

The comparison between the levels of pro-inflammatory cytokines in ADHD children with healthy subjects

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Soltanifar A, Massoudian H, Salimi Z, et al. The comparison between the levels of pro-inflammatory cytokines in ADHD children with healthy subjects. *J Child Adolesc Psych* 2018;2(3):03-06.

ABSTRACT

Objective: ADHD is a neuropsychiatric disorder (neuropsychiatric disorder) associated with inattentiveness, impulsivity and hyperactivity. Different studies have identified the role of the immune system and pro-inflammatory cytokines in psychiatric illnesses, such as ADHD. This study aimed to compare the level of pro-inflammatory cytokines in ADHD children with healthy subjects.

Methods & Materials: 20 children ages 10-2 years, referred to the outpatient clinic of the Ibn-e-sina psychiatric hospital during 2015-2016 who diagnosed with ADHD based on DSM-5 criteria and psychiatric structural interview enrolled in our study. For the control group, 20 children without a history of psychiatric illness, after the structural interview by a psychiatric assistant and obtaining inclusion criteria were enrolled in the trial as well. After obtaining

consent from the parents or legal guardians from both groups, a 2.5 cc blood sample has been taken and serum levels of IL-2, TNF α were measured by ELISA method. Data were analyzed with SPSS software (version 11.5). The significance level was considered p-value <0.05.

Results: The mean age in the ADHD group was 8.2 ± 1.36 years old while in the control group was 6.5 ± 1.35 . The mean TNF α level was 7.75 with a standard deviation of 5.22. The level of TNF α in the ADHD group was greater than the control group, but this is not statistically significant between the two groups. (P-value: 0.24) Mean interleukin level was 139.45 ± 161.51 with a standard deviation. The level of interleukin in the case group was lower than the control group, which was not statistically significant. (P-value: 0.28)

Conclusion: The results showed that there is no significant difference between ADHD children's pro-inflammatory cytokines level compared with healthy subjects. These factors are not considered as direct causes of ADHD disorder.

Key Words: Attention deficit hyperactivity disorder; Impulsive behavior; Neuropsychiatric disorders

Attention Deficit Hyperactivity Disorder (ADHD) is a Sustained Attention deficit and/or hyperactivity and impulsive behavior patterns that are more severe and more common than it is commonly seen in children and adolescents with similar growth levels. In the past it was believed that hyperactivity is the most common sign of impairment in this disorder, but today it is considered to be secondary to impulse control impairment. For considering this disorder clinicians must pay attention that symptoms should appear until the age of 12 [1].

Attention deficit is the most serious and specific problem that children with this disorder have. It's difficult for these children to pay constant attention. Constant attention helps the person to control the interactions and respond only to one stimulus [2,3]. These children have many Educational and behavioral problems, and in some cases, the child or adolescent suffering from the disorder is unable to complete the educational process [4,5].

The incidence of this disorder varies between 2% and 20% for middle-aged children in the United States as well as the incidence of 9% to 14.2% in Iran [6-9]

At this point, there is a consensus that the etiology of this disorder is related to the interaction of neuro-anatomical and neuro-chemical systems. This consensus is based on studies done on Family genetics, twins and adoption, dopamine vector gene, brain imaging studies, and neurotransmitter data.

The lifelong progress of ADHD is very variable. Symptoms have been found to continue in about 50% of cases until adolescence or adulthood. Among other 50%, symptoms subsided during puberty or early adulthood [10].

Pharmacotherapy is the cornerstone for treating ADHD [11-13]. Central nervous system stimulants are the drug of choice due to their high effectiveness and low side effects. These drugs have a positive effect on both the cognitive and non-cognitive domains of the disorder [6,14].

These drugs include methylphenidate, dextroamphetamine, amphetamine, etc. [15-18].

Non-stimulant drugs are also used in the treatment of ADHD like atomoxetine hydrochloride, bupropion products, venlafaxine, and alpha-adrenergic agonists such as clonidine and guanfacine [19-28].

There has recently been a lot of data on the role of cytokines; glycoproteins secreted by the intrinsic and acquired immune system and interfere with many immune functions in neurobiological processes like peripheral immune system impairment in patients with neurological and neuropsychiatric disorders [29].

Pro-inflammatory cytokines include IL-2, And TNF is the main factor of various types of perinatal pathologies leading to severe mental and neurological disorders [30]. The effect of IL-2 on brain development affects its function, the impairment occurs as a result of its effect on the peripheral immune system and the central nervous system [29].

