Study Title

The Effect of Methylphenidate Treatment on Oxidative Stress Levels in Children Diagnosed With Attention Deficit Hyperactivity Disorder (ADHD)

Observational Study

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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 the study is to investigate the effect of methylphenidate treatment on oxidative stress by measuring the levels of Total Oxidant Status (TOS), Total Antioxidant Status (TAS), Oxidative Stress Index (OSI = TOS/TAS), Malondialdehyde (MDA), Oxidized LDL (Ox-LDL), and Superoxide Dismutase (SOD) in serum samples from patients diagnosed with Attention Deficit Hyperactivity Disorder (ADHD) who have either started or are planned to start methylphenidate treatment. Measurements will be taken at baseline (before the start of treatment) and at the 3rd month after the treatment begins.

Material and Methods of the Study

The study population will consist of patients who are diagnosed with "Attention Deficit Hyperactivity Disorder (ADHD)" according to DSM-5-TR, who have 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 have been started or planned to start methylphenidate treatment by the physician they have been examined by. After an in-clinic announcement, these patients will be directed to the researcher, Enes Faruk Altunkılıç. After being informed both verbally and in writing through the informed consent process, those who agree to participate, sign the informed consent form, and meet the inclusion and exclusion criteria will be included in the study. It will also be explained to the patients and their families that their participation or non-participation in this study will not affect the treatment they will receive.

After informed consent, a structured clinical interview for DSM-5-TR will be conducted using the "Mood Disorders and Schizophrenia Form for School-Aged Children - Now and Lifetime DSM-5 - Turkish Adaptation (ÇDŞG-ŞY-DSM-5-T)." To obtain sociodemographic and clinical data for the participants, the "Sociodemographic and Clinical Data Form" created by the researchers will be completed. For the ADHD diagnosed group, the "Conners Parent Rating Scale - Revised Short" and "Conners Teacher Rating Scale - Revised Short" will be applied to determine the severity of the disorder, symptoms, and predominant subtyping. After the diagnosis is made and evaluated according to the exclusion criteria, blood samples will be taken from the patient after a 10-12 hour fasting period, between 9-12 AM, in a yellow-capped tube, before the routine methylphenidate treatment is used. After waiting for 10-20 minutes at room temperature, the sample will be centrifuged at 3000 RPM for 20 minutes, and the serum will be collected in Eppendorf tubes and stored at -80°C until analysis. After 3 months of treatment, blood samples will be taken again in the same manner and stored. Once all the samples are collected, the serum samples will be analyzed for total antioxidant status (TAS), total oxidant status (TOS), malondialdehyde (MDA), superoxide dismutase (SOD), and oxidized LDL levels according to the human ELISA kit protocols at the Biochemistry Laboratory of Bakırköy Dr. Sadi Konuk Training and Research Hospital by biochemist Dr. Hacer Eroğlu İçli. After 3 months of treatment, the Conners Parent Rating Scale - Revised Short and Conners Teacher Rating Scale - Revised Short forms will be applied again. In addition to the markers, the Oxidative Stress Index (OSI = TOS/TAS) will be calculated and included in the evaluation before and 3 months after the treatment.

Inclusion criteria for the study:

• A diagnosis of "Attention Deficit Hyperactivity Disorder (ADHD)" according to DSM-5-TR and the initiation or planned initiation of methylphenidate treatment as part of routine care.
• Age between 6-11 years.
• Agreement to participate in the study after being informed about the study.

Exclusion criteria for the study

• Presence of any psychiatric disorder other than "Attention Deficit Hyperactivity Disorder (ADHD)."
• Diagnosis of "Attention Deficit Hyperactivity Disorder (ADHD)" without the initiation or planned initiation of methylphenidate treatment.
• Age below 6 years or above 11 years.
• Presence of organic brain damage, mental retardation, autism spectrum disorder, neurological diseases, or physical illnesses affecting neurocognitive functions.
• History of alcohol and/or psychoactive substance use.
• Ongoing active infection, allergic diseases, or chronic illnesses.
• Previous use of psychiatric medication.
• Presence of chronic diseases.
• Use of regular medications.

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.

First of all, descriptive statistics (mean, standard deviation, median, lowest-highest values, percentage) of the variables in the study were determined and these statistics were given as tables or bar graphs.

The normality of the variables was checked with the 'Shapiro-Wilk' test. In paired samples analyses, if the differences of the variables before and after the normality test were observed to fit the normal distribution, 'paired sample t-test' was performed; if it was observed that it did not fit the normal distribution, 'Wilcoxon Signed Ranks' test was performed. In SOD measurement, 2 samples did not react and did not change color and these values could not be read. For this reason, statistics were made over 37 sample numbers for SOD.

In order to determine the relationship between clinical change before and after treatment and changes in biomarkers, correlations were examined between the changes in the total score and subscale scores (before and after) and the changes in the biomarkers (before and after) of CADÖ-YK and CÖDÖ-YK.

In correlation analyses, the 'Pearson Correlation' test was performed for normally distributed variables, and the 'Spearman Correlation' test was used for variables that did not fit the normal distribution.

