Study Title

THE EFFECT OF METHYLPHENIDATE TREATMENT ON NEUROINFLAMMATION LEVELS IN CHILDREN WITH ATTENTION DEFICIT HYPERACTIVITY DISORDER

Observational Study

Content

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Research Protocol

Location of the Study

Health Sciences University Istanbul Bakırköy Prof. Dr. Mazhar Osman Mental Health and Neurological Diseases Training and Research Hospital.

Purpose of the Study.

The aim of this study is to examine the effect of methylphenidate treatment on neuroinflammation in patients diagnosed with attention deficit hyperactivity disorder (ADHD) who have started or are planning to start methylphenidate treatment as part of their routine treatment, by measuring the levels of Interleukin-6 (IL-6), S100B, and Claudin-5 in serum samples taken at 0 months before starting methylphenidate treatment and at 3 months after starting treatment. The aim of this study is to investigate the effect of methylphenidate treatment on neuroinflammation in ADHD.

Material and Methods of the Study

The study population consisted of patients who applied to the child and adolescent psychiatry outpatient clinics of Bakırköy Prof. Dr. Mazhar Osman Mental Health and Neurological Diseases Training and Research Hospital and were diagnosed with "Attention Deficit Hyperactivity Disorder (ADHD)" according to DSM-5-TR and who were routinely started on methylphenidate or planned to be started on methylphenidate by the examining physician will be referred to researcher Enes Faruk Altunkılıç and other assistant researchers following an in-clinic announcement. After being informed about the study both verbally and in writing through an informed consent form, patients who agree to participate, sign the informed consent form themselves, and meet the inclusion and exclusion criteria will be included in the study. Patients and their families will also be informed that their decision to participate or not participate in this study will not affect the treatment they receive.

After informed consent, a structured clinical interview for DSM-5-TR will be conducted using the "Emotional Disorders and Schizophrenia Form for School-Age Children - Present and Lifetime Form DSM-5 - Turkish Adaptation (ÇDŞG-ŞY-DSM-5-T)". The "Sociodemographic

and Clinical Data Form" created by the researchers will be completed to obtain sociodemographic and clinical data for the participants. The "Conners Parent Rating Scale-Revised Short Form" and "Conners Teacher Rating Scale-Revised Short Form" will be administered to the group diagnosed with ADHD to determine the severity of the disease, symptoms, and predominant subtypes. After the diagnosis is made and evaluated according to the exclusion criteria, peripheral venous blood will be collected in yellow-capped tubes between 9 and 12 a.m. after a 10-12 hour fast, prior to the routine planned methylphenidate treatment. After being left at room temperature for 10-20 minutes, the blood will be centrifuged at 3000 RPM for 20 minutes, and the serum portion will be collected into Eppendorf tubes and stored at -80 degrees until the samples are analyzed. Three months after starting treatment, blood will be collected and stored in the same manner. After all samples have been collected, the serum samples will be analyzed in a single batch for Interleukin-6 (IL-6), S100B, and Claudin-5 levels using human ELISA kit protocols at the Medical Biochemistry Laboratory of Bakırköy Dr. Sadi Konuk Training and Research Hospital by biochemistry specialist Dr. Hacer Eroğlu İçli. Three months after the start of treatment, the Conners Parent Rating Scale – Revised Short Form and the Conners Teacher Rating Scale – Revised Short Form will be administered again.
This is not an intervention study in terms of methodology. Patients who are already deemed suitable for methylphenidate treatment based on their diagnosis and clinical condition will be included in the study. No intervention will be made in the treatment they will receive during the study. In accordance with general medical ethics, the treatment process routinely applied in the outpatient clinic will be implemented, taking into account the clinical condition independent of the study, the risk-benefit ratio, and current guidelines. If necessary, treatment will be discontinued or the dose adjusted as required. The patient's treatment will not be interfered with for the sake of this study. In the study, patient recruitment will be completed by collecting blood samples and completing the scale forms at 0 and 3 months for 45 patients.

