



# Assessment of Serum Levels of Iron and Zinc in Children with ADHD Compared to Healthy Controls: A Case-Control Study

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## Abstract

**Background:** Attention deficit hyperactivity disorder (ADHD) is one of the most prevalent psychiatric disorders in both developed and developing countries. Deficiency of vital elements such as iron and zinc is thought to interrupt the function of dopaminergic pathways.

**Objectives:** This study aimed to assess the serum levels of iron and zinc among children with ADHD who referred to a psychiatric clinic in Mashhad, compared to healthy controls.

**Methods:** This case-control study was conducted on 51 subjects (36 ADHD cases and 15 healthy controls) aged 6 - 12 years. The serum levels of ferritin and zinc, total iron-binding capacity (TIBC), and hemoglobin were assessed and compared between the groups. P values of less than 0.05 were considered statistically significant.

**Results:** The mean age was  $7.8 \pm 2.12$  and  $8.4 \pm 3.11$  years in ADHD and control children, respectively. There were significant differences between the groups regarding mean corpuscular volume (MCV) ( $P = 0.003$ ), mean corpuscular hemoglobin concentration (MCHC) ( $P = 0.002$ ), and zinc level ( $P = 0.015$ ). However, no significant difference was observed in hemoglobin, iron, TIBC, and ferritin between the two groups ( $P > 0.05$ ).

**Conclusions:** The levels of serum iron and ferritin did not differ significantly between children with and without ADHD. Surprisingly, we found the serum zinc level to be higher in ADHD children than in healthy controls. Further studies with larger sample sizes are needed to draw reliable conclusions.

**Keywords:** Attention Deficit Hyperactivity Disorder, DSM-IV-TR criteria, Iron, Ferritin, Zinc

## 1. Background

The literature suggests a relationship between the symptoms of attention deficit hyperactivity disorder (ADHD) and the serum concentration of metals such as zinc (1). Zinc deficiency has been reported to play a role in the pathophysiology of ADHD (2). Besides, zinc is an essential element in the production of melatonin, the hormone that regulates dopamine function and is important in the treatment of ADHD (1).

## 2. Objectives

This study aimed to assess the serum levels of iron and zinc in children diagnosed with ADHD, compared to matched controls.

## 3. Materials and Methods

This case-control study was carried out on 51 children who referred to the Child and Adolescent Psychiatry Clinic of Ibn-e-Sina Psychiatric Hospital in Mashhad, Iran, from March 2014 to April 2015. The study was approved by the Ethics Committee of Mashhad University of Medical Sciences (approval code: IR.MUMS.REC.1391.96).

Due to the lack of studies in the region on similar populations, we performed this study as a pilot study and the sample size was determined to be 40 subjects in each group. However, due to the lack of cooperation and poor compliance, several parents who initially consented that their children participate in the study refused to give blood samples and thus, we had a high rate of dropouts. Eventually, 36 ADHD children (case group) and 15 healthy subjects (control group) were recruited using the simple non-

random convenience sampling method.

The case group included 36 ADHD children aged 6 to 12 years who referred to the Child and Adolescent Psychiatry Clinic of Ibn-e-Sina Psychiatric Hospital in the study period. They were diagnosed with ADHD based on the DSM-IV criteria by a child and adolescent psychiatrist. All patients in the case group were newly diagnosed cases of ADHD and were included only if they had not been under any treatment for ADHD before referring to us. The control group was selected from among the children of Ibn-e-Sina Hospital staff who did not have any mental disorders according to interviews with the child and adolescent psychiatrist. The subjects in the control group were matched to those in the case group in terms of age, gender, and family history of anemia.

The exclusion criteria were having any documented mental or physical illness (such as autism, depression, malnutrition, cardiovascular diseases, organic brain disorders, seizures, etc.) and taking supplementary drugs during the month before the study. Mental disorders other than ADHD were excluded by visiting the child and adolescent psychiatrist thorough examination and interviews with patients.

Parents completed written consent forms for entering the study. A demographic information checklist was completed by the researcher for each participant. The checklist collected data regarding developmental history, complete drug history especially for supplementary drugs, complete family history especially for anemia in first-degree relatives, and the number of children in the family. Then, all the subjects were referred to a single specific laboratory, where a single operator obtained 5-ml blood samples from their brachial veins at the same time of the day in the same standard conditions. The samples were analyzed to measure the serum concentration of zinc by an atomic absorption spectrophotometer (model 3030, Perkin Elmer instruments, Shelton, CT, USA) using the heated graphite atomization technique. The ferritin concentration was measured by radioimmunoassay. To analyze the normal or deficient status of different variables, standard cutoff points were used to analyze the data according to Nelson textbook (3).

Data analysis was performed using SPSS version 11.5 software (IBM Statistics, Chicago, IL, USA). The Kolmogorov-Smirnov test was used to assess the normal distribution of the data. The independent samples *t* test, Fisher exact test, and chi-square test were used, as appropriate, to compare different variables between the study groups. *P* values of less than 0.05 were considered statistically significant.

#### 4. Results

Overall, 51 subjects (36 ADHD cases and 15 controls) were studied. The ADHD group included 30 boys and 6 girls with the mean age of  $7.8 \pm 2.12$  years while the control group included 9 boys and 6 girls with the mean age of  $8.4 \pm 3.11$  years. The groups did not differ significantly in terms of age and sex ( $P > 0.05$ ).

