# The Impact of Copper in Children with Attention Deficit Hyperactivity Disorder

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*During one year (september 2013 – september 2014), 50 children from the residential institution for children SOS Satele Copiilor Bucharest, where included in our research. The children were distributed in two groups: Group A which consisted of 25 children (12 girls, 13 boys) who were not diagnosed with attention deficit hyperactivity disorder (ADHD) and Group B which consisted of 25 children (14 boys, 11 girls) who were diagnosed with ADHD. Initially, the two groups were subjected at a psycho-diagnostic battery of tests, one of them being the Evaluating the Health State of children with ADHD questionnaire elaborated by us, particularly for this research, with the purpose of highlighting the direct link between the children health state and their ADHD symptoms. This study sought to determine the concentration of copper in urine samples from a group of children with ADHD and a group of normal children, to highlight the role of copper in the development of ADHD symptoms. Levels of copper concentration in urine samples from the two groups were processed by Student's t-Test. Statistical analysis showed that the arithmetic averages of copper concentration in urine samples, of the two groups do not differ significantly, so copper may be causing ADHD symptoms occur. The copper content in urine samples has been determined using atomic absorption spectrometry with graphite furnace.*

*Keywords: copper concentration in urine samples, attention deficit hyperactivity disorder (ADHD), Graphyte Furnace - Atomic Absorption Spectrometry (GF-AAS).*

During the last two decades the incidence of *attention deficit hyperactivity disorder (ADHD),* increased among children due to some favorable factors like food comprising, containing additives or lack of exercise [1]. But there are voice that said that these are also other factors, that can lead to ADHD such as the presence of heavy metals in situ environments.

On the other hand, ADHD is underestimated in medical establishments and often ignored in schools. Most of the times pupils suffering of ADHD are often marginalized because there is not an suitable method of identification and their integration within the educational community.

Often, kids suffering from ADHD get mislabeled and are seen as uneducated, disobedient and even cocky, therefore family and school blame each other and both sides wait for the other to solve the problem. These restless, aggressive, naughty and incapable of concentration kids are a nightmare for those who they get in contact with, have problems respecting rules and regulations and often get expelled from schools [2].

ADHD is often described as a controversial disorder. Some people claim ADHD is due to nowadays modern lifestyle or lack of education, others believe that it should be treated with specific attention and medication. In 2000 the *American Academy of Pediatrics* published for the first time clinical recommendations for diagnosis and ADHD evaluation, followed by treatment indications in 2001 [3].

The diagnosis is clinically established, being sustained by medical comprehensive, developed, educational and psychological evaluations.

The differential diagnosis between ADHD and other disruptive behavior disorders can sometimes be difficult. Most of ADHD symptoms expressed during kindergarten can be associated with difficulties in communication that can appear in other developmental disorders, some specific learning disabilities, anxiety, depression or behavioral disorders [4]. During childhood ADHD symptomatology becomes more obvious: constant movement of the lower limb, motor instability, impulsive speech, lack of awareness.

The medical evaluation is based on the identification of potential treatable conditions that can improve or worsen one's symptoms. The development and evaluation of ADHD disorder summons the beginning and evolution of symptoms, while the educational evaluation relies on the description of central symptoms which suppose educational history review, use of evaluation scales and list of medical symptoms. Nevertheless, evaluation scales and list of medical symptoms cannot differentiate ADHD from other development or comportamental disorders. *Pure ADHD* diagnosis is rather an exception than a rule [5].

The clinical picture of children diagnosed with ADHD is frequently complicated with other pathologies (in 85% of cases, ADHD comes along with other pathologies, 50% - 90% of cases present at least one pathology associated, 50% of the kids with ADHD are diagnosed with 2 other associated disorders) [6].

ADHD diagnosis is based on a pluridisciplinary evaluation, because of the variety of symptoms and the risk to be mistaken for other symptoms. Before establishing the ADHD diagnosis, the physician will take into

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consideration other differential diagnoses that can interfere with ADHD symptoms. The presence of different associated pathologies has been clinically and epidemiologically confirmed.

The association between ADHD and metabolic disorders of microelements such as lead, selenium, aluminum, copper and cobalt, described in the medical literature, may play an important role in the outbreak of the disease or in it's treatment [7].