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, please sign this form. If you choose not to participate, there will be no interruption in your treatment. Your signature on this form indicates that you have been informed about the study and that you have freely made your decision. If you do not understand any of the terms in the form, please ask the doctors involved in the study for clarification. The title of the study is "The Effect of Methylphenidate Treatment on Oxidative Stress Levels in Children with Attention Deficit Hyperactivity Disorder (ADHD)."

Your clinical diagnosis has already been established, and the treatment plan prescribed by your physician has been determined. Therefore, your participation or non-participation in this study will not affect the treatment you will receive. This study aims to investigate the potential role of methylphenidate on oxidative stress. Ethical approval for the research has been obtained. Dr. Enes Faruk ALTUNKILIÇ will assist you with any questions or problems you may have (Phone: 05077362191).

For the research, a psychiatric interview will be conducted, and certain scales will be applied. There will be a follow-up for 3 months. The follow-up will occur at most once a month, depending on your clinical condition. Before starting methylphenidate treatment, venous blood will be drawn from the inner side of your elbow between 9 AM and 12 PM after a 10-12 hour fasting period. This will be done again 3 months after the treatment begins. A total of two blood samples will be collected. Your participation in the research will take a maximum of 30 minutes, which is the duration required during your routine tests. You will not have any additional responsibilities. This research does not offer any direct benefit to your treatment at this time, and it is not expected to alter the course of your treatment. The results obtained from this research are expected to contribute to the scientific community and potentially benefit the treatment of others in the future. There will be no costs for the procedures involved, and no costs will be charged to you or any institution under your insurance. No payment will be made to you for participating in this research. Your personal and medical information will remain confidential. Even if the research is published, your identity will not be shared. However, the research observers, record keepers, ethics committees, and official authorities may have access to your medical information when necessary. You may also access your own medical information upon request. The total number of volunteers for this research will be 39 individuals.

You may withdraw from the study at any time without penalty or loss of any rights. If you fail to comply with the treatment protocol, interrupt the research program, or experience any adverse effects related or unrelated to the research, the responsible researchers may remove you from the

study without your consent. The results of the study will be used for scientific purposes. In case of withdrawal or removal from the study, your medical data may be used for scientific purposes if necessary.

Participant's Statement

I have read the above information and fully understand the scope and purpose of the study I am being asked to participate in. I have been informed that the research results will be used for educational and scientific purposes, and that my personal information will remain confidential. Written and verbal explanations about the study have been provided by the researcher mentioned below, and I have asked questions and received satisfactory answers. The potential risks and benefits of the study have been explained to me. I understand that I may leave this study at any time without giving a reason and without facing any negative consequences. Under these conditions, I voluntarily agree to participate in this study without any pressure or coercion.

For minors or those under guardianship or custody, a parent or legal guardian must sign:

Name-Surname:
Phone:
Date and Signature:

Witness of the researcher's explanation:
Name-Surname:
Phone:
Date and Signature:

Researcher's information:
Name-Surname: Enes Faruk ALTUNKILIÇ
Position: Assistant Doctor
Phone: 05077362191
Date and Signature:

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

PARTICIPANT'S STATEMENT:

I have been informed by Assistant Doctor Enes Faruk ALTUNKILIÇ, that a medical research study will be conducted at the Children and Adolescent Psychiatry Clinic of Bakırköy Prof. Dr. Mazhar Osman Psychiatry and Neurology Training and Research Hospital of the Health Sciences University. The above information regarding this study was provided to me. After receiving this information, I have been invited to participate as a "participant" in this research study.

If I participate in this research, I believe that confidentiality will be maintained regarding my personal information during the study, and I am assured that my personal information will be carefully protected in any educational or scientific use of the study results.

I understand that I can withdraw from the study at any time without providing a reason. However, I am aware that it is appropriate to inform the researchers in advance of my decision to withdraw. I also understand that I may be removed from the study by the researchers for my medical well-being. I have no financial responsibility for the costs associated with the study, and no payment will be made to me. In the event of any health issue arising from the study, either directly or indirectly, I am assured that all necessary medical intervention will be provided, and I will not incur any financial burden related to these interventions.

If I encounter a health issue during the study, I know that I can contact Assistant Doctor Enes Faruk ALTUNKILIÇ at the Children and Adolescent Psychiatry Clinic of Bakırköy Prof. Dr. Mazhar Osman Psychiatry and Neurology Training and Research Hospital, or reach him at (0212) 4091515-3510. I understand that I am not obligated to participate in this study and that I may choose not to participate. I have not encountered any coercion to participate. If I refuse to participate, I am aware that this will not affect my medical care or relationship with my doctor.

I have read all the explanations in the Informed Consent Form. A written and verbal explanation about the study, its purpose, and the details mentioned above has been provided to me by the physician named below. I understand all the explanations provided to me. After taking adequate time to think, I have decided to participate as a "participant" in this research project. I am voluntarily participating in this study, and I am aware that I can withdraw at any time, with or without reason. I agree to participate in the study without any pressure or coercion.

Participant’s Name/Signature
Date

Researcher's Name/Signature
Date

Witness Name/Signature
Date