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## Serum interleukin-6 level in unmedicated adults with attention-deficit/hyperactivity disorder – preliminary report

Stężenie interleukiny 6 u nieleczonych dorosłych z rozpoznaniem zespołu nadpobudliwości psychoruchowej z deficytem uwagi – doniesienie wstępne

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### Abstract

**Aim:** The aim of the study was to compare adults suffering from attention-deficit/hyperactivity disorder with healthy controls, in terms of their serum concentrations of inflammatory cytokine interleukin-6. **Materials and methods:** The diagnosis of attention-deficit/hyperactivity disorder was established during the interview with the clinician on the basis of the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition. A total of 36 participants with attention-deficit/hyperactivity disorder and 28 in the control group were included. The age range was 21–55 years. Individuals with a psychiatric comorbidity, allergy, chronic inflammatory disease, and receiving psychostimulant treatment were excluded. **Results:** Adults diagnosed with attention-deficit/hyperactivity disorder had higher serum concentrations of interleukin-6 in comparison to healthy controls. **Conclusions:** The aetiology of attention-deficit/hyperactivity disorder may be influenced by the concentration of inflammatory factors.

**Keywords:** ADHD, inflammation, interleukin-6

### Streszczenie

**Cel:** Celem badania było porównanie osób dorosłych cierpiących na zespół nadpobudliwości psychoruchowej z deficytem uwagi z osobami zdrowymi pod względem stężenia w surowicy krwi cytokiny zapalnej interleukiny 6. **Materiał i metody:** Rozpoznanie zespołu nadpobudliwości psychoruchowej z deficytem uwagi potwierdzono na podstawie wywiadu z klinicystą w oparciu o piątą edycję Diagnostic and Statistical Manual of Mental Disorders. Do badania włączono 36 uczestników z zespołem nadpobudliwości psychoruchowej z deficytem uwagi oraz 28 osób z grupy kontrolnej. Przedział wiekowy wynosił 21–55 lat. Z udziału w badaniu wykluczono osoby przyjmujące leczenie psychostymulujące oraz z współwystępującymi zaburzeniami psychicznymi, alergią i przewlekłymi chorobami zapalnymi. **Wyniki:** Osoby dorosłe z rozpoznaniem zespołu nadpobudliwości psychoruchowej z deficytem uwagi charakteryzowały się wyższym stężeniem interleukiny 6 w surowicy krwi w porównaniu z osobami zdrowymi. **Wnioski:** Stężenie czynników zapalnych może wpływać w sposób istotny na etiologię zespołu nadpobudliwości psychoruchowej z deficytem uwagi.

**Słowa kluczowe:** ADHD, stan zapalny, interleukina 6

## INTRODUCTION

Attention-deficit/hyperactivity disorder (ADHD) is a neurodevelopmental condition that has its onset in childhood and often persists into adulthood. The main symptoms of ADHD include attention deficits, hyperactivity, and impulsivity (Faraone et al., 2015). It has been shown that the aetiology of this developmental disorder is influenced by the concentration of inflammatory factors (McEwen, 2012). Epidemiological studies report an increased co-occurrence of ADHD with inflammatory and autoimmune diseases, which may indicate a common pathomechanism, such as changes in immune response, as well as common genetic and environmental risk factors (Leffa et al., 2018). A high risk of developing ADHD has been found among patients suffering from allergic rhinitis, asthma, and dermatitis (Chang et al., 2022). According to a meta-analysis conducted in 2022, ADHD symptoms may be exacerbated by atopic diseases (Chuang et al., 2022). ADHD is also a risk factor for the development of obesity (Li et al., 2020), and the presence of inflammation is associated with obesity (Moghbeli et al., 2021).

The role of inflammatory factors in the aetiology of ADHD has been investigated mainly in children and adolescents. The exploration of biomarkers that influence symptoms of ADHD in adults is a compelling area of research. The aim of this study was to determine if the serum levels of pro-inflammatory interleukin-6 (IL-6) were significantly different in adults with ADHD in comparison to healthy controls.