The relationship between inflammatory environmental functions, oxidative stress and neutrophils roles as toxic systemic mediators with the activity of the disease has been well established. In some meta-analysis studies, the increase in the serum level of these pro-inflammatory cytokines compared with the control group [6,7,31,32].

Because few studies about ADHD and its relationship with pro-inflammatory cytokines have been performed [32], hence finding the etiology and the pathogenesis of the disorder may be so helpful for finding new effective therapeutic ways to overcome the disorder and cause no pre-clinical studies have been done to investigate this issue, we decided to check whether Inflammation is playing a dominant role in the pathogenesis of ADHD, and whether there is a relationship between the levels of these cytokines and the severity of the disease or not.

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Received: November 23, 2018, Accepted: December 18, 2018, Published: December 21, 2018



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MATERIALS AND METHODS

This case-control study was conducted in 2016-2017 after approving the thesis and approval of the ethics committee of Mashhad University of Medical Sciences. A total number of 40 children including; 20 children ages 10-2 years, referred to the outpatient clinic of the Ibne-Sina psychiatric hospital diagnosed with ADHD based on DSM-5 criteria and psychiatric structural interview and 20 children without a history of psychiatric illness, after the structural interview by a psychiatric assistant and obtaining inclusion criteria enrolled in our study. A detailed explanation was provided to the parents about how the study was conducted.

Inclusion criteria:

1. There is no other comorbid psychiatry disorder in axis I.
2. Age of 12- 2 years old.
3. Completion of informed consent by parents or legal guardians.
4. The absence of active microbial disease in the last two months.
5. Not taking a vitamin supplement and other supplements in the last two months.

Exclusion criteria

1. Taking anti-inflammatory drugs like NSAIDs.
2. Having a particular diet or receiving any supplement.
3. Using any drug that has proven effects on mentioned inflammatory. Cytokines

The control group was matching by age and sex with case group.

Since this research was done for the first time due to lack of research, Therefore this pilot study was conducted with a sample size of 20 cases in each group.

The study was conducted by obtaining written informed consent from the parents of the children. The benefits, disadvantages and methods of implementing the study's plan are fully explained. The name and characteristics of the patient were kept confidential and, if the patient's parents were unwilling to continue the study, the patient's would be excluded from the study at any time.

After obtaining consent from the parents or legal guardians from both groups, a 2.5 cc blood sample has been obtained early in the morning and serum levels of IL-2, TNF α were measured by ELISA method.

Statistical analysis

For describing the descriptive data statistics, including frequency tables, diagrams, and statistical indicators were employed, and data analysis was done through the use of SPSS software version 11.5.

To analyze the data, the Kolmogorov-Smirnov test, the independent T-test, the Mann-Whitney test and the Pearson chi-square were applied. A p-value less than 0.05 were taken to mean statistical significance.

RESULTS

The mean age in the ADHD group of patients was 8.2 with a standard deviation of 1.36 and the age range was 6 to 12 years old. In the control group, the mean age was 6.5 with a standard deviation of 1.35 and a range from 6 to 12 years old (Table 1).

TABLE 1

Mean age of patients in both control and intervention groups

Groups	Mean age	Standard deviation
Control group	6.5	1.35
Intervention group	8.2	1.36

The mean TNF- α level was 7.75 with a standard deviation of 5.22. The lowest levels of TNF- α were 2.30 in the ADHD group and 3.20 in the control group and the highest levels of TNF- α were 29.60 in the ADHD group and 14.30 in the control group. Using the Mann-Whitney test, the level of TNF- α in the ADHD group was greater than the control group, but this was not statistically significant.

The mean interleukin level was 139.45 with a standard deviation of 161.51. Also, the lowest level of interleukin 2 in the ADHD group was 52.10, which

was 52.10 in the control group and the highest level in the ADHD group was 538.80 and comparing to the control group (766.40) (Table 2).

TABLE 2

Average inflammatory factors in both intervention and control groups

Control group N=20	Intervention group N=20	Inflammatory factors
6.44 (\pm 2.72)	9.06 (\pm 6.70)	TNF
145.03 (\pm 190.60)	133.87 (\pm 139.95)	IL2

The level of interleukin in the ADHD group was lower than the control group, which was not statistically significant

The coefficient effect of age was 0.24, which was not significant. This means that age has no significant effect on the TNF- α level and is not predictive of its level

DISCUSSION

ADHD is a neuropsychiatric disorder of childhood that is associated with hyperactivity, impulsivity and attention deficit [33,34].

