Zn/Cu Levels in the Field of Autism Disorders: A Systematic Review and Meta-analysis

How to Cite This Article: Sayehmiri F, Babaknejad N, Bahrami S, Sayehmiri K, Darabi M, Rezaei-Tavirani M. Zn/Cu Levels in the Field of Autism Disorders: A Systematic Review and Meta-analysis. Iran J Child Neurol. Autumn 2015;9(4):1-9.

Abstract

Objective

There is probably a relationship between zinc/copper concentration in individuals with autism. The present review was written to estimate this probability by using meta-analysis method.

Materials & Methods

In this meta-analysis of Fixed Effect Model, by searching PubMed, Scopus and Google scholar databases, 11 articles were selected and verified published in 1978 to 2012. *I*² statistics were calculated to examine heterogeneity. The information was analyzed by R and STATA Ver. 11.2.

Results

Due to non-uniform measurement methods of Zn/Cu concentrations, the concentration of these elements was measured in various subgroups (plasma, hair and general) in both study cases and controls. There was a significant statistical difference between plasma OR=0.252 (95% CI: -0.001-0.504) and hair OR=0.27(95% CI: 0.059-0.481, \( P=0.01 \)) concentrations of Zn/Cu statuses between controls and autistic patients. Using a Fixed Effects Model, the overall integration of data from the two groups was significant as risk factor OR=0.31(95% CI:0.16-0.46, \( P=0.001 \)).

Conclusion

Significant correlation existed between Zn/Cu levels and the development of autistic disorders in general analysis. Therefore, Zn/Cu levels could be mentioned as a pathogenesis reason of autism spectrum disorders.

Keywords: Zinc; Cupper; Concentration; Autism spectrum disorders; Meta-analysis

Introduction

Autism is a long-term disability characterized by social deficits, social imagination, language impairments, and repetitive behaviors (1). It is rare, but data indicates the prevalence of this disorder is 20/10,000 births (2). Autism is a neural development disorder with characteristics of impaired social interaction, verbal or non-verbal communication disorders, and repetitive restricted behavior. In most cases, the diagnostic criteria involve early diagnosis of symptoms before the child is three year old (3). This disease alters the connections and organization of synapses and nerve cells, which in turn alters information processing in the brain. Nonetheless, the way this phenomenon occurs is not yet fully understood (4).
Despite having complex and vague genetics, autism has a strong genetic root, which can be described as scarce mutations or rare combinations of common variants of genes (5). In very few cases, agents, which cause birth defects, are considered responsible for autism (6). There are, however, controversies about other proposed environmental factors such as exposure to heavy metals, pesticides, or early vaccinations (7). The vaccine hypotheses are biologically implausible and lack convincing scientific evidence (8). The prevalence of autism is about 1–2/1,000 people worldwide, and occurs about four times more often in boys than girls (9). The environmental factors, which can prove important in future research, include particular foods, infectious diseases, heavy metals, solvents, diesel exhaust, PCBs, phthalates and phenols used in plastic products, pesticides, brominated flame retardants, alcohol, smoking, illicit drugs, vaccines (9) and prenatal stress (10).

Zinc levels in plasma, hair, and nails in autistic patients have been measured which were not normal (11). The reported findings are not the same, so that in some cases zinc deficiency has been reported in individuals diagnosed with autistic spectrum disorders, but in other studies, autistic children had similar plasma zinc levels to neurotypical children (2, 11, 12). Plasma copper values were also determined on each blood specimen, and low plasma zinc was associated with an increased plasma copper level (13). Probably, there is a correlation between plasma, hair and teeth zinc and copper, and severity of symptoms associated with autism (14-17).

Several studies have suggested a disturbance in the copper (Cu) and zinc (Zn) metabolism in ASDs (autism spectrum disorders) (6,8,14,16,18-27). Zinc deficiency, excess Cu levels, and low Zn/Cu ratio are common in children diagnosed with ASD.

Due to the lack of uniform results and considering the impact of this element in symptoms and improvement of this disease, this meta-analysis study was conducted.

**Martials & Methods**

**Study method**

A systematic review and Meta-analysis using PubMed, Google scholar and Scopus databases was undertaken to identify any study published in 1978-2012, in English, reporting Zn and Cu concentrations in individuals with autism. Databases were searched using the keywords ‘Autism Spectrum Disorders’, ‘autism’, ‘Zinc concentration’, ‘Copper concentration’, ‘Copper/Zinc concentration’, ‘Cu/Zn concentration’, ‘trace element concentrations’ and their combinations. Eligible studies, including epidemiologic manuscripts, were analyzed for Zn and Cu levels in autistic patients by measuring the concentration of these elements in any of the following biological sample specimens: blood/ serum, nails, hair and teeth. All papers, with keywords presented in their titles or abstracts, were used in the initial list and other unrelated articles were eliminated.