Copper is an essential element for the existence of life. It can be found in small amount in a big variety of eatable such as: vegetables, nuts, cereals, avocado, mussels, meat (especially organs such as liver) and even in very small quantities in drinking water. Breast milk contains a high concentration of copper (400 - 800μg/L). Inside the human body copper plays a very important role in several cell mechanisms. Copper also represents a compound of some enzymes: cytochrome oxidase, monoamin oxidase, superoxide dismutase, tyrosinase and ascorbic oxidase. Ceruloplasmine transports approximately 70% of the total serum copper. This mineral is involved in the regulation of genes expression, mitochondrial functions, cell metabolism, growth of connective tissue and iron's absorption, stocking or metabolism. A third of the ingested copper is absorbed in the stomach, duodenum and jejunum. Copper absorption is enhanced by an acidic *pH* and it is diminished by calcium, zinc, iron; minerals that compete with iron regarding the absorption mechanism. Copper is a high concentration in water and food can become toxic, causing various health problems.

In case of metabolic disorders, the level of copper can increase and thus, generate restlessness and other neurological symptoms that can be found in ADHD patients. This group of symptoms is called Wilson's syndrome [8]. Low levels of ceruloplasmine, positive tests for penicillamine, high levels of hepatic copper and ATP7B genetic mutation confirm Wilsons's disease [9]. But, also low levels of zinc and copper have been reported in diets among ADHD patients [10].

A total lack of copper in one's diet could be associated with Alzheimer's disease, because copper along manganese, zinc and other minerals, participate in different enzymatic mechanisms involved in the protection against free radicals. The genetic potential of a child during his growth period and mental development can be compromised, even subclinicall, by lack of micronutrients, including copper. Behavior and mental dysfunctions are more likely to occur among children and teenagers with diminished nutritional status and can be rectified by suitable diets [11].

Regarding trials of patients with ADHD, the results are contradictory: some trials witnessed high levels of serum copper [12] while others haven't noticed any changes [13].

The purpose of this trial was to determinate the influence of copper urinary levels of children diagnosed with ADHD, to compare the results with those obtained from a control group and to highlight the sex influence.

The measurements of copper concentration in urine samples were made by Atomic Absorption Spectrometry with Graphite Furnace [14].

## Experimental part

*Method and materials*

During years period (2013-2014), 50 children were selected from the *SOS Satele copiilor* orphanage and divided in a 2 groups, like as:

-*group A* - including 25 children without ADHD (12 boys and 13 girls);

-*group B* - including 25 children diagnosed with ADHD (14 boys and 11 girls).

Inclusion criteria of children in *group A* were the following: age between 7 and 15 years old.

Exclusion criteria of children in *group A* were the following:

-existence of psychiatrique diagnosis, mental disorders, autism, psychosis etc;

-existence of neurological chronic diseases, infantile cerebral palsy etc.

Inclusion criteria of children in *group B* were the following:

-age between 7 and 15 years old;

-ADHD diagnosis: hyperkinetic disorder, lack of awareness, aggressiveness.

-lenght of pharmacological treatment before group inclusion, $\leq 6$ months;

-possibility to follow ambulatory care;

Sex didn't represent a criteria selection.

Exclusion criteria of children in *group B*:

-children with ADHD who present other chronic pathologies that can affect health and induce neurosis, anxiety, organic disease;

-mental deficiency (mild, moderate, severe);

-lack of compliance;

-neurogenic language disorders.

10 mL urinary samples were collected from every patient included in the trial.

The apparatus used in specific analyses for measurement of copper concentration are:

-Atomic absorption spectrometer AAS-880-Varian [15];

-Graphite tube atomization GTA-100 [15];

-Programmable sample dispenser PSD [15];

-Watercooler Neslab CFT 22 [15];

-Dominick Hunter nitrogen generator [15].

-Reagents and specific instruments of an analytical toxicology: Argon - Gas cylinder, purity 99.9999%.

In order to measure copper levels concentrations in the onset of some specific ADHD symptoms, the following specific method of the analytical laboratory for urine sample preparation: in every to 10 mL of urine samples taken from each child participating at the trial, 1 mL $HNO_3$ 65% have been added. The solution is left for 20 min in test tubes and eventually centrifuged at 2500 rpm during 10 min [16-21].

The supernatant represents the matrix for to analysis using GF – AAS system. The GF-AAS spectral method was used for the determination of copper concentration in the injection matrix and it's presented in table 1 and table 2.

## Results and discussions

The average of copper concentration in urine samples of the entire group is 101.2 μg/L (standard deviation, SD = 42.85; mean standard deviation, MSD = 6.32). The average of copper concentration in urine samples recorded from the boys group is 102.5μg/L (SD = 44.61, MSD = 9.11), while the one from the girls group is 91.84μg/L (SD = 48.13, MSD = 9.83).

