente desconhece);
	Enganar a criança para alguma atividade específica;
	Ameaça-la para obter sossego;
	Dar o celular ou tablet para obter sossego;
	Fazer promessas para obter determinados comportamentos da criança;
	Ser incapaz de contê-la com firmeza para determinados procedimentos;
	Ignorar o exame ou as orientações realizadas pelo profissional;
	Agressão física ou emocional à criança;
	Permitir excesso de mimos para a criança;

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Entrevista inicial com a criança ou adolescente (entendendo o problema): Data:

Das pessoas da sua família, ou entre as pessoas com quem você convive, de quem diria ser mais próximo (a)? Marque um X.			
	Ambos os pais biológicos		Conselho tutelar
	Mãe biológica		Cuidador de Casa de Acolhimento
	Pai biológico		Babá
	Avó materna		Mãe adotiva
	Avó paterna		Pai adotivo
	Avó materno		Madrasta
	Avó paterno		Padrasto
	Tia/tio maternos		Outro
	Tia/tio paternos		Nome:

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Anexo VI: Anamnese padronizada de primeira consulta – Página 19

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Exame físico: Dados de nascimento – consultar cartão da criança e questionar responsável

Peso ao nascer (Kg)		Estatura ao nascer (cm)		PC ao nascer (cm)	
Apgar 5'		Temperatura °C			
Peso atual (kg)		PA MSE (mmHg)		PA MSD (mmHg)	
Estatura atual (cm)		Saturação O2 (%)			
IMC Kg/m²		Ausculta cardíaca	O Não alterada O Alterada Descreva:		
Cintura atual (cm)		Frequência Cardíaca (bpm)	Esquerda:	Direita:	
Sinais de autolesão e/ou agressões? Descreva:					

Classificacão IMC	Classificacão PA
O Baixo Peso O IMC adequado ou eutrófico O Sobrepeso O Obesidade	O Normotenso: PA < p90 O PA elevada: PA ≥ p90 e < p95 O Hipertensão: PA ≥ p95

Exame neurológico Deve ser realizado se a história clínica sugerir quadro neurológico ou na suspeita.	Pupilas:	O Não alterado	O Alterado	O Não realizado / Não se aplica
	Pares cranianos:	O Não alterado	O Alterado	O Não realizado / Não se aplica
	Força muscular:	O Não alterado	O Alterado	O Não realizado / Não se aplica
	Manobra dedo-nariz:	O Não alterado	O Alterado	O Não realizado / Não se aplica
	Reflexos profundos:	O Não alterado	O Alterado	O Não realizado / Não se aplica
	Marcha:	O Não alterado	O Alterado	O Não realizado / Não se aplica
	Movimentos involuntários:	O Não alterado	O Alterado	O Não realizado / Não se aplica
	(EX. tremores, tiques, mioclonias, coreia, atetose, balismo, distonias, espasmos, fasciculacoes, mioquimias, alterações cerebrais e cerebelares, parkinsonismo, distonia, acatisia, discinesia)			
	Se ALTERADO em algum item, descreva:			

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Exame Psicopatológico

Aparência	O Cuidada O Parcialmente cuidada O Descuidada O Bizarra/Extravagante (fisionomia, cuidados e vestimenta, higiene, adequação para idade)		
Atitude com o entrevistador	O cooperativo O não cooperativo O de oposição O hostil O de fuga O suspicaz O querelante O reivindicativa O arrogante O evasiva O invasiva	O de esquiva O inibida O desinibida O jocosa O irônica O lamuriosa O dramática O teatral O sedutora O pueril	O gliscroide O simuladora O dissimuladora O indiferente O manipuladora O submissa O expansiva O amaneirada O reação de último momento.
Consciência	O Hipervigil O Vígil O Sonolento O Torporoso O Coma		
Atenção	O Hipoprosexia O Aprosexia O Rigidez de Atenção O Labilidade de Atenção		
Memória (curto e longo prazo)	O Sem alterações O Alterações		
Orientação	Tempo O Total O Parcial O Ausente Espaço O Total O Parcial O Ausente Pessoa O Tota O Parcial O Ausente		
Comportamento empático			
Contato visual			
Comunicação gestual/mímica			
Fala	Prosódia O Normoprosódica O A/hipoprosódica O Hiperprosódica Velocidade O Normolálica O Bradilalia O Taquilalia Quantidade O Mutismo O Oligolalia O Logorreia Tonalidade O Normofônica O Hipofônica O Hiperfônica Latência de resposta O Sem alteração O Aumentada O Diminuída Qualidade O Sem alterações O Com alterações:		
Leitura (nível, fluidez, entendimento)			
Escrita (letra, ortografia)			
Cálculos			
Pensamento **Juízo de realidade** O Preservado totalmente O Prejudicado parcialmente O Prejudicado totalmente/Ausente	Curso: O normal O acelerado O alentecido O interrompido Forma: O Sem alterações O Fuga de ideias O Desagregação O Prolixo O Minucioso O perseverante O Tangencial Conteúdo: O Sem alterações. O Concreto/ empobrecido O Confabulações	O Ideias obsessivas O Ideias depressivas O Ideias ansiosas O Ideias de morte O Ideias autoagressivas O suicidas O autolesão O Ideias heteroagressivas O dirigidas a pessoas O dirigidas a objetos O Ideia delirante (delírio primário) – tipo : ..... O Ideia deliroide (delírio secundário) – tipo : ..... O Ideia sobrevalorada (prevalente) – tipo :	