Inclusion criteria for the study:

- Diagnosis of "Attention Deficit Hyperactivity Disorder" according to DSM-5 TR and routine treatment with methylphenidate has been started/is planned to be started.
- Aged between 6 and 11 years old
- Agrees to participate in the study after being informed about it

Exclusion criteria for the study

• Presence of a psychiatric disorder other than Attention Deficit Hyperactivity Disorder
• Diagnosis of Attention Deficit Hyperactivity Disorder but no plans to start methylphenidate treatment
• Age under 6 or over 11
• Presence of organic brain damage, mental retardation, autism spectrum disorder, neurological disease, or physical illness affecting neurocognitive functions
• History of alcohol and/or psychoactive substance use
• Presence of ongoing active infection, allergic disease, and chronic illness
• Previous use of psychiatric medication

Research Questionnaire

1. **Informed Patient Consent Form: It is prepared to verify the patient's consent and to inform the participant.**

2. **Sociodemographic and Clinical Data Form:** This is a form prepared by the researchers to record the sociodemographic and clinical data of the patient volunteers for the purpose of the study. It includes questions about participants' sociodemographic characteristics such as age, gender, and education level, as well as questions regarding family history, any chronic illness or psychiatric disorder history, and inquiries about smoking and alcohol/substance use.

3. **Mood Disorders and Schizophrenia Form for School-Aged Children - Current and Lifetime Version DSM-5 - Turkish Adaptation (ÇDŞG-ŞY-DSM-5-T):** The first version of this form was created by Chambers et al. in 1985 according to DSM-3. It was updated based on DSM-5 diagnostic criteria by Kaufman et al. In the first section, the sociodemographic information of the child and family, complaints, developmental and general health status, and functionality are questioned. The second section contains screening questions covering over 200 specific symptoms from both the past and the last two months. The third section consists of evaluation and observation results by the clinician to verify DSM-5 diagnoses.

Ünal and colleagues conducted the Turkish translation and validity-reliability studies in 2019.

4. **Conners Parent Rating Scale-Revised Short Form (CPRS-RS):** The Conners Parent Rating Scale-Revised Short Form (CPRS-RS) was developed by Conners et al. in 1997 to measure the severity of ADHD symptoms and behavioral problems, and it is administered by parents. In 2013, Kaner and colleagues conducted the Turkish validity and reliability study. The scale consists of 27 items, including 3 subscales (Oppositional (KG), Cognitive Problems-Inattention (BP-D), Hyperactivity (H)) and 1 additional scale (ADHD index to assess children at risk for ADHD) answered by the parent.

5. **Conners Teacher Rating Scale-Revised Short Form (CTRS-RS):** The Conners Teacher Rating Scale-Revised Short Form (CTRS-RS) was developed by Conners et al. in 1997 to measure the severity of ADHD symptoms and behavioral problems, and it is administered by the teacher. In 2013, Kaner and colleagues conducted the Turkish validity and reliability study. The scale consists of 28 items, including 3 subscales (Oppositional (KG), Cognitive Problems-Inattention (BP-D), Hyperactivity (H)) and 1 additional scale (ADHD index to assess children at risk for ADHD) answered by the teacher.

Statistical Analysis

The statistics of this study were analyzed using the JAMOVI (The jamovi project (2024). jamovi (Version 2.5) [Computer Software]. Retrieved from https://www.jamovi.org) package program and SPSS 26.0 (IBM Corp. Released 2019. IBM SPSS Statistics for Windows, Version 26.0. Armonk, NY: IBM Corp) package program.

In evaluating the data, descriptive statistical methods (mean, standard deviation, median, interquartile range) will be used, and the Shapiro-Wilk test will be used to examine the distribution of the variables. For variables showing a normal distribution, the paired t-test will be used to evaluate repeated measurements; for variables not showing a normal distribution, the Wilcoxon test will be used to evaluate repeated measurements; and the Pearson correlation test and linear regression analysis will be used to determine the relationships between variables. Results will be evaluated at a significance level of $p<0.05$.