The distribution of the copper concentration in urine samples among the entire group are shown in figure *1.*

These results showed a small distribution of the values of the copper urinary levels. The normal copper concentration in urine samples for children aged between 0 – 15, represents a conversation topic between physicians. The upper normal limit of the copper concentration in urine samples is admitted at 120μg/L. After the age of 15, copper

**Table 1**
GENERAL PARAMETERS OF AAS SYSTEM FOR THE DETERMINATION OF COPPER

| Nr. | Parameters | Programming |
|---|---|---|
| 1. | Type of injection | Auto dilution |
| 2. | Type of calibration | Concentration |
| 3. | Type of measurement | Height peak |
| 4. | Standard replicates | 2 |
| 5. | Sample replicates | 2 |
| 6. | Smoothing | 9 |
| 7. | Wavelength | 327.4 nm |
| 8. | Slit width | 0.5 nm |
| 9. | Lamp current | 4 mA |
| 10. | Background correction | Yes |
| 11. | Standard 1 | 20 µg/L |
| 12. | Standard 2 | 60 µg/L |
| 13. | Standard 3 | 100 µg/L |
| 14. | Recalibration rate | 20 |
| 15. | Reslope rate | 10 |
| 16. | Reslope standard no. | 2 |
| 17. | Expansion factor | 1 |
| 18. | Concentration decimal places | 2 |
| 19. | Calibration algorithm | New Rational |
| 20. | Replicate % RSD limit | 5% |
| 21. | Correlation coefficient limit | 0.998 |
| 22. | Required detection limit | 1 µg/L |
| 23. | Instrument detection limit | 0.6 µg/L |
| 24. | Injected volume | 15 µL |
| 25. | Probe volume | 10 µL |
| 26. | Dilution coefficient | 2 |

**Table 2**
GENERAL PARAMETERS OF GF SYSTEM FOR THE DETERMINATION OF COPPER

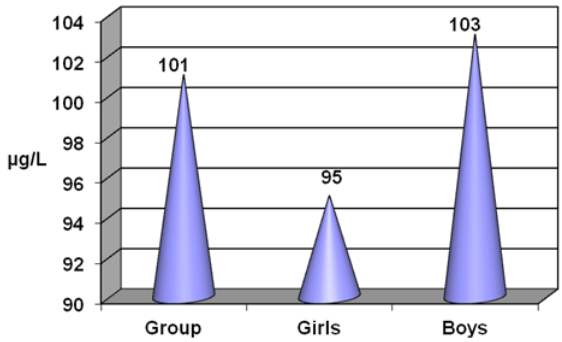
| PARAMETER/ STEP | Temp. (°C) | Time (s) | Gas | Flow (ml/min) | Read | Memory |
|---|---|---|---|---|---|---|
| **Step 1** | 40 | 5 | **N** | 3 | - | - |
| **Step 2** | 85 | 5 | **N** | 3 | - | - |
| **Step 3** | 85 | 5 | **N** | 3 | - | - |
| **Step 4** | 95 | 40 | N | 3 | - | - |
| **Step 5** | 120 | 10 | N | 3 | - | - |
| **Step 6** | 120 | 10 | Ar | 3 | - | - |
| **Step 7** | 800 | 5 | Ar | 3 | - | - |
| **Step 8** | 800 | 1 | Ar | 3 | - | - |
| **Step 9** | 800 | 2 | - | 0 | - | Yes |
| **Step 10** | 2300 | 1.1 | Ar | 0 | Yes | Yes |
| **Step 11** | 2300 | 2 | Ar | 0 | Yes | Yes |
| **Step 12** | 2500 | 0.1 | N | 3 | - | - |
| **Step 13** | 2500 | 2 | N | 3 | - | - |
| **Step 14** | 40 | 21.3 | N | 3 | - | - |
| **Step 15** | 40 | 5 | N | 0 | - | - |

concentration in urine samples higher than 80µg/L indicate the possibility of Wilson's disease [21]. Sex analysis indicates10.66µg/L less copper concentration in urine samples for the girls than for the boys. Nevertheless, the *Student's t-Test* shows that both group averages of the copper concentration in urine samples aren't significantly different, from a statistical perspective ($p>0.8$) . None of the recorded values exceed the upper limit of 120µg/L.

Among *group A*, the average of the copper concentration in urine samples is 99.06µg/L (SD = 43.95, MSD = 9.37). The average of copper concentration in urine samples recorded from the boys group is 94.48µg/L (SD = 43.05; MSD = 12.98), while the one from the girls group was 103.65µg/L (SD = 46.43; MSD = 14.00). The results among the group diagnosed with ADHD are shown in figure 2.

