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Study of serum neopterin in children with attention deficit hyperactivity disorder and autistic spectrum disorder: Fayoum Governorate, Egypt

Hadeer Mahmoud Gamal El-Deen Abdel Ghaffar[1], Noha Khalifa Abdelghaffar[2], Hanaa Hanafi Ahmed[1] and Mariam Ehab Dawoud[3*]

Abstract

Background: There is evidence supporting that cellular immunity may play a role in the pathophysiology of autism spectrum disorder (ASD) and attention deficit hyperactivity disorder (ADHD). Neopterin, a pteridine mainly synthesized by activated macrophages, is a marker of inflammation, immune system activation that may be involved in the pathophysiology of both disorders.

Methods: Fifty drug-naïve patients were diagnosed according to DSM-5 (25 with ASD, 25 with ADHD), in addition to 25 healthy volunteers matched in age and gender with the patients were included. The CARS, Conners' scales used to assess the severity of the disorders, respectively. Serum neopterin level was measured using ELISA technique for all participants.

Results: Statistically nonsignificant difference in mean neopterin level between control and both patients groups with significant negative correlation between neopterin level and younger ages in ASD group were found. Statistically nonsignificant difference also was found between its levels among subtypes of ADHD as well and with the degree of ASD symptoms severity.

Conclusions: There was no statistically significant difference between serum neopterin level in ADHD, ASD patients groups and control group reference.

Keywords: ASD, ADHD, Neopterin

Background

Attention deficit hyperactivity disorder (ADHD) is a neurodevelopmental disorder characterized by pervasive and persistent behavioral symptoms of inattention, hyperactivity, and/or impulsivity that are extreme for age and interfere with social or academic functioning. Diagnosis requires the emergence of symptoms before 12 years of age and that symptoms present in two or more settings. Autism spectrum disorder (ASD) refers to a neurodevelopment disorder that is characterized by difficulties with social communication and social interaction and restricted and repetitive patterns in behaviors, interests, and activities. By definition, the symptoms are present early in development and affect daily functioning. The term 'spectrum' is used because of the heterogeneity in the presentation and severity of ASD symptoms, as well as in the skills and level of functioning of individuals who have ASD [1].

*Correspondence: med11@fayoum.edu.eg
[3] Psychiatry Department, Faculty of Medicine, Fayoum University, P.O. Box: 63514, El-fayoum, Egypt
Full list of author information is available at the end of the article

Neopterin is a product secreted by activated macrophages and dendritic cells and can be detected in serum and other fluids using routine immunoassay methods [2].

Increased serum neopterin levels have been found in neuropsychiatric disorders in which inflammatory processes might be activated, such as ASD and Tourette's syndrome [3].

Methods

This study was a cross-sectional case–control study.

Sample size calculated using G-Power© software version 3.1.7 (Institute of experimental psychology, Heinrich Heine University, Dusseldorf, Germany), two-sided (two tails) type I error 0.05 and power of 80%, each group should include at least 25 subjects for each group.

Participants included in this study were 25 patients with ADHD diagnosed according to the DSM-5 criteria, and 25 patients with ASD diagnosed according to DSM-5 (American Psychiatric Association, 2013) [1] who were recruited from the Neuro Pediatric and Psychiatric outpatient clinics, Fayoum University Hospitals, as well as 25 healthy controls matched in age, gender among relatives of medical and paramedical personnel staff of Fayoum University hospitals through the period of first June 2020 to end of December 2020. The Arabic version of Conner's Parent Rating Scale- Revised- Long version [4] and the childhood autism rating scale (CARS) [5] were used to detect the severity of ADHD and autism, respectively.

All the scales showed absence of psychopathology in the control group. Patients with a comorbid psychiatric, neuro-metabolic, neuro-degenerative and neuro-cutaneous diseases were excluded, as well as patients with a history of chronic systemic diseases, such as endocrinologic and allergic diseases. Patients who previously had used psychotropic drugs within last 6 months were excluded as well.

All patients were drug naive to avoid effect of medication on neuroinflammatory markers, as supported by Valvassori et al. 2019 who reported that atomoxetine has an anti-inflammatory effect on CNS [6].