Anexo VI: Anamnese padronizada de primeira consulta – Página 21

Psicomotricidade Descrever	O Hipocinesia O Hipercinesia O Estereotipias
Humor	O Normofórico O Hipofórico O Euforia O Disforia
Afeto	Quantidade O Preservado (sem alterações) O Achatado O Embotado O Exaltado Congruência O Congruente O Incongruente Modulação O Normomodulado O Lábil O Incontinente O Rigidez afetiva Conteúdo O Sem alterações O Paratimia O Ambivalência afetiva O Neotimia
Processamento sensorial (audição, paladar, tato, dor, temperatura, visão, olfato, vestibular, interocepção – sensações interiores)	O Sem alterações O Com alterações. Descreva:
Sensopercepção **Descrever o tipo de alteração.**	O Sem alterações O Alucinações O Alucinoses O Ilusões
Consciência do Eu	O Sem alterações O Com alterações: O Despersonalização O Desrealização O Outros:
Inteligência (Aparentemente)	O Mediana (sem alterações) O Baixa O Alta O Duvidosa
Tolerância a frustrações	O Mediana O Baixa O Alta O Duvidosa
Conação (Vontade) (livremente praticar ou deixar de praticar algum ato, desejo, intenção, etc. está sob domínio da inteligência e afetividade). 4 fases: intenção, deliberação, ato, execução)	O Sem alterações O Hipobulia ou abulia O Enfraquecimento de impulsos específicos (anorexia, da sede, insônia, redução da libido) O Intensificação de impulsos específicos (bulimia, potomania, hipersonia, hiperssexualização) O Ato impulsivo O Ato compulsivo O Ambitendência (ambivalência volitiva) O Outro: .....
Impulsividade	O Mediana O Baixa O Alta O Duvidosa
Pragmatismo	O Normopragmático O Hipopragmatismo O Apragmatismo Capacidade de colocar em prática, de realizar de forma eficaz, aquilo que se deseja ou que foi planejado. Implica identificar os interesses e objetivos do paciente, e avaliar a adequação do comportamento quanto à realização de tais objetivos.)
Insight (Consciência de Morbidade)	O Mediano O Baixo O Alto O Duvidoso
Confiabilidade das informações	O Mediana O Baixa O Alta O Duvidosa Verificar: O Simulação O Dissimulação
Planos futuros	O Presentes e adequados O Presentes e inadequados O Ausentes
Nível de esperança	O Mediano O Baixo O Alto O Duvidoso

Anexo VI: Anamnese padronizada de primeira consulta – Página 22

Hipótese (s) Diagnóstica (s):

Diagnóstico (s) Sindrômico (s):

Diagnóstico (s) Nosológico (s):

Diagnósticos Clínicos e/ou dados de saúde relevantes:

Problemas psicossociais relevantes:

22

Conduta:

Indicado **notificação compulsória de violência interpessoal ou autoprovocada** (casos suspeitos ou confirmados de violência doméstica/intrafamiliar, sexual, autoprovocada (automutilação, tentativa de suicídio), trabalho escravo, trabalho infantil, tráfico de pessoas, tortura, intervenção legal e violências homofóbicas contra mulheres e homens em todas as idades. No caso de violência extrafamiliar/comunitária, somente serão objetos de notificação as violências contra crianças, adolescentes, mulheres, pessoas idosas, pessoa com deficiência, indígenas e população LGBT)

O Sim O Não Se sim: Foi realizada O Sim O Não
Anexar cópia no prontuário e encaminhar original à coordenadora da enfermagem da UAES.

Se adolescente do sexo feminino, avaliar:
DUM: / / . Uso de contraceptivo (Qual?): .....

Gestante ou risco de gravidez atual? O Sim O Não O Duvidoso.

Se duvidoso, pedir teste gravidez e avaliar condutas mais seguras. Se gestante, avaliar condutas mais seguras e oferecer orientações gerais, além de verificar se iniciou pré-natal. Iniciar ácido fólico no primeiro trimestre se for o caso.
Avaliar necessidade de oferecer orientações sobre sexualidade para adolescentes (ambos os sexos).

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8.7. Anexo VII: Anamnese padronizada de consultas de seguimento – Página 1.

