MMP-2, MMP-9, TNF-α Levels In Relation To Subtypes of Attention Deficit Hyperactivity Disorder

Rania A. Hamed¹, Heba A. Elmalt², Abeer A.A. Salama³, Safaa M. Hammouda¹, Eman R. Youness²*, Naglaa A. Abd-Allah⁴, Fatma A. AlZaree⁵, Sarah Y. Abozaid ⁶ and Hala S. Ashour⁷

¹Department of Psychiatry, Faculty of Medicine for Girls, Al-Azhar University, Cairo, Egypt. ²Medical Biochemistry Department, Medical Research Division, National Research Centre, Giza, Egypt. ³Pharmacology Department, National Research Centre, Giza, Egypt. ⁴Department of Pediatric, Faculty of Medicine for Girls, Al-Azhar University, Cairo, Egypt. ⁵Child Health, Medical Research Division, National Research Centre, Giza, Egypt. ⁶Department of Clinical Pathology, Faculty of Medicine for Girls, Al-Azhar University, Cairo, Egypt. ⁷Department of Psychiatry, Faculty of Medicine, Mansoura University, Mansoura, Egypt.

*Corresponding author E-mail: hoctober2000@yahoo.com

https://dx.doi.org/10.13005/bpj/2156

(Received: 04 May 2020; accepted: 17 April 2021)

Many authors have suggested the association between Attention Deficit Hyperactivity Disorder (ADHD) and inflammation through various mechanisms among which increased serum cytokines. 30 newly diagnosed ADHD children, aged 6-12 and of both sexes were collected from outpatient clinic, Psychiatry Department, Al Zahraa University Hospital and a matched control group of 30 children. They were subjected to Clinical assessment, Wechsler Intelligence Scale for children (WISC), Conners’ Parent Rating Scale-Revised & serum MMP-2, MMP-9, TNF-α levels were determined. There was statistical significant difference between patient and control groups regarding MMP-2 level (648.50 ± 81.94 vs 344.13 ± 32.02), MMP-9 level (143.00±16.98 vs 102.90 ± 4.13) & TNF-α level (345 ± 7.1 vs 202 ± 22.3). Hyperactive/impulsive subtype represented 16(53.3%), Inattentive subtype represented 6(20%), Combined subtype represented 8(26.7%) of the ADHD group. MMP-2, MMP-9, TNF-α levels were all higher among the Hyperactive/impulsive subtype, followed by the combined subtype then the Inattentive subtype with high statistical significant difference. A high statistical significant difference was found in all subscales of Conners’ scale among the 3 subtypes of ADHD. A positive correlation was found between TNF-α level and age, whereas, a negative correlation exists between MMP-2, MMP-9, TNF-α level and IQ. In addition, correlation was found between MMP-2, MMP-9 levels and cognitive problems, TNF-α level and inattention. Our study illustrates the co-occurrence of inflammatory process and ADHD, but further studies on larger sample are needed.

Keywords: Attention Deficit Hyperactivity Disorder, MMP-2, MMP-9, TNF-α.

Attention Deficit Hyperactivity Disorder (ADHD) is the most common neurodevelopmental disorder in childhood, it can persist into adolescence and adulthood, it is characterized by sustained symptoms of inattention and/or hyperactivity and impulsivity, based on symptoms, 3 presentations of ADHD can occur: Combined Presentation, Predominantly Inattentive Presentation,
and Predominantly Hyperactive/ Impulsive Presentation.

The incidence of ADHD shows large variability, it is estimated to range between 3 - 11% & reached 20% in school aged children in USA & 20.5% in Egypt. ADHD symptoms lead to significant impact on the children’s quality of life as shown by poor academic performance, low self-esteem and progressive social deterioration.

The exact pathogenesis of ADHD is not fully understood, but a growing body of evidence for the role of the immune process and inflammatory mechanisms has been hypothesized.