Blood sampling

Blood samples of participants were collected between 9:00 AM and 12:00 AM. The blood samples were centrifuged at 3000 rpm for 20 min to obtain sera. The samples were stored frozen at − 80 °C before analysis in Fayoum University Hospital labs. The biochemical analysis was performed after all the blood samples were collected. Serum neopterin levels were measured by using commercially enzyme linked immunoassay (ELISA) kit.

Written informed consent was obtained from all the parents after they were provided with a complete description of the study.

Statistical methods

Data management will be performed using the statistical package for social science (version 15.0; SPSS Inc., Chicago, IL, USA). Compute standard descriptive statistics (e.g., mean, standard deviation) will be used to summarize the data.

Nominal data will be analyzed using simple × 2 test, while independent sample *T*-test procedure will be used to compare means for two groups of cases; for more than two groups, data will be evaluated with one-way analysis of variance (ANOVA). A probability value (*P* value) less than 0.05 was considered significant.

Results

In this study, the range for age of patients was 3–15 years with the mean 8 years in ADHD group and 5.5 years in ASD.

Male represented 76% in ADHD group while female represented 24%.

Male represented 52% of ASD group while female represented 48% in ASD group (Table 1).

In this study, we found that among ADHD group 88% showed combined presentation, while 8% showed

Table 1 Socio-demographic data

	Control (*N* = 25)		ADHD (*N* = 25)		ASD (*N* = 25)		*P*-value[#]
	Mean	SD	Mean	SD	Mean	SD	
Age	7.9	2.7	8	2.3	5.5	2	< 0.0001
Gender							
Male	14	56.0%	19	76.0%	13	52.0%	0.175
Female	11	44.0%	6	24.0%	12	48.0%	

[#] One-way ANOVA [##]Chi-squared test

ADHD attention deficit hyperactivity disorder, *ASD* autism spectrum disorder

Table 2 Clinical characters of ADHD group

	N	%
Combined	22	88.0%
Predominantly hyperactivity	1	4.0%
Predominantly inattention	2	8.0%

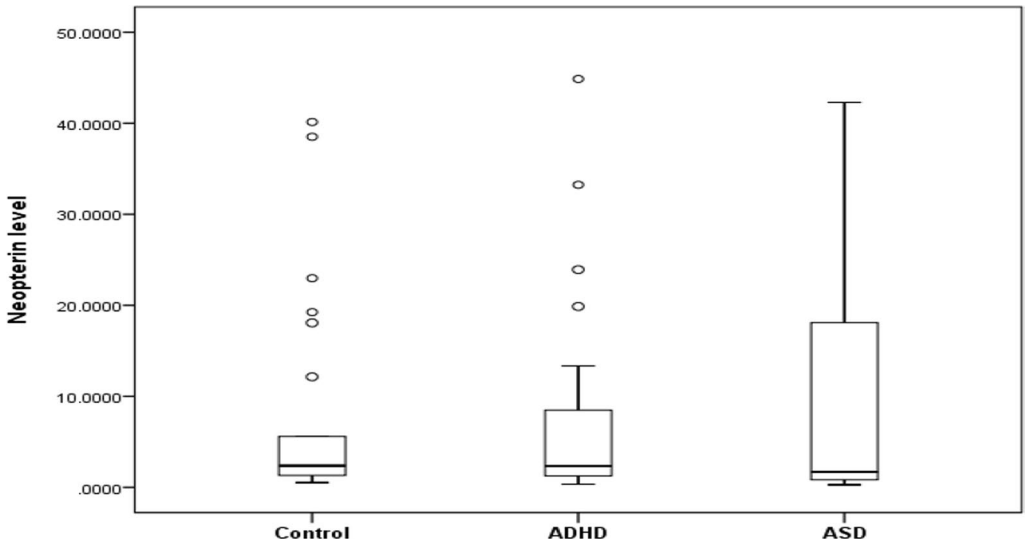

Fig. 1 Neopterin level in control group and patient groups

Table 3 Comparison of neopterin level in control group and patient groups

	Control (*N* = 25) Neopterin level	ADHD (*N* = 25)	ASD (*N* = 25)	*P*-value[#]
Mean	7.7	7.7	9.4	0.785
SD	11.4	11.4	13.2	
Median	2.4	2.3	1.7	
Minimum	0.5	0.3	0.3	
Maximum	40.1	44.9	42.3	

[#] Kruskal–Wallis test

ADHD attention deficit hyperactivity disorder, *ASD* autism spectrum disorder

predominantly inattentive presentation, 4% showed predominantly hyperactive presentation (Table 2).