At this point, there is a consensus that the cause of attention deficit-hyperactivity disorder is related to the interaction of neuro-anatomical and neuro-chemical systems. This consensus is based on studies on family genetics, twins and adoption, the vector of dopamine gene, neuro-imaging and neurotransmitter studies. Most children with ADHD show no evidence of obvious damage to the anatomic structure of the central nervous system [1].

Currently, a lot of data about the role of cytokines in neurobiological processes has been gained and there are many studies that have demonstrated the peripheral immune system impairment in patients with Neuropsychiatric disorders [29].

Since there are only few and limited studies on investigating the role of these cytokines in ADHD, we found no really similar studies to compare ours finding with. Considering the fact that our study is the very first pilot one to be done in this field we take a look to other studies' results for this chapter.

Pro-inflammatory cytokines include IL-2, And TNF are a pathogenic part of a variety of perinatal pathologies leading to severe mental and neurological disorders [30].

Since finding the etiology and pathogenesis of the disease is helpful in finding effective treatment strategies. Considering the unknown pathogenesis of ADHD, we decided to study the serum levels of some pro-inflammatory cytokines such as IL-2 and TNF in ADHD children, and Compare the results with the control group.

It should be noted that studies that have investigated the role of cytokines in patients suffering from ADHD are very limited and we will try to compare our findings with these studies in this part.

The results of our study showed that the level of TNF in the ADHD group was more than the control one, which is not statistically significant.

In the most recent study that was conducted in ADHD patients during 2017 with the aim of measuring the level of pre-inflammatory cytokines; there was no significant difference between the ADHD and control groups in terms of the fact that the patients did not have any chronic inflammatory disease or infectious disease. This finding confirms ours [35]. It can be said that the level of this factor can be similar to the control group and normal population, and maybe serum inflammatory cytokines do not directly play a role in the pathogenesis of the disease, which requires higher sample size studies to confirm these results.

Of course, different studies in the years 2009 and 2010 have focused on the difference between serum levels and the brain blood levels of these factors, which were similar to the results of experimental studies with animals these findings have completely different results comparing to ours [36-39].

In a recent systematic review about the level of pro-inflammatory cytokines in ADHD conducted in 2017, the results showed that 6 studies examined the serum cytokine levels while 7 studies evaluated the gene polymorphism and 1 study examined the level of cytokines in CSF of ADHD patients and control group [39,40].

Out of these six studies that looked at the level of these cytokines in serum, Oades et al. Showed no significant difference between the control group (21 cases) comparing to the patient group (14 cases), which is aligned with the

results of our study

while other studies have reported different results comparing to ours: A study was conducted in 2010 on the association between serum levels of these cytokines and ADHD symptoms in patients, reporting that there is a correlation between serum cytokines and hyperactivity symptoms of ADHD patients; so that the decrease in pro-inflammatory levels of interleukin-2 play a role in the coping impairment symptoms of Patients as well as the decreased TNF levels and the symptoms of commission errors [40].

The relationship between the severity of symptoms and the level of cytokines can also be related to environmental and genetic stressors that require longitudinal studies. The results of this study are contrary to our current study.

CONCLUSION

The results of this study showed that the level of pro-inflammatory factors, such as IL-2 and TNF- α , in patients suffering from hyperactivity-attention deficit disorder is little bit different compared to the healthy control group, but this difference is not statistically significant which suggested that inflammatory processes leading associated with these cytokines did not play an important role in the ADHD baseline etiology. This study was a pilot one and it should be confirmed with other large sample size studies in the future.

LIMITATIONS

The limitations of this research are largely due to the small sample size, and this is due to the time limit for the special assistant to collect sample size. On the other hand, families of these patients are less willing to participate in such projects because of the disappointment and frustration of numerous and expensive treatments methods. Since such studies require high sample sizes to validate the results, and the cost of these studies is relatively high, considering the perspective gained by this study, it is recommended that subsequent studies with higher sample sizes be conducted in collaboration with other research centres.

In many previous studies, changes in the serum levels of various cytokines have been seen, which was not possible due to the high cost of the measurement plan, and this is another limitation of our study.

SUGGESTIONS

- It is recommended that further studies be carried out with a larger sample size and in collaboration with other research centres in the country.
- It would be better to study inpatient settings as well for more accurate results.
- Control of confounding factors such as age should be considered.

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