Many authors have suggested the association between ADHD and inflammation. The inflammatory pathways are related to the pathophysiology of ADHD through various mechanisms, among which increased serum cytokines as indicated by several studies.

TNF-α is a proinflammatory cytokine, produced by macrophages and other cells such as B cells, T cells and activated monocytes in response to various stimuli, it is involved in controlling a broad variety of biological processes including the proliferation, differentiation, apoptosis of cells. It has also been documented that TNF-α plays a key role in tryptophan metabolism and dopaminergic pathways which are also involved in ADHD. Moreover, TNF-α is a potent stimulant for metalloproteinases expression in the brain through their release and activation.

Metalloproteinases (MMPs) are extracellular matrix (ECM)-degrading enzymes involved in inflammatory processes and remodeling of tissues. Thus, metalloproteinases have a role in the production of brain injury through either their proteolytic activity on the ECM or, moreover, their ability to increase the levels of soluble TNF-α levels as well.

Although many studies have investigated prevalence, characteristics and pathogenesis of ADHD patients, yet, no sufficient informations are available for evaluation of MMP-2, MMP-9, TNF-α in such patients.

Aim

Since the detection of ADHD pathogenesis is essential for the development of new therapeutic approaches, we aimed to measure serum matrix MMP-2, MMP-9 and TNF-α in ADHD and correlate them with subtypes of ADHD.

SUBJECTS AND METHODS

A case control study was conducted on a convenience sample of 30 newly diagnosed children with ADHD based on DSM 5 criteria, aged between 6-12 and of both sexes, they were collected from outpatient clinic, Psychiatry Department, Al Zahraa University Hospital, Cairo, Egypt. Patients with other comorbid psychiatric, neurological, autoimmune disorders or intellectual disability or having history of infectious disease within the last 3 months were excluded from the study. A control group of 30 children matched for age and sex were recruited from typically developing children having no history of psychiatric, neurological, autoimmune disorders or intellectual disability or infectious disease within the last 3 months.

All participants were subjected to the following:

2. Wechsler Intelligence Scale for children (WISC) Arabic version: To assess different categories of IQ. Children with IQ < 70 were excluded from the study.
3. Conners’ Parent Rating Scale-Revised: To diagnose ADHD in children and adolescents (3-18 years old), it comprises of 5 subscales: Cognitive problems, hyperactivity, inattention, liability and Hyperactivity/impulsivity.
4. Determination of serum MMP-2, MMP-9, TNF-α levels with the use of Enzyme-Linked Immunosorbent Assay (ELISA):

The human serum MMP-2, MMP-9 and TNF-α were assessed by using the RayBio® Human ELISA (Enzyme-Linked Immunosorbent Assay). Instructions were followed for obtaining results. Samples were pipetted with MMP-2, MMP-9 and TNF-α human antibodies, they were incubated & then washed and biotinylated antihuman MMP-2, MMP-9 and TNF-α antibody were added. Biotinylated antibody and horseradish peroxidase-conjugated streptavidin were pipetted and washed again. Tetramethylbenzidine substrate solution was added & intensity of the developed color was measured.
Data analysis
Statistical analysis was performed using SPSS (Statistical Package of Social Science) version 21.0 for the collected and coded data. Statistical significant difference were considered at P value < 0.05.

RESULTS
The sociodemographic analysis of both ADHD and control groups showed that they were matching regarding age, sex, IQ assessment with no significant difference at P value. High statistical significant difference was present between both groups regarding MMP-2 level (648.50 ± 81.94, 344.13 ± 32.02 respectively), MMP-9 level (143.00 ± 16.98, 102.90 ± 4.13 respectively) & TNF-α level (345 ± 7.1, 202 ± 22.3 respectively).

ADHD group were further subdivided into 3 subtypes
Hyperactive/impulsive subtype representing 16(53.3%), Inattentive subtype representing 6(20%), Combined subtype representing 8(26.7%) of the patient group (Table 1).