On comparing both groups, patients and controls, we did not find a statistically significant difference regarding mean neopterin serum level (Fig. 1) (Table 3) and in between different ADHD subtypes ($P = 0.618$) (Table 4).

As regards the ASD group, 52% had mild-to-moderate presentation, 48% had severe presentation. There was no statistically significant difference in mean neopterin level in relation with severity of ASD symptoms ($P = 0.769$) (Table 5), with a statistically significant difference in mean neopterin level in younger ages of ASD group ($P = 0.026$) (Fig. 2) (Table 6).

Table 4 Difference in neopterin level according to characteristics of ADHD cases

	Neopterin level				
	Mean	**SD**	**Median**	**Minimum**	**Maximum**
Combined	**8.3**	12	**2.5**	0.3	44.9
Hyperactivity	**1.2**	0	**1.2**	1.2	1.2
Inattention	**4.9**	5	**4.9**	1.4	8.5
P-value[##]	0.618 (NS)				

[#] Mann–Whitney *U* test [##]Kruskal–Wallis test

Table 5 Difference in neopterin level according to severity of ASD cases

	Neopterin level				
	Mean	**SD**	**Median**	**Minimum**	**Maximum**
P-value[#]	0.642 (NS)				
Severity					
Mild to moderate	**8.9**	13.3	**1.7**	0.3	42.3
Severe	**10**	13.8	**1.6**	0.3	37.4
P-value[#]	0.769 (NS)				

[#] Mann–Whitney *U* test

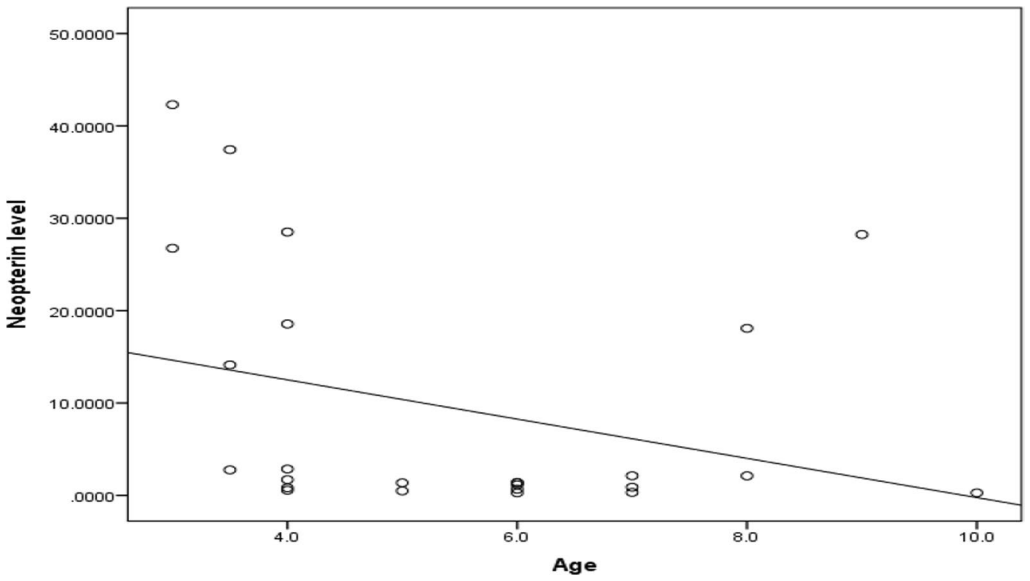

Fig. 2 Correlation between neopterin level and age

Table 6 Correlation between neopterin level and age

	Neopterin level					
	Control		ADHD		Autism	
	r	*P*-value	*r*	*P*-value	*r*	*P*-value
Age	0.07	0.739 (NS)	− 0.087	0.678 (NS)	− 0.444	0.026 (S)†

† Significantly higher in younger age

Discussion

Central nervous system and immune system are systems which are in interaction with each other [7]. Immune cells and molecules play a crucial role in forming the brain functions by affecting the cognitive and emotional processes [8].

In this study, the range for age of patients was 3 -15 years with the mean 8 years in ADHD group this supported by CDC, 2016 that reported the average age of ADHD is 7 years. The mean age in ASD was 5.5 years, this was supported by American Academy of Pediatrics, 2017 that reported that the average age of diagnosis is about 4.5 years [9, 10].

