Serum Interleukin-2 Level in Children with Attention-Deficit Hyperactivity Disorder (ADHD)

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Abstract

Background: Attention deficit hyperactivity disorder (ADHD) etiology is not completely understood, but common comorbid dysfunction of immune system suggest that these systems may be affected by common genetic background and molecular mechanism, for example, increase levels of specific cytokines are observed in ADHD. The aim of this study was to evaluate the level of interleukin-2 (IL-2) as a biomarker of ADHD and find out the relation between interleukin-2 and ADHD symptoms. Patients and Methods: A case control study carried out on 90 children who assigned into two equal groups: ADHD patients: met diagnosis of ADHD according to DSM V criteria and a control group. All groups were subjected to full history taken, thorough clinical examination, and measurement of Human IL-2 by ELISA technique. Results: The mean value of IL2 level was statistically significantly higher among ADHD cases than control group (p<0.001). There was statistically significant positive correlation between IL2 level (ng/L) and Duration Of illness and Conner Parent Scale, there was statistically significant negative correlation between IL2 level (ng/L) and IQ level. While there was statistically nonsignificant correlation between IL2 level (ng/L) and age and Order of ADHD child among his siblings. Conclusion: Serum IL-2 levels were elevated in ADHD children and correlated with the severity of ADHD.

Keywords: Interleukin-2; IL-2; ADHD, biomarkers

Introduction
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 (1).

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 before the age of 12 (2).

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 (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).

The etiology of ADHD is not yet fully understood and is likely attributable to complex interactions between genetic and environmental risk factors, although genetic factors play an important role considering heritability rates of 70–80% (5). In addition to genetic factors, current evidence indicates that a number of environmental insults during the prenatal period increase the risk of ADHD in offspring. These include maternal pre-pregnancy obesity, hypertensive disorders during pregnancy, pre-eclampsia and maternal exposure to acetaminophen during pregnancy (6).

Interleukin (IL)-2 is a 15– 20 kDa glycoprotein that modulates central nervous system (CNS) activity. IL-2 is present and modulates activity in the mesolimbic and mesostriatal systems. IL-2 mRNA and receptors are expressed in the cortex, mesencephalon, and striatum (7).

The proinflammatory cytokines that were assessed in ADHD include the interleukins IL-1β, IL-2, IL-6, tumour necrosis factor-α (TNF-α) and interferon-gamma (IFN-γ) for contrast with the anti-inflammatory cytokines IL-10 and IL-13 (8). Also included in the analyses were IL-4 (where levels proved to be below detection limits) and IL-16 that stimulates proinflammatory cytokine production but has been reported also to have antiinflammatory properties in different disorders (9).
The aim of this study was to evaluate the level of interleukin-2 (IL-2) in children with ADHD and compare them with a group of healthy matching children and find out the relation between interleukin-2 and different sociodemographic factors and ADHD symptoms.

**Patients and methods**

This case-control study was carried out on 90 children who were assigned into two equal groups: ADHD group and control group. Children with ADHD met diagnosis of ADHD according to DSM V criteria (10), patients were recruited from the pediatric and psychiatric clinics, Benha University Hospital, during the period from 1st April 2021 to 1st February 2022. Control group consists of 45 healthy children clinically free and have no history of other psychiatric or neurological disorders and were matching with the ADHD regarding age, residence and gender.

**Exclusion criteria:**

- Patients with chronic medical illness: renal, hepatic, thyroid, connective tissue diseases, neurological diseases, diabetes mellitus,…..etc.
- Co-morbid psychiatric disorders: conduct disorder, oppositional defiant disorder, depression, mood disorders and anxiety disorders (mental disorders).

Written informed consent was obtained from the parents of all subjects of the study. The study was approved by the ethics committee of faculty of medicine, Banha University.

All groups were subjected to full history taken, thorough clinical examination including developmental, neurologic, and psychometric evaluation. Conners’ Parent Rating Scale used for the assessment of ADHD and its comorbid disorders in children (11). An Arabic version of Conners’ Parent Rating Scale was used. Stanford Binet scale (fifth edition) is used for assessment of intelligent quotient (IQ) (12).