Studies were excluded if they were not written in English; had insufficient data; if they were reviews; or if they were not epidemiologic studies. All the abstracts were reviewed and duplicates excluded. The following variables were extracted from each paper: sample characteristics (first author’s last name, year of publication, sample size, sample age, location), Zinc concentrations, Copper concentration, zinc to copper ratio, Mean difference, Zinc and Copper screening method and sample specimens (Figure 1).

**Statistical analysis**

Studies were combined based on the sample size, mean and standard deviation. The difference between the average variance of the normal distribution was calculated using the formula of two integrated variance. To assess heterogeneity of the studies, Cochran test and the I2 index were used. Due to significant heterogeneity in the studies, random effects model was used. To examine publication bias, Begg Plot and regressions method were used. P-value less than 5% was considered as a significant heterogeneity test. Sensitivity analyses were pre-specified. Statistical analyses were performed using STATA version 12.

**Results**

The initial search returned 60 citations. Of these, 29 studies were discarded after reviewing the abstracts while the full text of the remaining 21 citations was examined in more detail; among those, 11 were appropriate for inclusion in the meta-analysis. The standard unit for measuring Zn and Cu concentrations in many articles
was microgram per gram (µg/gr). However, all studies 1). Zn/Cu concentrations were measured in plasma, hair and total. Due to non-uniform measurement methods of Zn and Cu concentration, the levels of these elements were measured in various subgroups in both cases and controls. There was significance statistical difference between plasma OR=0.252 (95% CI: -0.001-0.504); hair and nail OR=0.27(95% CI: 0.059-0.481, P=0.01) and Zn/Cu statuses analysis between controls and autistic patients after sensitivity analysis and removal of Al-Farsi’s study. The overall integration of data from the two groups showed there was no significance statistical difference between Zn/Cu concentrations in autistic patients and healthy controls (0.25 (95% CI: 0.00 - 0.504)) by using a random effects model. However, the error is significant at 0.06 (P=0.051). Zn/Cu mean levels in healthy subjects were higher than autistic individuals were. The results obtained from the hair and nail has clarified that there was no significant difference of Zn/Cu concentrations between autistics and healthy subjects (P=0.13). In all the twelve studied analysis, which applied individual levels of Zn/Cu measured in plasma and hair, there was no significant difference in Zn/Cu levels between autistic patients and healthy individuals OR=-0.12(95% CI:-0.63 - 0.37, P=0.61). After deleting Al-farsi studies there was significant association between Zn/Cu and autism (OR=0.31(95% CI:0.16 - 0.46, P=0.000). Nonetheless, these differences were often weak and some studies showed positive results while others showed negative results (16-31).

Low Zn/Cu ratio is common in children diagnosed with ASD (21). Zinc is in a balance with Cu in the blood, and changes in these two trace elements are in an inverse relationship. This can, in large scale, be explained because of cytokine regulation of the metabolism of the two elements, with the same cytokines causing enhancement of the cellular uptake of Zn and enhancement of the production of ceruloplasmin in the liver. A low plasma Zn concentration is nearly always associated with a high serum Cu concentration. The normal Zn to Cu ratio in children and adults is close to 1:1, besides, the plasma Zn/serum Cu ratio may be used as a rapid method of determining the functional state of the metallothionein system (18, 19).

Low Zn/Cu ratios can be associated with total body Zn deficiency or accumulation of toxic metals, which can act as Zn-antagonists. Hg toxicity may be a major cause of MT dysfunction in children diagnosed with ASD, which may be reflected in the Zn/Cu ratio (19, 20). The toxic metals Hg and Cd, similar to that proposed above for oxidative stress due to genetic disturbances, might have opposite net effects on Zn and Cu metabolism, as enhanced MT induction in the liver might affect Cu excretion via the bile more than it affects the mobilization of this element from the liver to the blood, while for Zn it is the rate of mobilization to the blood which is more strongly affected (21).