Males represented 76% in ADHD group while females represented 24%, this is in line with Danielson et al. 2018 who reported that boys being over-represented, on average, approximately 2:1 [7].

Males represented 52% of the ASD group while females represented 48% in ASD group, this supported by current estimates of CDC, 2018 that ASD is more than 4 times among boys than among girls [11].

In this study, we found that among ADHD group 88% showed combined presentation (ADHD-CT), 8% showed predominantly inattentive presentation (ADHD-PI), 4% showed predominantly hyperactive presentation (ADHD-H). This is in line with clinic samples prevalence which reported ADHD-CT is approximately one and one half times more prevalent than ADHD-PI. Although ADHD-PI appears to be more prevalent in the general population, children diagnosed with ADHD-CT are more likely to be referred for treatment probably reflecting the greater amount of disruptive behavior associated with the ADHD-CT symptoms [12].

In ASD group, 32% ($N=8$) had mild-to-moderate presentation, 68% ($N=17$) had severe presentation. This is consistent with Nguyen et al. 2021 who found more Vietnamese children were diagnosed with severe autism (59.4%) than mild and moderate autism [13].

In this study, there was no statistically significant difference in mean neopterin level between controls and both ASD and ADHD group. This is inconsistent with Zhao et al. 2015 who studied neopterin level among 80 patients

with autism and 80 typically matched healthy participants and Sweeten et al. 2003 who measured neopterin level among 23 participants with autism and 21 healthy subjects. In both previously mentioned studies, plasma neopterin levels were significantly higher in the autistic group than in the comparison subjects [14, 15]. Also inconsistent with Ceylan et al. 2014 who found that neopterin levels were significantly higher in the ADHD group than in the comparison subjects [16]. This inconsistency may reflect the need for further studies among those patients with multiple sampling and genetic assessments to detect role of neopterin and other immunologic markers.

In this study, no statistically significant difference was found between mean neopterin levels in ADHD subtypes. This is consistent with Ceylan et al. 2014 who found no significant differences in neopterin levels among the subtypes of ADHD [16].

There was no statistically significant difference in mean neopterin level in relation with severity of ASD symptoms. This is inconsistent with Zhao et al. 2015 who reported a significant positive association between plasma neopterin levels and CARS scores [15].

There is statistically significant difference in mean neopterin level in younger ages of ASD group. This may suggest that immune system activation may occur early in the course of the disease. This is supported by Mazzone et al. 2018 who suggested that neuroanatomical and neurochemical events occur relatively early in the development of the central nervous system (CNS), this may help in early pharmacological intervention that helps to cure and maybe even preclude some of the severe behavioral symptoms of ASD [17].

Limitations of the study

Neopterin level was measured in plasma, not in cerebral spinal fluid. It is still uncertain whether peripheral neopterin levels could reflect similar changes in the central nervous system. Without serial measurement of the circulating neopterin levels, this study yielded no data regarding when and how the levels changed in these children.

Conclusions

Autistic spectrum disorders (ASD) and attention deficit/hyperactivity disorder (ADHD) are the most frequently occurring neuropsychiatric disorders in childhood with an etiology that is not fully understood. Despite the insignificance in neopterin levels in ASD group, neopterin level was statistically significantly higher in young ages of ASD, we can recommend for more research to use it as a screening test for early detection of children at risk of ASD before development of symptoms.

Abbreviations

ADHD: Attention deficit hyperactive disorder; ADHD-CT: ADHD combined presentation; ADHD-H: ADHD predominantly hyperactive presentation; ADHD-PI: ADHD predominantly inattentive presentation; APA: American Psychiatric Association; ASD: Autism spectrum disorder; CARS: Childhood Autism Rating Scale; CDC: Center for Disease Control and Prevention; ELISA: Enzyme linked immunoassay.

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Authors' contributions

HG prepared the main idea, and was the main supervisor on editing the manuscript. MD and HA prepared the collected samples, questionnaire's data, analyzed and interpreted the patient data regarding the clinical data and psychometric tools and was a major contributor in writing the manuscript. NA was the major contributor in interpreting labs data. All authors read and approved the final manuscript.

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Availability of data and materials

Not applicable.

Declarations

Ethics approval and consent to participate

This study was approved by the Research Ethics Committee, Faculty of Medicine, Fayom University. The number of approval is M490 (10 May 2020). Contents of consent were clarified prior to participation.