The results of the laboratory study are compared between the groups in Table 1. As seen, the mean concentration of zinc was significantly higher in the ADHD group than in the control group ( $P = 0.015$ ). After careful evaluation of drug history, we found 13 subjects (36.1%) in the ADHD group and one subject (6.7%) in the control group who took multivitamins/minerals, folic acid supplements, and Ritalin in the six-month period before the study. After excluding those subjects from the analysis, the mean serum levels of zinc were  $82.11 \pm 3.69 \mu\text{g/dL}$  in the case group and  $73.15 \pm 8.17 \mu\text{g/dL}$  in the control group, which still indicated a higher zinc level in ADHD children, but the difference did not remain significant ( $P = 0.064$ ).

#### 5. Discussion

Ferritin, as a reliable indicator of iron storage in tissues such as the brain, has been linked to ADHD regardless of anemia status (4). Sever et al. assessed the relationship between iron deficiency and ADHD and reported that 5 mg/kg iron supplement in 30 days can increase serum ferritin and decrease ADHD symptoms (5). We found no significant difference in ferritin levels between the ADHD and control groups. This is consistent with the results of similar studies by Menegassi et al. and Donfrancesco et al. (6, 7). One possible cause of this inconsistency might be the medications. Psychostimulants or other psychotropic treatments can affect appetite and subsequently the ferritin level. Juneja et al. did not assess recent medications used by patients. Thus, the mentioned difference in the ferritin level can possibly be due to using psychostimulants. Besides, Menegassi et al. reported lower ferritin levels in ADHD children under treatment with stimulants (6). The length of medication use is also important. Both Konofal et al. and Menegassi et al. studies excluded subjects who received prior psychostimulants during the past two months (4, 6). This period might not be enough for removing the effects of drugs on micronutrient levels.

Another possible reason for this inconsistency might be the difference in ferritin measurement techniques between our study and other studies. Moreover, the lower sample size in our study than in the study by Konfol et al. who studied 53 ADHD children and 27 controls might have been a cause of inconsistency (4).

**Table 1.** The Results of Laboratory Tests in the Case and Control Groups<sup>a</sup>

Variable	ADHD	Control	Total	P Value <sup>b</sup>
Hb, g/dL	13.0 ± 1.03	13.2 ± 0.63	13.1 ± 0.92	0.558
Iron, µg/dL	82.9 ± 35.26	73.2 ± 35.90	80.0 ± 35.38	0.374
Ferritin, ng/mL	43.4 ± 28.60	37.8 ± 22.91	41.8 ± 26.95	0.506
Zinc, µg/dL	85.1 ± 12.59	74.8 ± 15.09	82.1 ± 14.05	0.015

<sup>a</sup>Values are expressed as mean ± SD.

<sup>b</sup>Independent samples *t*-test was used and P values of less than 0.05 were considered significant.

Surprisingly, we found that the mean serum zinc level was significantly higher in ADHD subjects than in controls. Even after the exclusion of 14 subjects who received supplementary drugs or Ritalin during the past six months, the zinc level was higher in ADHD patients than in controls; however, the difference became insignificant.

Incongruently, nearly all previous studies reported lower levels of zinc in ADHD patients than in healthy subjects (8, 9). For instance, Bekaroglu et al. found that the mean level of zinc was significantly higher in ADHD patients (60.6 ± 9.9 mcg/dL) than in controls (105.8 ± 13.2 mcg/dL) and concluded that zinc might be involved in the pathophysiology of ADHD (8). Arnold et al. found a significant negative relationship ( $r = -0.45$ ,  $P = 0.004$ ) between the zinc level and the score of Conner's Continuous Performance Test, an inattention symptom score that is scored by teachers and parents (9).

In our study, the level of hemoglobin was not significantly different between the groups while MCV was significantly higher in controls than in ADHD patients. Among the ADHD patients in our study, only were two anemic patients (hemoglobin below normal level), both of whom had normal iron and ferritin. Congruently, Oner et al. found no relationship between the severity of ADHD and the level of hemoglobin and MCV (10). The relationship between ADHD and different indicators of anemia needs to be assessed by further studies with the appropriate sample size.

Our major limitation was the small sample size, especially in the control group. The main reason for this problem was the lack of cooperation and poor compliance of parents. We had fewer subjects in the control group because their parents thought their healthy children do not need invasive procedures such as blood sampling and it was hard to convince them for taking blood samples. Besides, despite we excluded subjects who took medications one month before the study, we observed that the serum zinc level was relatively affected by medications taken in the six months before the study.

In conclusion, based on our findings, serum iron and ferritin levels did not differ significantly between healthy

controls and ADHD patients. However, more studies with larger sample sizes are necessary to assess this link. Surprisingly, the zinc level was significantly higher in ADHD patients than in controls, which might have been affected by the long-term use of medications. Although we did not find significant differences in the levels of hemoglobin and the prevalence of anemia between the groups, since iron deficiency is a common cause of anemia, all anemic ADHD patients should be carefully assessed.

## Footnotes

**Authors' Contribution:** Farnaz Zahedi Avval studied the concept, collected the data and analyzed the blood samples, laboratory results and edited the manuscript. Atefeh Soltanifar studied the concept, the sampling and collected the data. Fatemeh Moharreri studied the sampling collected the data, and edited the manuscript. Maedeh Kamrani studied the sampling, collected the data, wrote the manuscript, and edited the manuscript. Mohamad Mahdi Mohamadi Rad studied the concept, obtained the consent forms, trained the participant, and edited the manuscript.

**Clinical Trial Registration Code:** It is not declared by the authors.

**Conflict of Interests:** The authors have no conflict of interest to declare.

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