Comparison between MMP-2 and MMP-9, TNF-α level in subtypes of ADHD shows that all levels were higher among the Hyperactive/impulsive subtype, followed by the combined subtype then the Inattentive subtype with high statistical significant difference.

Comparing between subtypes of ADHD regarding WISC, no significant difference was found regarding total, verbal, performance IQ, however, a high statistical significant difference was present in all subscales of Conners’ scale.

A positive correlation was found between TNF-α level and age, whereas, a negative correlation exists between MMP-2, MMP-9, TNF-α level and IQ. In addition, correlation was found between MMP-2, MMP-9 levels and cognitive problems, TNF-α level and inattention, Hyperactivity/impulsivity.

DISCUSSION
Recent studies have suggested the link between many psychiatric disorders and inflammatory processes such as schizophrenia and mood disorders. Despite hundreds of studies conducted on ADHD, yet, its pathogenesis remains a controversial issue among researchers & the contribution of inflammation is still unclear. The exact role of MMP-2 in ADHD remains ambiguous due to lack of data in various studies, however, few studies are available on the relation between MMP-9, TNF-α and ADHD but none of them was conducted in Egypt. So, it seems necessary to us to

| Table 1. Comparison between patient and control group regarding various parameters, MMP-2, MMP-9 and TNF-α levels |
|----------------|----------------|----------------|----------------|----------------|
|                | Patient group  | Control group  | t test         | P value        |
| (N=30)         | (N=30)         |                |                |                |
| Age(mean ± SD) | 9.37 ± 1.88    | 9.20 ± 1.75    | t = 0.3625     | 0.7183         |
| Sex            |                |                |                |                |
| Male           | 19             | 18             | X²=0.07        | 0.79           |
| Female         | 11             | 12             |                |                |
| WISC(mean ± SD)|                |                |                |                |
| Total IQ       | 96.80 ± 3.24   | 97.80 ± 1.75   | t = 1.4874     | 0.1423         |
| Verbal IQ      | 96.00 ± 3.25   | 97.1 ± 2.03    | t = 1.5723     | 0.1213         |
| Performance IQ | 96.70 ± 2.37   | 97.70 ± 2.22   | t = 1.6867     | 0.0970         |
| MMP-2(pg/ml)(mean ± SD) | 648.50±81.94   | 344.13±32.02   | t = 18.9499    | <0.0001        |
| MMP-9(pg/ml)(mean ± SD) | 143.00±16.98   | 102.90±4.13    | t =12.5686     | <0.0001        |
| TNF-α(pg/ml)(mean ± SD) | 345 ± 7.1      | 202 ± 22.3     | t =33.4677     | <0.0001        |
| ADHD subtype   |                |                |                |                |
| (N,%)          | Hyperactive/impulsive | 16(53.3%)   |                |                |
|                | Inattentive     | 6(20%)         |                |                |
|                | Combined        | 8(26.7%)       |                |                |
Table 2. Comparison between MMP-2 and MMP-9, TNF-α level in subtypes of ADHD

<table>
<thead>
<tr>
<th></th>
<th>Hyperactive/Impulsive (N=16)</th>
<th>Inattentive (N=6)</th>
<th>Combined (N=8)</th>
<th>F test</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMP-2 (pg/ml)(mean ± SD)</td>
<td>157±15.38a</td>
<td>118.17±1.94b</td>
<td>133.07±14.24c</td>
<td>20.63</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>MMP-9 (pg/ml)(mean ± SD)</td>
<td>713.75±38.47a</td>
<td>571.17±8.61b</td>
<td>621.00±44.90c</td>
<td>39.13</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>TNF-α (pg/ml)(mean ± SD)</td>
<td>357±7.4a</td>
<td>341±1.6b</td>
<td>345±2.3c</td>
<td>22.61</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Same letter means non-significant difference, while different letter means high significant difference at (P <0.001).

carry out this study to explore the eventual role of MMP-2, MMP-9 and TNF-α as possible markers of ADHD.