The frequency of zinc deficiency, copper toxicity and low Zn/Cu ratio in children with autism spectrum disorders may indicate decrement in metallothionein system functioning attributed to MTs including the sequestration and dispersal of metal ions, primarily in zinc and copper homeostasis specifically in regulation of the biosynthesis and activity of zinc metalloproteins,
most notably, zinc-dependent transcription factors. A retrospective review of plasma zinc, serum copper and Zn/Cu was performed. The entire cohort’s mean zinc level was 77.2 microg dl (-1), mean copper level was 131.5 microg dl(-1), and mean Zn/Cu was 0.608, which was below the 0.7 cut-off of the lowest 2.5% of healthy children. The plasma zinc/serum copper ratio may be a biomarker of heavy metal, particularly mercury, toxicity in children with ASDs (22, 23).

Medical nutrition therapy and use of dietary supplements is a suggestion for curing this disease (26). However, many risk factors are included in the etiology of autism. One of them is essential elements deficiency. Therefore, it is important to determine the trace elements concentrations in humans to monitor and assess their impact on health (24, 25). The major limitation of this study is the conduct of a meta-analysis in the presence of high heterogeneity among the studies. Fixed effects model was used to try to mitigate the heterogeneity as an issue, and sensitivity analyses changed the results. Other limitations and weaknesses were commonly related to the methodology of reviewed studies. Some of these weaknesses are as follows:

1) Lack of a same method of measurement for the variances.
2) Selection of cases from women referred to the health centers compared to a random selection.
3) Lack of information about nutrition and lifestyle of participants.
4) Various kinds of screening methods and lack of same standard unit for measuring selenium concentrations in different articles.

In conclusion, this meta-analysis, which was based

---

**Fig 1. Study flowchart**

60 of study identified through Google scholar, PubMed and Scopus database searching

30 of records after duplicates removed

30 of records screened

6 of records excluded

24 of studies included in qualitative synthesis

13 excluded
Lack of enough information

11 of studies included in quantitative synthesis (meta-analysis)
on fixed effect model, indicated that in the etiology of autism, significant correlation existed between Zn/Cu levels based on sensitivity analysis by excluding al-Farsi et al. (19). Zn/Cu supplements can be used in randomized clinical trials for the nutritional therapy of autistic patients.

Acknowledgement
We would like to acknowledge the Students Researches Committee of the Ilam University of Medical Sciences for financial support of this project.

Conflict of interests: No conflict of interests.

Authors’ contribution
All authors contributed extensively to the work presented in this paper. Sayehmiri F and Babaknejad N designed the experiment; Darabi M and Bahrami S, exclude data; Sayehmiri K, Sayehmiri F analyzed and described the analytic model. Sayehmiri F and Babaknejad N wrote the manuscript. Tavirani M gave technical support and conceptual advice.

Fig 2. Forest plots for the hair and nail Zn/Cu statues difference between autistic patients and healthy individuals. The area of each square is proportional to the percentage weight of each individual study in the meta-analysis (CI 95%). In this chart studies are stored in order of year publication and author’s names, based on a fixed effects model.
Zn/Cu Levels in the Field of Autism Disorders: A Systematic Review and Meta-analysis

**Fig 3.** Forest plots for the plasma Zn/Cu statues difference between autistic patients and healthy individuals. The area of each square is proportional to the percentage weight of each individual study in the meta-analysis (CI 95%). In this chart studies are stored in order of year publication and author’s names, based on a fixed effects model.

**Fig 4.** Forest plots for the studies Zn/Cu statuses combination difference between autistic patients and healthy individuals. The area of each square is proportional to the percentage weight of each individual study in the meta-analysis (CI 95%). In this chart studies are stored in order of year publication and author’s names, based on a random effects model.
Zn/Cu Levels in the Field of Autism Disorders: A Systematic Review and Meta-analysis

Fig 5. Sensitivity analyses forest plots for the studies Zn/Cu statuses combination difference between autistic patients and healthy individuals. The area of each square is proportional to the percentage weight of each individual study in the meta-analysis (CI 95%). In this chart, studies are stored in order of year publication and author’s names, based on a fixed effects model.

![Forest plot of sensitivity analyses](image)

Table 1. Study characteristics.

| Study ID | Percentage weight | SMD (95% CI) | Weight |
|----------|-------------------|--------------|--------|
| Shearer T(1982) | -0.01 (-0.81, 0.79) | 4.75 |
| Wecker. L (1985) | -0.32 (-1.03, 0.39) | 5.70 |
| Jackson. M (1987) | 0.13 (-0.54, 0.80) | 6.28 |
| Adams. J (2006) | -0.02 (-0.43, 0.39) | 11.86 |
| Lakshmi Prriya. M.D (2011) | 0.69 (0.27, 1.10) | 11.84 |
| Bakurock busch. E (2011) | 0.67 (0.26, 1.09) | 11.85 |
| Russo. A. J (2011) | 0.62 (0.07, 1.17) | 8.38 |
| Russo. A. J (2011) | 0.30 (-0.21, 0.82) | 9.15 |
| Elsheshtaway. E (2011) | 0.48 (-0.02, 0.96) | 9.55 |
| Blaurock busch. E (2011) | 0.28 (-0.28, 0.83) | 8.21 |
| Adams. J. B (2011) | 0.07 (-0.33, 0.47) | 12.42 |
| Overall (I-squared = 35.7%, p = 0.113) | 0.31 (0.11, 0.50) | 100.00 |

NOTE: Weights are from random effects analysis.