Measurement of Human IL-2 was done by ELISA technique. Three milliliters of venous blood were collected from each subject by the use of disposable sterilized plastic syringes. The needle of the syringes was then removed and each sample was allowed to pass gently along the wall of a clean dry centrifuge tube labeled with the code number. The blood was allowed to clot for half an hour in water bath at 37ºC then it was centrifuged for 15 min at 3000 r.p.m for
separation of serum by means of clean dry tubes for determination of IL-2 by enzyme linked immunosorbent assay. The kit uses a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) to assay the level of Human Interleukin 2(IL-2) in samples.

**Statistical analysis**

The data were recorded on an “Investigation report form”. These data were tabulated, coded then analyzed using the computer program SPSS (Statistical package for social science) version 26. Descriptive statistics were calculated for the data in the form of mean and standard deviation (±SD) for quantitative data and number and percent for qualitative data. In the statistical comparison between the different groups, Student’s *t*-test was used to compare between mean of two groups of numerical (parametric) data, for continuous non-parametric data, MannWhitney *U*-test was used for inter-group analysis. Pearson correlation coefficient (r) test was used correlating different parameters. Inter-group comparison of categorical data was performed by using chi square test (*X*²-value). Some investigated parameters were entered into regression analysis model to determine which of these factors is considered as a significant predictor. So, p-value considered significant as the following: *P >*0.05: statistically insignificant, *P <*0.05: statistically significant, *P <*0.01: highly significant.

**Results**

This case-control study was carried on 90 children who assigned into two equal groups: ADHD group and control group. There was no statistically significant difference between ADHD patients’ group and control group regarding age, sex, Consanguinity, family history of ADHD and order among siblings. There was a very high statistically significant difference. Regarding residence (*p*<0.001) (Table 1).
Table (1): Comparison of the ADHD and control group regarding sociodemographic data
Sociodemographic data of the studied ADHD and control groups.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>ADHD patients (n=45)</th>
<th>Control group (n=45)</th>
<th>Test of sig.</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age/months (mean ± SD)</td>
<td>9.01 ± 1.6</td>
<td>9.3 ± 2.5</td>
<td>0.7</td>
<td>0.2</td>
</tr>
<tr>
<td>Sex No. (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>16 (35.6%)</td>
<td>11 (24.4%)</td>
<td>1.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Male</td>
<td>29 (64.4%)</td>
<td>34 (75.6%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Residence No. (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urban</td>
<td>18 (40%)</td>
<td>38 (84.4%)</td>
<td>18.9</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Rural</td>
<td>27 (60%)</td>
<td>7 (15.6%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Consanguinity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>37 (82.2%)</td>
<td>38 (84.4%)</td>
<td>0.1</td>
<td>0.7</td>
</tr>
<tr>
<td>Positive</td>
<td>8 (17.8%)</td>
<td>7 (15.6%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Family history</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>38 (84.4%)</td>
<td>41 (91.1%)</td>
<td>0.9</td>
<td>0.3</td>
</tr>
<tr>
<td>Positive</td>
<td>7 (15.6%)</td>
<td>4 (8.9%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Order among siblings</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>13 (28.9%)</td>
<td>17 (37.8%)</td>
<td>5.1</td>
<td>0.2</td>
</tr>
<tr>
<td>2</td>
<td>24 (53.3%)</td>
<td>15 (33.3%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>8 (17.8%)</td>
<td>11 (24.4%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0 (0%)</td>
<td>2 (4.4%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duration Of illness (mean ± SD)</td>
<td>2.5 ± 1.04</td>
<td>---</td>
<td>---</td>
<td>---</td>
</tr>
</tbody>
</table>

Table (1) shows that there was no statistically significant difference between ADHD patients’ group and control group regarding age, sex, Consanguinity, family history of ADHD and order among siblings. There was a very high statistically significant difference Regarding residence (p<0.001).

Table (2): Comparison of the studied ADHD and control groups regarding IL2 LEVEL (ng/L)

<table>
<thead>
<tr>
<th></th>
<th>ADHD patients (n=45)</th>
<th>Control group (n=45)</th>
<th>Mann-Whitney U</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>IL2 level (ng/L)</td>
<td>50.54</td>
<td>34.18</td>
<td>15.11</td>
<td>5.85</td>
</tr>
</tbody>
</table>

Table (2) shows that mean value of IL2 level (ng/L) was very highly statistically significant among ADHD cases than control group (p<0.001).