These results show a small distribution of the values of the copper concentration in urine samples. Sex analysis indicates that, for the girls copper concentration in urine samples have 9.17µg/L, more than those of the boys. The odd *Student's t-Test* indicates that both averages of the copper concentration in urine samples aren't significantly different ($(p>0.6)$).

Among *group B*, the average of the copper concentration in urine samples is 103.13µg/L (SD= 42.68; MSD = 12.9). Sex analysis indicates that boys copper concentration in urine samples is 109.3µg/L (SD = 46.47; MSD =12.9)




ig. 1. Distribution of the copper concentration (µg/L) in urine samples among the entire group.

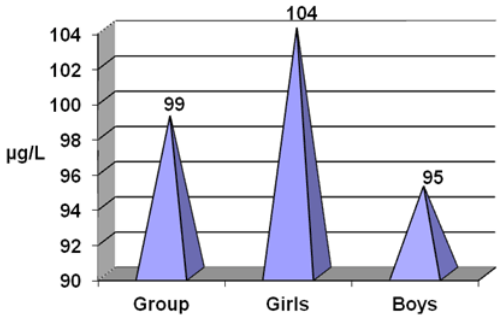



Fig. 2. Distribution of the copper concentration (µg/L) in urine samples among the group A.

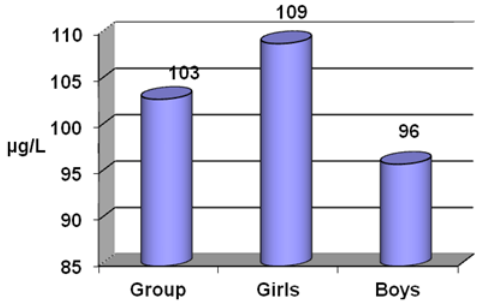



Fig. 3. Distribution of the copper concentration (µg/L) in urine samples among the group B

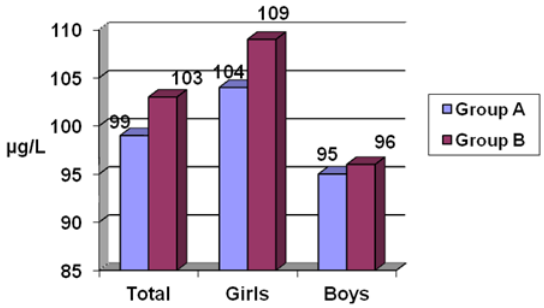



Fig.4. Distribution of the copper concentration (µg/L) in urine samples between subgroups.

while the girls one is 95.83µg/L (SD = 38.59; MSD = 11.63).

Figure 3 presents the results which showed a small distribution of the values of the copper concentration in urine samples. Sex analysis, indicates that girls copper urinary level has 13.47µg/L less than the boys one. The odd Student's t-Test indicates that both averages of the copper concentration in urine samples aren't significantly different ($p>0.6$).

The difference between the two groups, regarding the average of the copper concentration in urine samples, is 4.07µg/L. Sex analysis indicates that the boys of group B average of the copper concentration in urine samples has 14.82µg/L, more than the one from the boys of *group A*. The girls of *group A* average of the copper concentration in urine samples has 7.82µg/L, more than the one of the girls from group B. The results are shown in figure 4.

The odd Student's t-Test indicates the following:

-both group averages of the copper concentration in urine samples aren't significantly different ($p>0.7$). The difference between both group averages is 4.07µg/L;

-both groups of boys averages of the copper concentration in urine samples aren't significantly statistically different ($p>0.4$). The difference between the two averages is 14.82µg/L;

-both groups of girls averages of the copper concentration in urine samples aren't significantly statistically different ($p>0.6$). The difference between the two averages is 7.82µg/L.

There isn't any significant difference between the two subgroups averages of the copper concentration in urine samples.

## Conclusions

Although the levels of copper in urine samples from the control group is 4 µg/L higher than give a group of children with ADHD, this difference is not statistically significant.

The concentration of copper for boys in urine samples is higher than 5µg/L obtained in the control group comparative to the group with ADHD. Neither this difference is statistically significant.

Girls copper concentration in urine samples is higher than 1 µg/L in the control group compared to the group with ADHD. This difference is not statistically significant.

In the group of children with ADHD, the levels of copper in urine samples is higher by 9 µg/L in the control group compared to the group with ADHD. This difference is not statistically significant, however.

The children's nutritional status of the two groups (A and B) was identically and the copper concentrations in urine samples are the same in both groups.

At the same time, there is no correlation between copper concentration in urine samples and ADHD diagnosis, according with patient's sex.

GF- AAS method for determining copper concentration in urine samples is sensitive, reproducible and easier method to be used, with acceptable cost price.

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