INFORMED CONSENT FORM

Bakırköy Prof. Dr. Mazhar Osman Mental Health and Neurological Diseases Training and Research Hospital

You have been invited to participate in a clinical study. After reading the information below, if you wish to participate in the study, please sign this form. If you do not wish to participate in the study, there will be no interruption in your treatment. Signing this form indicates that you have been informed about the scope of the study and that you have made your decision freely. If there are any terms in the form that you do not understand, please ask the doctors involved in the study for clarification. The name of the study is "The Effect of Methylphenidate Treatment on Neuroinflammation Levels in Children Diagnosed with Attention Deficit Hyperactivity Disorder."

The treatment and plan you will receive from your physician, who has previously diagnosed you clinically, have already been determined. Therefore, your participation or non-participation in the study will not affect the treatment you will receive. The aim of this study is to investigate the possible role of methylphenidate on oxidative stress. Ethical committee approval has been obtained for the research. Dr. Enes Faruk ALTUNKILIÇ will assist you with answering your questions and/or resolving any potential issues. (Phone: 05077362191)

For the study, you will undergo a psychiatric interview, and some scales will be applied. You will be followed up for 3 months. The frequency of follow-up will be determined based on your clinical condition, with a maximum of once per month. During this 3-month follow-up, venous (vein) blood will be drawn from the inner side of the elbow after a 10-12 hour fast between 9-12 AM, prior to the initiation of the methylphenidate-containing medication. The same procedure will be performed 3 months after the start of treatment. Blood will be drawn a total of 2 times. Your participation in the study will take a maximum of 30 minutes. This time is already required for your routine tests. You have no liability. This study is not expected to provide any additional benefit to your treatment at this time, and no change in the course of your treatment is anticipated. It is hoped that the results obtained from this study will contribute to the scientific community and be used for the benefit of other people's treatment in the future. You will not be charged for the procedures performed or any other expenses, nor will any private or public institution under your insurance be charged. You will not receive any payment for participating in this research. Your personal information and all medical information will be kept confidential. Even if the research is published, your personal information will not be shared. However, research observers, auditors, ethics committees, and official authorities may access your medical information when necessary. You may also access your own medical information at any time. The number of volunteers participating in this research is 45.

You may withdraw from the study at any time without penalty or loss of rights. If you fail to comply with the requirements of the treatment regimen, disrupt the study program, or experience an adverse effect related to or unrelated to the study, the responsible researchers may exclude you from the study without your consent. The results of the study will be used for scientific purposes; if you withdraw from the study or are excluded by the researcher, your medical data may also be used for scientific purposes if necessary.

Participant's Statement

I have read the information provided above, which must be given to the participant/volunteer prior to the research, and I fully understand the scope and purpose of the study I am being asked to participate in, as well as the responsibilities that fall upon me as a volunteer. I have been provided with sufficient information and assurance that the research results will be used for educational and scientific purposes and that my personal information will be kept confidential during this process. Written and verbal explanations about the study were provided by the researcher whose name is listed below, I asked my questions, and I received satisfactory answers. The potential risks and benefits of the study were also explained to me verbally. I understand that I can withdraw from this study at any time and for any reason without having to give a reason, and that I will not encounter any negative consequences if I withdraw. Under these conditions, I agree to participate in the study of my own free will, without any pressure or coercion.

For participants under guardianship or custody, the guardian or custodian:

Name-Surname:

Tel:

Date and Signature:

The organization official/interview witness who witnessed the researcher's approval process:

Name-Surname:

Phone:

Date and Signature:

The researcher:

Name-Surname: Enes Faruk ALTUNKILIÇ

Position: Assistant Doctor

Position: Assistant doctor

Phone: 0507736****

Date and Signature:

Note: This form is prepared in two copies. One copy is given to the volunteer in exchange for their signature, and the other is retained by the researcher.