## MATERIALS AND METHODS

Volunteers were recruited and examined by two clinicians between August 2018 and December 2019. The participants applied for the study via the Internet in response to an online advertisement. Every volunteer received a message with a detailed description of the study. In the next step, the clinicians performed the assessment of eligibility and invited patients who fulfilled the inclusion criteria for further procedures. A detailed description of the procedure is presented in Fig. 1.

The study participants comprised 36 subjects with ADHD (six with predominantly inattentive subtype and 30 with combined subtype) and 28 healthy controls. The control group was recruited from the general population. In order to obtain reliable results, we adopted the following exclusion criteria: 1) active inflammation in the body caused by allergies; acute and chronic inflammatory diseases (bacterial, viral, and autoimmune); history of recent trauma or infection, excess weight, and obesity, 2) anti-inflammatory drug treatment, 3) psychostimulant treatment, 4) pregnancy and breastfeeding, 5) occurrence of any psychiatric disorder (except ADHD in the study group). None of the enrolled patients had any prior exposure to stimulant or non-stimulant medications, or any other form of medication specifically

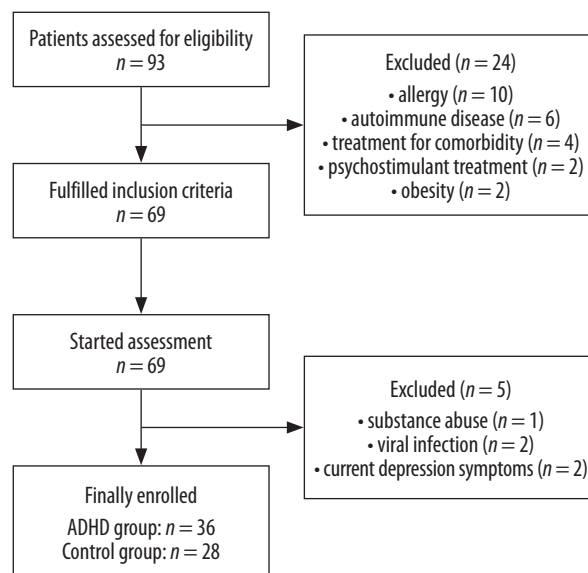


Fig. 1. Recruitment process flowchart

intended for treating ADHD. The study was conducted following the guidelines outlined in the Helsinki Declaration and received approval from the Medical University of Lodz ethics committee (No. RNN/349/17/KE).

Written informed consent was obtained from all adult participants involved in the study. Each participant completed a demographic survey (age, gender, education, place of residence, marital status, occupational status, and contact address in case of a request for research results), and the Adult ADHD Self-Report Screening Scale for DSM-5 (ASRS-5). The diagnosis of ADHD was established during the interview with the clinician on the basis of the Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-5) criteria for ADHD. The diagnosis of comorbid psychiatric disorders was performed by the structured Mini International Neuropsychiatric Interview (MINI). A 10 mL sample of blood was collected from each participant in the Department of Geriatric Psychiatry and Psychotic Disorders. Blood was centrifuged at 7,000 rpm for 20 min. Separated serum was placed in three Eppendorf tubes and refrigerated at  $-80^{\circ}\text{C}$  until biochemical parameters were determined at the Research Laboratory CoreLab of the Medical University of Lodz, with the use of high-sensitivity ELISA enzyme-linked immunosorbent assay (BioVendor, Czech Republic).

The distribution of the variable was determined using the Shapiro–Wilk test. IL-6 concentration was significantly different from normal distribution, so a non-parametric test was selected. To compare the average level of IL-6 in the two subgroups analysed, a non-parametric Mann–Whitney test was used. Categorical variables were compared using Fischer’s exact test. Spearman’s rank correlation coefficient was used to assess the correlation between IL-6 concentration and ASRS-5 score. Statistically significant differences were found for  $p < 0.05$ . Statistical analysis was performed using IBM SPSS 22.0.