Fig 6. Begg’s funnel plot for publication bias in the risk difference (RD) analysis. The diameter of each circle represents the weight in the metaanalysis.

![Begg's funnel plot](image)
### Table 1. Study characteristics.

| References | Year of publication | Country | City | Mean age | Case Control | Mean difference | type of Zinc and Copper measurement |
|------------|---------------------|---------|------|----------|---------------|----------------|-------------------------------------|
| 23         | 1978                | London  | 7-16 | 7-17     | 20 30 plasma | -1.162 -0.007 -0.584 | Atomic Absorption Spectroscopy |
| 26         | 1985                | USA     | 2-11 | 2-11     | 12 21 Hair   | -1.408 0.050 -0.679 | Atomic Absorption Spectroscopy |
| 25         | 1982                | USA     | 8    | 8        | 12 12 Hair   | -0.841 0.786 -0.014 | Atomic Absorption Spectroscopy |
| 28         | 2006                | USA     | 3-6  | 3-6      | 51 40 Hair   | -0.149 0.683 0.267 | Inductively Coupled Plasma–Mass spectrometry (ICP-MS) |
| 29         | 2011                | India   | 4-12 | 4-12     | 45 50 Hair   | -1.229 -0.390 -0.810 | Atomic Absorption Spectroscopy |
| 29         | 2011                | India   | 4-12 | 4-12     | 45 50 Nail   | -1.189 -0.354 -0.772 | Atomic Absorption Spectroscopy |
| 16         | 2011                | USA     | 38   | 42       | 73 16 Plasma | -0.831 0.254 -0.288 | ICP-MS |
| 16         | 2011                | USA     | 11.7±5.62 | 11.7 ± 5.62 | 79 18 Plasma | -0.801 0.226 -0.288 | ICP-MS |
| 30         | 2011                | Egypt   | 4.1 ± 0.8 | 4.1 ± 0.8 | 32 32 Hair   | -3.802 -2.343 -0.073 | Atomic Absorption Spectrophotometer |
| 31         | 2011                | Egypt   | 5.29±1.9 | 6.25 ± 2.39 | 25 25 Hair   | -1.464 -0.300 -0.882 | ICP-MS |
| 14         | 2011                | USA     | 5.16 | 5.16     | 55 44 Plasma | -0.543 0.340 -0.057 | ICP-MS |
| 19         | 2012                | Oman    | 3_14 | 3_14     | 27 27 Hair   | 0.899 2.113 1.506 | ICP-MS |

### References

1. Arnold LE, Farmer C, Kraemer HC et al. Moderators, Mediators, and Other Predictors of Risperidone Response in Children with Autistic Disorder and Irritability. J Child Adolesc Psychopharmacol 2010;20(2):83-92.
2. Karimzadeh P. Recent finding about etiology of autism. Rehabilitation 2000; 1(2):58-63.
3. American Psychiatric Association. Diagnostic and statistical manual of mental disorders: DSM-IV. 4th ed. Washington, DC: American Psychiatric Association; 2000. ISBN 0-89042-025-4. OCLC 768475353. Diagnostic criteria for 299.00 Autistic Disorder.
4. Levy SE, Mandell DS, Schultz RT. Autism. Lancet 2009;374 (9701):1627–38.
5. Abrahams BS, Geschwind DH. Advances in autism genetics: on the threshold of a new neurobiology. Nat Rev Genet 2008;9(5):341–55.
6. Arndt TL, Stodgell CJ, Rodier PM. The teratology of
Zn/Cu Levels in the Field of Autism Disorders: A Systematic Review and Meta-analysis

autism. Int J Dev Neurosci 2005;23(2–3):189–99.

7. Rutter M. Incidence of autism spectrum disorders: changes over time and their meaning. Acta Paediatr 2005;94(1):2-15.

8. Gerber JS, Offit PA. Vaccines and autism: a tale of shifting hypotheses. Clin Infect Dis 2009;48(4):456-61.

9. Newschaffer CJ, Croen LA, Daniels J, et al. The epidemiology of autism spectrum disorders. Ann Rev Public Health 2007; 28:235–58.