Table (3): Relation between IL2 LEVEL (ng/L) and type of ADHD in the studied cases

<table>
<thead>
<tr>
<th></th>
<th>Combined (n=30)</th>
<th>Hyperactive &amp; impulsive (n=9)</th>
<th>Inattention (n=6)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
</tr>
<tr>
<td>IL2 level (ng/L)</td>
<td>57.90</td>
<td>39.20</td>
<td>38.64</td>
<td>9.43</td>
</tr>
</tbody>
</table>

Table (3) shows that the mean value of IL2 level (ng/L) was higher among combined ADHD cases (mean 57.9) followed by Hyperactive & impulsive (mean 38.64) then Inattention cases (mean 31.6) and the difference between them was statistically insignificant (p=0.07).
Table (4): Relation between IL2 LEVEL (ng/L) and the severity of the ADHD in the studied cases

<table>
<thead>
<tr>
<th>Mild (n=13)</th>
<th>Moderate (n=23)</th>
<th>Sever (n=9)</th>
<th>F test</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>Mean</td>
<td>Mean</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL2 level</td>
<td>26.66</td>
<td>46.03</td>
<td>96.58</td>
<td>23.2</td>
</tr>
<tr>
<td>(ng/L)</td>
<td>4.90</td>
<td>5.51</td>
<td>54.19</td>
<td></td>
</tr>
</tbody>
</table>

Table (4) and figure () shows that the mean value of IL2 level (ng/L) was very highly statistically significant higher regarding the severity (p<0.001).

Table (5): Correlation between IL2 level (ng/L) and different variables

<table>
<thead>
<tr>
<th></th>
<th>r</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>-0.04</td>
<td>0.79</td>
</tr>
<tr>
<td>Duration Of illness</td>
<td>0.38</td>
<td>0.01*</td>
</tr>
<tr>
<td>IQ</td>
<td>-0.61</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Severity of ADHD</td>
<td>0.69</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Order among siblings</td>
<td>-0.10</td>
<td>0.51</td>
</tr>
</tbody>
</table>

Table (5) shows that there was statistically significant positive correlation between IL2 level (ng/L) and the Duration Of illness and the severity of ADHD measured with Conner Parent Rating Scale, there was statistically significant negative correlation between IL2 level (ng/L) and IQ level. While there was statistically non-significant correlation between IL2 level (ng/L) and age and Order of ADHD child among his siblings

Discussion
The etiology of ADHD is not fully understood, however concomitant immunological dysfunction suggests that both systems may share a shared genetic foundation and molecular processes. For instance, elevated levels of certain cytokines have been seen in ADHD, whereas several genes associated with ADHD have immunological functions. It is hypothesized that an immunological imbalance, necessitating a predisposed genetic background, contributes to the pathophysiology of ADHD (13).

Pro-inflammatory cytokines include IL-2, is the main factor of various types of perinatal pathologies leading to severe mental and neurological disorders. 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 (2).

In the current study, regarding residence 60% of ADHD group were from rural areas. ADHD is statistically higher among resident in rural areas than urban areas (p<0.001).
This could be due to the location of hospital, which is in Benha city and this hospital not only serves the city but the surrounding villages. In addition, this may be explained by that children living in rural areas are more likely to experience family adversity, potentially in the form of poor parental mental health and financial difficulties. This, in combination with a lack of individual and community level resources for treatment, may lead to higher rates of persistent behavioral problems.

In the same line with our study, (14) showed that 70% of the studied ADHD patients were from rural areas. Similarly, (15) reported that the rural areas showed significantly higher prevalence estimates than urban areas. In consistency with our findings, the study from the center’s National Center for Health Statistics found that almost 20 percent of children ages 3 to 17 in rural areas qualified for a developmental disability diagnosis, compared to roughly 17 percent of children who live in urban areas. Of the selected developmental disabilities examined, children living in rural areas (compared to urban) were more likely to be diagnosed with ADHD (11.4% compared with 9.2%, p < 0.001) (16).

In disagreement with the current study, (17) reported that 30% of the studied ADHD patients were from rural areas and 70% were from urban areas.

In the present study, positive family history was 17.8% and negative was 82.2% in ADHD group. Family history was no statistically significant difference between ADHD patients’ group and control group. This could be explained as most of the cases where from rural areas and psychiatric symptoms could be overlooked or neglected.

In contrary with the present work, (18) found a significant higher percent of family history of ADHD among their cases (P<0.001).

In the current study, the highest percentage of ADHD was combined (66.7%) followed by Hyperactive & Impulsive (20%) while the lowest one was Inattention (13.3%). This may be as the combined type has both problems with hyperactivity and inattention, so the parents are more alert to the problem.