In our study, WISC assessment of IQ in both ADHD group and control group was statistically non-significant. In many studies, intellectual ability of children with ADHD was investigated & discrepancies between results were obtained. Although, some studies revealed differences in cognitive variables between ADHD children compared with healthy ones, yet, many studies were in accordance with our study, amongst which the study of Naglieri et al. who examined the relation between WISC and the scores of Conners’ Scale (parent form) and found non-significant correlation23. Also, Kaplan et al. tested 63 ADHD children with WISC & found the majority of ADHD children to have the average range of scoring 24.

In our study, There was high statistical significant difference between ADHD group and control group regarding MMP-2, MMP-9 & TNF-α levels. Our result was in accordance with the study of Kadziela-Olech et al. who studied a group of 37 boys with ADHD and found the level of MMP-9 was associated significantly with symptoms of ADHD25. Another study done by Soltanifar et al. on 20 children aged between 2-10 were diagnosed with ADHD revealed an increase in the level of TNF-α in these children than in the control group 26. These findings indicate a correlation between the risk of ADHD occurrence and inflammatory process.

To the contrary to our study, Oades et al. detected no differences in serum TNF-α in 21 ADHD treatment-naïve patients compared to the control group, however, they found significant differences between 14 medicated children and the non-medicated ones27. A larger sample group is mandatory to reach a final conclusion.

In our study, we also correlated MMP-2, MMP-9 & TNF-α levels with subtypes of ADHD. The highest level were found among the Hyperactive/impulsive subtype. The same result was obtained by Kadziela-Olech et al. who found a correlation between serum MMP-9 and impulsivity25. Also, Cortese et al. assessed symptoms of ADHD in 52 obese children/adolescents & revealed a significant correlation between TNF-α & hyperactivity/impulsivity 28.

This can be explained by the fact that inflammatory markers have been proved to be one of the key markers linked to impulsive behavior 29. In addition, many studies have established a correlation between impulsivity and inflammatory processes 30, 31.

In our study, by comparing between subtypes of ADHD, no significant difference was found regarding total, verbal, performance IQ, however, a high statistical significant difference was found in all subscales of Conners’ scale.

These findings were similar to that of Oner et al. who evaluated 537 ADHD patients aged between 6-15 and both combined and inattentive subtypes, using WISC, they found that total and performance IQ were not significantly different 32. Moreover, similarly to our result, they found that Verbal IQ was higher among the inattentive subtype, but also not statistically significant.

Also, in the study of Grizenko et al. ADHD children, Combined/Hyperactive versus Inattentive subtypes were evaluated & no IQ difference was found between both groups33. This can be explained by the fact that relation between
Table 3. Comparison between subtypes of ADHD regarding WISC, Conner’s test

<table>
<thead>
<tr>
<th>Control</th>
<th>Hyperactive /Impulsive (N=16)</th>
<th>Inattentive (N=8)</th>
<th>Combined (N=16)</th>
<th>F test</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>WISC(mean ± SD)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total IQ</td>
<td>97.94 ± 1.81</td>
<td>98.00 ± 1.79</td>
<td>97.38 ± 1.77</td>
<td>0.3</td>
<td>0.73</td>
</tr>
<tr>
<td>Verbal IQ</td>
<td>98.00 ± 1.86</td>
<td>98.67 ± 1.03</td>
<td>96.63 ± 2.56</td>
<td>2.1</td>
<td>0.14</td>
</tr>
<tr>
<td>Performance IQ</td>
<td>97.44 ± 2.48</td>
<td>98.50 ± 1.87</td>
<td>97.63 ± 2.00</td>
<td>0.48</td>
<td>0.62</td>
</tr>
</tbody>
</table>