10. Kinney DK, Munir KM, Crowley DJ, Miller AM. Prenatal stress and risk for autism. Neurosci Biobehav Rev 2008;32(8):1519–32.

11. Cornish E. Gluten and casein free diets in autism: a study of the effects on food choice and nutrition. J Hum Nutr Dietet 2002; 15:261-268.

12. De Palma G, Catalani S, Franco A, Brighenti M, Apostoli P, et al. Lack of Correlation Between Metallic Elements Analyzed in Hair by ICP-MS and Autism. J Autism Dev Disord 2012; 42(3):342–353.

13. Halsted JA, Hackley BM, Smith JC. Plasma zinc and copper in pregnancy and after oral contraceptives. Lancet 1968; 2:278.

14. Adams JB, Romdalvik J, Ramanujam VM, Legator MS. Mercury, Lead, and Zinc in Baby Teeth of Children with Autism Versus Controls J Toxicol Environ Health A 2011;70(12):1046-51.

15. Blaurock-Busch E, Amin OR, Dessoki HH, Rabah T. Toxic Metals and Essential Elements in Hair and Severity of Symptoms among Children with Autism. Maedica (Buchar) 2012;7(1):38-48.

16. Russo AJ. Increased Copper in Individuals with Autism Normalizes Post Zinc Therapy More Efficiently in Individuals with Concurrent GI Disease. Nutrition and Metabolic Insights 2011;4: 49–54.

17. Russo AJ, Bazin AP, Bigega R, et al. Plasma Copper and Zinc Concentration in Individuals with Autism Correlate with Selected Symptom Severity. Nutr Metab Insights 2012;28(5):41–7.

18. Van Weyenbergh J, Santana G, Oliveira DA, et al. Zinc/copper imbalance reflects immune dysfunction in human leishmaniasis: and ex vivo and in vitro study. BMC Infect Dis 2004; 4: 50.

19. Al-Farsi, Waly MI, Al-Sharbati MM, et al. Levels of Heavy Metals and Essential Minerals in Hair Samples of Children with Autism in Oman: a Case–Control Study. Biol Trace Elem Res 2013;151(2):181-6.

20. Aschner M, Syversen T, Souza DO, Rocha JBT. Metallothioneins: mercury species-specific induction and their potential role in attenuating neurotoxicity. Exp Biol Med 2006; 231: 1468–1473.

21. Geir Bjørlund. The role of zinc and copper in autism spectrum disorders. Acta Neurobiol 2013;73: 225–236.

22. Faber S, Zinn GM, Kern JC, Kingston HM. The plasma zinc/serum copper ratio as a biomarker in children with autism spectrum disorders. Biomarkers 2009;14(3):171-80.

23. Jackson MJ, Garrod PJ. Plasma zinc, copper and Amino acids levels in the blood of autistic children. J Autism Child Schizophr 1978;8(2):203-8.

24. Russo AJ. Decreased Serum Cu/Zn SOD in Children with Autism. Nutr Metabol Insights 2009, 2, 27-35.

25. Shearer TR, Larson k, Neuschwander, Gedney B. Minerals in the hair and nutrient intake of autistic children. J Autism Develop Disord 1982;12:1

26. Wecker L, SHifra B, Miller S, et al. Trace element concentrations in hair from autistic children. J Ment Defic Res 1985; 29:15-22.

27. Xia W, Zhou Y, Sun C, Wang J, Wu L. A preliminary study on nutritional status and intake in Chinese children with autism. Eur J Pediatr 2010;169(10):1201-1205.

28. Adam JB, Holloway CE, George F, Quig D. Analyses of Toxic Metals and Essential Minerals in the Hair of Arizona Children with Autism and Associated Conditions, and Their Mothers. Biol Trace Element Res 2006;110:193-209.

29. Lakshmi Priya MD, Geetha A. Level of Trace Elements (Copper, Zinc, Magnesium and Selenium) and Toxic Elements (Lead and Mercury) in the Hair and Nail of Children with Autism. Biol Trace Elem Res 2011; 142:148–158.

30. Elsheshawy E, Tobar S, Sherra KH, Atallah S, Elkasaby R. Study of some biomarkers in hair of children with autism. Middle East Current Psychiatr 2011; 18:6–10

31. Busch EB, Amin OR, Rabah T, et al. Heavy Metals and Trace Elements in Hair and Urine of a Sample of Arab Children with Autistic Spectrum Disorder. J Clin Med 2011;6(4):247-57.