INFORMED CONSENT FORM FOR CHILDREN AND ADOLESCENTS:

Study Title: The Effect of Methylphenidate Treatment on Neuroinflammation Levels in Children Diagnosed with Attention-Deficit/Hyperactivity Disorder

You are being asked to take part in a research study. Before deciding whether or not you want to participate, it is important for you to understand why the research is being conducted, how your information will be used, what the study involves, and the potential benefits, risks, or discomforts it may entail. Please take time to read the following information carefully, and if you wish, feel free to discuss it with your family or personal doctor.

Your participation in this study is entirely voluntary. If you decide to take part, you will be asked to sign this Informed Consent Form. You are free to withdraw from the study at any time without giving a reason. Withdrawing from the study will not affect the quality of care or treatment you receive.

This study aims to investigate how methylphenidate treatment affects levels of neuroinflammatory markers in children diagnosed with Attention-Deficit/Hyperactivity Disorder (ADHD). The medication used in this study is already the first-line treatment for this condition, and you would receive it whether or not you participate in the research. In other words, you will not be exposed to any unproven treatments solely for the purpose of this study. If you choose not to participate, you will still be prescribed this medication based on the information obtained from previous clinical assessments.

Before starting treatment, a small amount of blood will be drawn from a superficial vein on your arm using a fine needle, as is commonly done. Additionally, your parents and teacher will be given forms to complete. These procedures will be repeated at the third month of treatment. The potential side effects and risks of this study do not exceed those of standard medical care or treatment for your condition. You will be followed up for a period of three months. In case of any side effects or concerns, your family has been given the phone number of the investigator, and you may contact them at any time. You will continue to receive whatever treatment is medically appropriate based on your current condition. This study will never override proven medical interventions or compromise your care.

There is no cost to participate in the study. New information that may affect your decision to participate will be shared with you as it becomes available during the course of the study. By participating, you will also be contributing to scientific knowledge and potentially helping other children and adolescents in similar situations. Thank you in advance for your contribution to this important research.

PARTICIPANT'S STATEMENT:

I have been informed by Assistant Doctor Enes Faruk ALTUNKILIÇ from the Child and Adolescent Psychiatry Clinic of Bakırköy Prof. Dr. Mazhar Osman Mental Health and Neurological Diseases Training and Research Hospital, affiliated with the University of Health Sciences, that a medical research study will be conducted. The information regarding this research, as outlined above, has been provided to me. I have been invited to take part as a

“participant” in this study.
I believe that my personal information, which should remain confidential between myself and my doctor, will be treated with the same level of care and respect during this research. I have been assured that my personal data will be protected if used for educational or scientific purposes.
I understand that I may withdraw from the study at any time without providing a reason. However, I acknowledge that informing the researchers in advance would be appropriate to avoid any inconvenience. I also understand that I may be withdrawn from the study by the researcher if necessary, to avoid any potential harm to my medical condition. I will not bear any financial responsibility for the costs related to this research, nor will I receive any compensation. I have been assured that if any health problems arise as a result of this research—either directly or indirectly—all necessary medical interventions will be provided at no cost to me.
If I experience any health issues during the study, I understand that I can contact Assistant Doctor Enes Faruk ALTUNKILIÇ at the Child and Adolescent Psychiatry Clinic of Bakırköy Prof. Dr. Mazhar Osman Mental Health and Neurological Diseases Training and Research Hospital or call (0212) 4091515–3417. I understand that participation in this study is voluntary and that I am under no obligation to take part. I have not been subjected to any pressure or coercion to participate. If I choose not to participate, it will not affect my medical care or relationship with my doctor in any way.
I have read all the statements in the informed consent form. The subject and aim of the research were explained to me both in writing and verbally by the physician named below. I fully understand all the explanations provided. After taking sufficient time to consider, I have decided to participate as a “volunteer” in the study mentioned. I understand that I can leave the study at any time, with or without giving a reason. I freely consent to participate in this study without any pressure or coercion.

Participant's Full Name / Date / Signature

Researcher’s Full Name / Date / Signature

Witness’s Full Name / Date / Signature