Conners’ scale:
- Cognitive problems: 70.69±11.25 vs. 78.83 ± 5.98 vs. 83.13 ± 6.25, F = 5.17, P = 0.012
- Hyperactivity: 84.94 ± 7.11 vs. 57.50 ± 3.26 vs. 81.13 ± 5.73, F < 0.0001
- Inattention: 54.93 ± 1.81 vs. 79.50 ± 7.05 vs. 77.25 ± 7.04, F = 67.8, P < 0.0001
- Liability: 63.69±11.25 vs. 53.00 ± 5.37 vs. 73.13±12.75, F = 5.91, P = 0.007
- Hyperactivity impulsivity: 77.88 ± 5.81 vs. 53.55 ± 6.52 vs. 76.38±12.31, F = 20.76, P < 0.0001

Table 4. Correlation between MMP-2, MMP-9 and TNF-α level and various parameters

<table>
<thead>
<tr>
<th>MMP-2</th>
<th>MMP-9</th>
<th>TNF-α</th>
</tr>
</thead>
<tbody>
<tr>
<td>R</td>
<td>P value</td>
<td>R</td>
</tr>
<tr>
<td>Age</td>
<td>0.16</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>IQ</td>
<td>-0.39</td>
<td>&lt; 0.001*</td>
</tr>
<tr>
<td>Cognitive problems</td>
<td>0.347</td>
<td>&lt; 0.001*</td>
</tr>
<tr>
<td>Hyperactivity</td>
<td>0.093</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Inattention</td>
<td>0.03</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Liability</td>
<td>0.053</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Hyperactivity impulsivity</td>
<td>0.064</td>
<td>&gt; 0.05</td>
</tr>
</tbody>
</table>

*High significance, P value < 0.001

the cognitive problems and behavioral difficulties in ADHD is bidirectional.

In our study, TNF-α level was positively correlated with age, whereas, no correlation was found between MMP-2, MMP-9 and age. Many studies suggest the effect of age on cytokines expressiveness. A study of 37 children aged between 1 and 17 years revealed that TNF-α concentration had a positive association with age. However, similarly to our result, Kadziela-Olech et al. [25] didn’t prove any relation between MMP-9 level and age in his study, to the contrary, Bonnema et al. found that MMP-2 and age were related positively whereas a negative relation exists between MMP-9 and age. Our study also revealed a negative correlation between MMP-2, MMP-9, TNF-α level and IQ.

Kudo et al. investigated the relation between MMP9 and cognitive function and found significant negative relation between MMP9 levels and verbal, performance and total IQ. Also, Jung et al. investigated the relation between intelligence and cytokines and found a negative association between TNF-α and vocabulary and full-scale scores.

A correlation was found between MMP-2, MMP-9 levels and cognitive problems. MMPs have been shown to have a vital role in various pathological as well as physiological processes in the brain particularly those influencing memory and the process of learning. MMP-9 was found to be necessary for hippocampus-dependent learning, as well as amygdala-dependent positive conditioning, interestingly, in human cognition.

Also, our study revealed a correlation between TNF-α and inattention, Hyperactivity impulsivity.
Recent studies show the role of TNF-α on the functions of the brain. It was found that the A allele of TNF-α affects cognitive functions. Also, TNF-α have a role in neurodegeneration, high TNF-α is associated with cognitive decline.

Moreover, Kim et al. found that TNF-α has strong correlation with frontal theta activity reflecting the presence of frontal dysfunction which is related to the impulsive behavior. Also, a recent study was held by Gassen et al. they figured out a correlation between difficulties in making decision as a result of impulsivity and immediate gratification and the release of cytokines including TNF-α. Their results point to the role of the immune system in impulsivity bringing new perspectives to future researchers.

CONCLUSION

ADHD is a chronic disorder affecting the developmental process in children. Though, scientific studies have enormously increased, yet, the underlying mechanism of ADHD remains elusive. So far, our study illustrates the co-occurrence of inflammatory process and ADHD, but further studies on larger sample are needed to reach a more definitive conclusion.

ACKNOWLEDGEMENTS

The authors express their appreciation to all children and their parents sharing in the study.

Conflict of interests

The authors declare that they have no conflict of interests.

Funding

There is no source of funding for the research.

REFERENCES