	Control group n = 28	ADHD group n = 36	p	Z	r <sub>pb</sub>
<b>Sex:</b>					
• female, n (%)	21 (75%)	17 (47%)	0.045		
• male, n (%)	7 (25%)	19 (53%)			
<b>Age (M ± SD)</b>	30.82 ± 10.30	32.25 ± 9.18	0.252	1.146	
<b>IL-6 [pg/mL] (Me [Q1; Q3])</b>	0.56 [0.33; 0.91]	0.99 [0.61; 1.95]	0.007	2.694	0.395
<b>ADHD</b> – attention-deficit/hyperactivity disorder; <b>IL-6</b> – interleukin-6; <b>r<sub>pb</sub></b> – point-biserial correlation coefficient.					

Tab. 1. Comparison of the groups regarding sex, age, and serum interleukin-6 level

	Control group		ADHD group	
	r	p	r	p
<b>ASRS-5</b>	-0.017	0.931	0.146	0.396
<b>ADHD</b> – attention-deficit/hyperactivity disorder; <b>ASRS-5</b> – Adult ADHD Self-Report Screening Scale for DSM-5.				

Tab. 2. Correlation between interleukin-6 level and ASRS-5 score in compared groups

## RESULTS

The study was conducted on 36 adults with ADHD and 28 healthy controls. The majority of the participants (84%) diagnosed with ADHD were of the combined subtype (15 women and 14 men), and 16% of them exhibited predominantly inattentive subtype (two women and five men). The control group consisted of seven men and 21 women. There was a statistically significant difference between the two groups regarding sex ( $p = 0.045$ ), and no difference regarding age ( $p = 0.252$ ). The age range in both groups was 21–55 years. The mean age in adults with ADHD was  $32.25 \pm 9.18$  years, and in the control group it was  $30.82 \pm 10.30$  years. At the time of sampling, none of the individuals included in the study were under any medication. According to the analysis, adults diagnosed with ADHD had significantly higher serum concentrations of IL-6 (pg/mL) ( $Me: 0.99 [0.61; 1.95]$ ;  $p = 0.007$ ) in comparison to healthy controls ( $Me: 0.56 [0.33; 0.91]$ ) (Tab. 1). ASRS-5 score did not correlate significantly with IL-6 concentration in the ADHD group ( $r = 0.146$ ;  $p = 0.396$ ) or in the control group ( $r = -0.017$ ;  $p = 0.931$ ) (Tab. 2).

## DISCUSSION

The aim of the current study was to investigate the inflammatory factor IL-6 in adult ADHD patients with regard to the serum level of this biomarker. We observed that the serum level of IL-6 was higher in unmedicated adults with ADHD in comparison to healthy controls. To avoid confounding factors, the participants were unmedicated and they did not suffer from any neuropsychiatric comorbidity nor from any chronic inflammatory disease. Our findings are not consistent with previous results reported by Corominas-Roso et al. (2017). This is the only