Consent for publication

Not applicable.

Competing interests

No competing interests were declared in this section.

Author details

[1]Department of Pediatrics, Faculty of Medicine, Fayoum University, El-fayoum, Egypt. [2]Department of Clinical Pathology, Faculty of Medicine, Fayoum University, El-fayoum, Egypt. [3]Psychiatry Department, Faculty of Medicine, Fayoum University, P.O. Box: 63514, El-fayoum, Egypt.

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References

1. American Psychiatric Association. Diagnostic and statistical manual of mental disorders 5th edition. 2013.
2. Castillo L, Carcillo J. Secondary hemophagocytic lymphohistiocytosis and severe sepsis systemic inflammatory response syndrome/multiorgan dysfunction syndrome/macrophage activation syndrome share common intermediate phenotypes on a spectrum of inflammation. Pediatr Crit Care Med. 2009;10(3):387–92.
3. Bodur S, Ceylon MF, Iseri E, Sener S, Yucel AA. Serum neopterin levels in patients with autism. Int J Dev Disabil. 2013. https://doi.org/10.1179/2047387713Y.0000000029.
4. El Sheikh M, Sadek A, Abd El Naser O, El Nahas G. Psychiatric morbidity in first degree relatives of a sample of ADHD Children. Ain Shams Univ Lib. 2003.
5. Schopler E, Reichler J. Toward objective classification of childhood autism: childhood autism rating scale (C.A.R.S.). J Autism Dev Disord. 1980;10:91–103.

6. Valvassori SS, Dal-Pont GC, Tonin PT, Varela RB, Ferreira CL, Gava FF, Andersen ML, Soares JC, Quevedo J. Coadministration of lithium and celecoxib attenuates the behavioral alterations and inflammatory processes induced by amphetamine in an animal model of mania. Pharmacol Biochem Behav. 2019;183:56–63.
7. Danielson ML, Bitsko RH, Ghandour RM, Holbrook JR, Kogan MD, Blumberg SJ. Prevalence of parent-reported ADHD diagnosis and associated treatment among US children and adolescents, 2016. J Clin Child Adolesc Psychol. 2018;47(2):199–212.
8. Yirmiya R, Goshen I. Immune modulation of learning, memory, neural plasticity and neurogenesis. Brain Behav Immun. 2011;25(2):181–213.
9. American Academy of Pediatrics, (2017). Periodic survey: cross-survey results and findings. 2018. Available at: https://www.aap.org/enus/professional-resources/Research/pediatrician-surveys/Pages/Periodic-Survey-List-of-Surveys-and-Summary-of-Findings.aspx. Accessed December 16, 2018.
10. CDC. Centers for disease and control prevention. 2016.
11. CDC. Centers for disease and control prevention. 2018.
12. Milich R, Balentine AC, Lynam DR. ADHD combined type and ADHD predominantly inattentive type are distinct and unrelated disorders. Clin Psychol Sci Pract. 2001;8(4):463–88.
13. Nguyen PM, Tran TT, Thach TNA, Van Nguyen T. An unexpected positive effect of social distancing measures on the care of children with autism in Vietnam. Asia Pac J Public Health. 2021;33(2–3):320–1. https://doi.org/10.1177/1010539521997717.
14. Sweeten TL, Posey DJ, McDougle CJ. High blood monocyte counts and neopterin levels in children with autistic disorder. Am J Psychiatry. 2003;2003(160):1691–3.
15. Zhao H, Yin S, Fan J. High plasma neopterin levels in Chinese children with autism spectrum disorders. Int J Dev Neurosci. 2015;41:92–7. https://doi.org/10.1016/j.ijdevneu.2015.02.002.
16. Ceylan MF, Uneri OS, Guney E, Ergin M, Alisik M, Goker Z, Dinc GS, Kara FK, Erel O. Increased levels of serum neopterin in attention deficit/hyperactivity disorder (ADHD). J Neuroimmunol. 2014;273(2014):111–4. https://doi.org/10.1016/j.jneuroim.2014.06.002.
17. Mazzone L, Postorino V, Siracusano M, Riccioni A, Curatolo P. The relationship between sleep problems, neurobiological alterations, core symptoms of autism spectrum disorder, and psychiatric comorbidities. J Clin Med. 2018;7(5):102.

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