In the same line with our result, (19) found that the combined type of ADHD was predominant in the sample (51.4%), followed by the inattentive and hyperactive types in descending order of frequencies.

In the present work, the mean value of IL2 level was 50.54±34.18 in ADHD group and
15.11±5.85 in the control group. The mean value of IL2 level was statistically significantly higher among ADHD cases than control group (p<0.001).

A plausible explanation for our findings is that proinflammatory cytokines, including IL-2 are the main factor of various types of perinatal pathologies leading to severe mental and neurological disorders (20). 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 (21).

In line with our study findings, two previous studies of (22) and (23) have reported an association between ADHD and the polymorphism of genes encoding some cytokines, mainly IL-2. In the same context. (24) reported that 90% of the children with ADHD had detectable IL-2. Also (25) reported that the increase in interleukins IL-16 and IL-13, were positively associated with hyperactivity and inattention, respectively; increase in IL-2 was associated with opposition ratings symptoms in ADHD.

In addition, (26) ,(27) and (25) reported higher levels of IL-2 (but with lack of statistical significance) in patients with ADHD than normal individuals. We believe that this does not preclude the importance of such findings as, despite that the higher levels of IL-2 in ADHD found in these studies did not reach the level of significance, and the clinical significance was ensured in the latter study which declared normalization of IL-2 levels after treatment of ADHD with psychostimulant medication. The lack of significance in the described study could be related to variabilities in the sample characteristics, technique of measuring the cytokine, or other medications affecting the IL-2 levels.

In the present study, the mean value of IL2 level (ng/L) was higher among combined ADHD cases (mean 57.9) followed by hyperactive & impulsive (mean 38.64) then Inattention cases (mean 31.6) and the difference between them was statistically insignificant (p=0.07). The mean value of IL2 level (ng/L) was very highly statistically significant higher regarding the severity (p<0.001). There was statistically significant association between cytokines and ADHD symptoms. This is consistent with (25) study that reported 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.
This study sheds the light on a simple feasible marker that could help in assessment of the disease prognosis and response to treatment in children with ADHD patients. A very few studies assessed this marker, which strengthen the current work, which provides a rationale for a prospective, larger sample, longitudinal studies to gain insight into inflammatory processes underpinning the link between ADHD and inflammatory cytokines. This line of research has the potential to lead to novel, pathophysiologically based management strategies for children with ADHD.

In our study, there was statistically significant positive correlation between IL2 level (ng/L) and the duration of illness and the severity of ADHD measured with Conner Parent Rating Scale, and there was statistically significant negative correlation between IL2 level (ng/L) and IQ level. All these observations could be explained by the association of higher levels of IL-2 with more neurological and brain affection, that, in turn, affect the severity of symptoms and is more overt in cases not receiving treatment. None of the few research studies addressing IL-2 levels in ADHD patients have assessed its relation with the disease severity or established a cutoff value for diagnosis. The negative association between IQ levels and ADHD has a unidirectional effect on intelligence in a number of ways. The impact of limited self-control and impaired sustained attention may, to a small degree, diminish the acquisition of intellectual skills (28).

In harmony with the current study (18),(14) and (17) in their studies revealed that IQ was lower in ADHD patients than controls (p=0.001). In harmony with the current study (29) studied two hundred and five children with ADHD combined type, they reported that lower IQs were found in participants with ADHD when compared to controls (p=0.010).

On the other hand (30) described an above average IQ scores and executive functioning in children having ADHD. However, this was described for patients diagnosed with adult-onset ADHD and their high intelligence provided them social adaptation abilities that might have masked ADHD behavioral characteristics, complicating the diagnosis during childhood.

This study was limited by the small sample size which didn’t allow for a better analysis. All the patients from the same class. We did not measure the level of the other cytokines.
Also, we excluded patients with chronic medical illness, mental retardation, co-morbid disorders.

Conclusion

Serum IL-2 seems to be a promising marker for the diagnosis and severity of ADHD. Positive family history, type of ADHD (severity more with combined type) and IL2 level was significant predictors for severity of ADHD cases.

Recommendations

Interleukin-2 could be used as a biological marker for ADHD. Need studies on more children with more variation, different age and different sociodemographic standard. Further studies are needed to support our finding in this study and to make Interleukin-2 one of the routine lab investigations in ADHD patients. Additional studies are needed to investigate the role of other cytokines in the pathogenesis of ADHD.

References


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