study that included healthy unmedicated adult participants with an age range similar to our population (18–55 years). The aforementioned analysis did not reveal any significant differences in serum IL-6 concentration between the ADHD group and the control group. The only study on IL-6 in adults that found the level of IL-6 to be elevated in patients with comorbid ADHD and asthma is a longitudinal study by Leffa et al. (2021), however, in this study, the cytokine was measured in younger participants (aged 18 and 22 years). More available data comes from studies on children. Some of them are in line with our results (Chang et al., 2020; Darwish et al., 2019; Donfrancesco et al., 2020; Elhady et al., 2022; Elsadek et al., 2020; Koç et al., 2023; Mahmoud et al., 2020), while others do not support our conclusion (Dursun et al., 2021; Puzino et al., 2022; Verlaet et al., 2019; Wang et al., 2022). Of note, our results are supported by the findings from a recent meta-analysis of 10 studies, showing higher concentrations of IL-6 in the peripheral blood of ADHD patients. However, eight of the included studies were conducted on children (Misiak et al., 2022). IL-6 is an inflammatory mediator of pleiotropic nature. It is involved in specific and non-specific immune responses (Murakami et al., 2019). It is released by lymphocytes, macrophages, osteoblasts, smooth muscle cells, neurons, microglia, and astrocytes. Helper T cells secrete cytokines that can cross the blood–brain barrier (Hunter and Jones, 2015). IL-6 exerts a number of actions in the central nervous system that may be relevant to neurodevelopmental disorders. It has been shown that IL-6 is involved in the regulation of the expression of neurotrophic factors, in the regulation of the circadian rhythm and food intake, as well as in learning and memory processes (Borovcanin et al., 2017). IL-6 affects neurogenesis, synaptic formation, and myelination (Tancredi et al., 2000). IL-6 can be related to ADHD symptoms through several mechanisms. Increased levels of IL-6 can affect the functioning of the prefrontal cortex (Trapero and Cauli, 2014), which is responsible for executive functions, including action planning, decision-making, attention, and motivational processes (Goto et al., 2010). Decreased executive functioning is believed to be a core impairment in ADHD patients (Mostert et al., 2015). Functional magnetic resonance imaging studies indicate changes in the activity of the prefrontal cortex and the anterior cingulate cortex under the influence of chronic inflammation caused by elevated levels of tumour necrosis factor (TNF- $\alpha$ ) and IL-6 (Rosenkranz et al., 2005). Inflammation at the peripheral level can cause changes in dopaminergic and noradrenergic neurotransmission (Anand et al., 2017). It has been proven that IL-6 affects the metabolism of neurotransmitters, disturbing the balance between the levels of norepinephrine and dopamine in the brain. These neurotransmitters are involved in the pathophysiology of ADHD (Cortese, 2012). Our study aimed to achieve a better understanding of the inflammatory mechanisms that contribute to the connection between ADHD and inflammatory cytokines.

However, its cross-sectional design is unable to establish a causal relationship between the elevated levels of cytokines and ADHD.

The study has some limitations. The ADHD group consisted of volunteers who suspected they might have ADHD and were motivated to verify it. Also, the control group was not a random sample and consisted of volunteers, resulting in a potential for selection bias. It is difficult to determine whether the obtained results reflect the actual state of the general population. The small sample size did not allow us to compare ADHD adults with regard to sex and ADHD subtypes. There was a significant gender disproportion in the compared groups, but the literature does not indicate significant differences in terms of serum concentrations of IL-6 between males and females in the general population (Sadeghi et al., 2005). The ADHD diagnosis was established based on interviews with patients, and there was no collateral information. The study protocol did not include a general health examination of the participants. We only relied on their declaration. We did not collect body mass index (BMI) data, though it would be worth comparing the BMI in examined groups. Other factors that could influence study results are diet, smoking, daily exercise, sleep, and psychological stress.

## CONCLUSIONS

An increasing body of evidence supports the notion that abnormalities within the immune system may play a role in the development and progression of ADHD. The presence of elevated IL-6 levels in adults diagnosed with ADHD suggests an immune-mediated basis for ADHD. The increased levels of IL-6 may potentially contribute to the underlying causes of ADHD, but we cannot assume the direct and causal nature of this relationship. The study needs a replication with a larger sample size.

### Conflict of interest

*The authors do not report any financial or personal connections with other persons or organisations that could negatively affect the content of this publication and claim authorship rights to this publication.*

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### Author contributions

*Original concept of study: ZR, JK. Collection, recording and/or compilation of data: ZR, JW, HJ. Analysis and interpretation of data: ZR, HJ. Writing of manuscript: ZR. Critical review of manuscript: JK. Final approval of manuscript: JK.*

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