Consulta de Acompanhamento – O 4 O 8 O 12 O 18 O 24 semanas

Data: ID: LUCCA		Acompanhante:
Diagnóstico: TDAH O apresentação desatenta O apresentação hiperativa/impulsiva O apresentação combinada	Comorbidade (s)	Data da última consulta:
Medicação em uso: O Ritalina 10mg (+ + +) O Metilfenidato10mg (+ + +) O Ritalina LA mg (+ + +) O Venvanse mg (+ + +)		
Trouxe cartelas da medicação para conferência do número de comprimidos?		SIM NÃO
Número de comprimidos que deveria ter tomado desde a última consulta:		Doses não tomadas:
A criança tomou o medicamento em dias de semana e fins de semana?		SIM NÃO
A criança tomou mais medicamentos do que o prescrito em algum dia?		SIM NÃO
Adesão ao uso da medicação:		SIM NÃO

Medida de Adesão ao Tratamento (MAT) – Versão Brasileira adaptada pela pesquisadora							
1		2		3		4	
5		6		7		8	
Resposta da questão 8:							

EXAME FÍSICO. Se PA alterada, fazer nova medida ao final da consulta. Questionar vontade de urinar (ir ao banheiro e medir novamente).

PA MSD sentado (mmHg)		PA MSE sentado (mmHg)		
FC (bpm)MSD/MSE Ver no aparelho PA	/	AUSCULTA CARDÍACA (Sopros?)	Ritmo cardíaco O regular O irregular	
TEPERATURA (°C)		PESO (Kg)		
ESTATURA (cm)		IMC (Kg/m²)		
CINTURA (cm)		Saturação O_2 (%)		
MENARCA	O SIM Se sim, quando?		O NÃO	O Não se aplica

Criança: Percepção de alguma melhora O Sim O Não

Efeitos adversos: O Sim O Não

Qual:	Sim	Não
Redução apetite		
Náusea		
Cefaléia		
Insônia		
Nasofaringite		
Tonteira		
Dor abdominal		
Irritabilidade		
Sonolência		
Taquicardia		
Aumento de PA		
Aumento de FC		
Perda de Peso		
TICs		
Sintomas psicóticos		
Alergia		
Outro. Especifique		

- Se houve melhora ou piora, questionar em uma escala de zero a 10, quanto o responsável acha que houve mudança. Sintomas de:

Desatenção O Melhor O Pior O Não se aplica

0 1 2 3 4 5 6 7 8 9 10

Hiperatividade/Impulsiv. O Melhor O Pior O Não se aplica

0 1 2 3 4 5 6 7 8 9 10

Desregulação emocional O Melhor O Pior O Não se aplica

0 1 2 3 4 5 6 7 8 9 10

Sintomas de TOD O Melhor O Pior O Não se aplica

0 1 2 3 4 5 6 7 8 9 10

Perguntar se já estava presente antes do uso da Ritalina ou se apareceu após início da medicação.

Anexo VII: Anamnese padronizada de consultas de seguimento – Página 2.

Responsável: Percepção de alguma melhora O Sim O Não

Efeitos adversos: O Sim O Não

Qual:	Sim	Não
Redução apetite		
Náusea		
Cefaléia		
Insônia		
Nasofaringite		
Tonteira		
Dor abdominal		
Irritabilidade		
Sonolência		
Taquicardia		
Aumento de PA		
Aumento de FC		
Perda de Peso		
TICs		
Sintomas psicóticos		
Alergia		
Outro. Especifique		

Perguntar se já estava presente antes do uso da medicação ou se apareceu após início da medicação.

Está fazendo uso de Ferro? O Sim O Não
Está fazendo uso de Vitaminas? O Sim O Não
Se sim, qual vitamina?

- Descreva como a criança evoluiu desde a última consulta. Se houve melhora ou piora, questionar em uma escala de zero a 10, quanto o responsável acha que houve mudança. Sintomas de:

Desatenção O Melhor O Pior O Não se aplica

0 1 2 3 4 5 6 7 8 9 10

Hiperatividade/Impulsiv. O Melhor O Pior O Não se aplica

0 1 2 3 4 5 6 7 8 9 10

Desregulação emocional O Melhor O Pior O Não se aplica

0 1 2 3 4 5 6 7 8 9 10

Sintomas de TOD O Melhor O Pior O Não se aplica

0 1 2 3 4 5 6 7 8 9 10

Entrevista com a criança e responsável – Descreva pontos importantes coletados:

Conduta:

Medicação: (+ + +)
Próxima consulta em: semanas.
Orientar trazer as cartelas do remédio na próxima consulta.
Se consulta de 12 ou 24 semanas orientar jejum de 10 horas para coleta de sangue na segunda feira pela manhã. Orientar que no dia da coleta, o remédio será tomado após a coleta de sangue na UAES. Neste dia, serão repetidos questionários e testes, consulta será um pouco mais demorada.

Lembrar de calcular IMC e colocar na curva de crescimento as medidas. Verificar na tabela ou aplicativo a PA

Escala de Impressão Clínica Global

Severidade da Doença	Melhora da Doença
1. Não avaliado	1. Não avaliado
2. Não está doente	2. Muito melhor
3. Muito leve	3. Moderadamente melhor
4. Leve	4. Levemente melhor
5. Moderada	5. Sem alterações
6. Acentuada	6. Levemente pior
7. Grave	7. Moderadamente pior
8. Extemamente grave	8. Muito pior