

A Research on the Level of Zinc and Copper in the Hair of Students with Lower IQ

Khadije Meghrazi,^{1*} Mojtaba Soleymani Sabet,² Fateme Kheradmand,¹ and Masoomeh Akhgar³

¹Department of Biochemistry, Faculty of Medicine, Urmia University of Medical Sciences, Urmia, Iran

²Department of English Language, Training and Education District 2, Urmia, Iran

³Solid Tumor Research center, Urmia University of Medical Sciences, Urmia, Iran

*Corresponding author: Khadije Meghrazi, Department of Biochemistry, Faculty of Medicine, Urmia University of Medical Sciences, Urmia, Iran. Tel: +98-9191266244, E-mail: kmeghrazi@gmail.com

Received 2017 April 12; Revised 2017 July 31; Accepted 2017 December 13.

Abstract

Background: The measurement of trace elements in human body has proved to be of great use in the evaluation of human health and the diagnosis of various diseases. No light, however, has been shed on these elements where they concern the human intelligence.

Objectives: The goal is to make a comparison between the students with a lower IQ and those having an ordinary IQ in terms of the amount of zinc and copper as two trace elements.

Methods: This is a case-control study and the subjects as the patients were chosen from mentally retarded students having no particular diseases. The sample size for the patient and control was 30 for each. The hair samples of the experimental group and the ordinary students (the control group) were digested with concentrated HNO₃ after being washed with water and acetone. After being digested, the values of zinc and copper in the samples were measured with atomic absorption spectroscopy. The obtained data were analyzed using SPSS-15 via two independent sample tests of significance tests.

Results: The levels of zinc in the hair of students with lower IQ was shown to be $141.70 \pm 88.56 \mu\text{g/g}$ and $198 \pm 90.90 \mu\text{g/g}$ for that of the ordinary students ($P = 0.01$). The copper level for the samples of the experimental group was $11.90 \pm 3.97 \mu\text{g/g}$ and $13.23 \pm 3.97 \mu\text{g/g}$ in that of the control group, indicating no significant difference ($P = 0.18$) between the two groups.

Conclusions: The findings suggest that improving nutrition with zinc supplements may help enhance the development of learning and education in the students with lower IQ.

Keywords: Trace Element, Hair, Lower IQ, Copper, Zinc

1. Background

By definition, essential elements are metabolic and functional food in gradients that are necessary for the sustenance of life [1]. Measuring of these elements can be useful in assessing the human health and in the diagnosis of various diseases [2]. The knowledge of trace element levels is of great significance in regards with the understanding of children's growth and development [3].

Cognition refers to the mental processes which are involved in knowledge acquisition and their integration into responses such as learning, attention, memory, IQ and consciousness [4]. Trace elements involvement in metabolic processes and oxidation-reduction reactions in the central nervous system (CNS) might have some effect on cognitive function [5]. Among these trace elements are zinc and copper. Zinc is essential for growth and development. Intermediary metabolism, immunity, DNA metabolism and repair, reproduction, taste, vision, and cognition/behavior can be referred to as examples of functions which require zinc [6]. In a study was found that the use of zinc supple-

ments in middle-aged and old women in 15 - 30 mg daily (at 3 months only) can improve their understanding and memory performance [7]. On the other hand in autism children the hair concentration of zinc was lower [8].

Copper is considered as an essential component of many enzymes (e.g. cytochrome oxidase, superoxide dismutase (SOD)) and a coenzyme. Copper deficiency results in hematological deficiencies, bone and joint abnormalities and psychomotor retardation [9]. Cu and Zn play an important role in the proper functioning of the whole body, including the central and peripheral nervous system. Lower serum concentration of Zn and higher Cu/Zn ratio in multiple sclerosis (MS) patients can suggest the relationship between MS and trace elements [10] and the mean values of Zn and Cu concentrations of hair in patients with Epilepsy were decreased in compared with control groups [11]. Mean plasma zinc values were significantly lower in criminal schizophrenic patients when compared to non-criminal schizophrenic patients, while mean serum copper values were significantly higher in criminal subjects

than noncriminal subjects [12].

Hair trace mineral analysis has been scientifically proven to be useful in the evaluation of general state of nutrient and health [3]. Also evaluation of the trace elements in the hair can be useful in the diagnosis of diseases such as cancer, check poisoning in humans when exposed to high doses of these elements and examination of malnutrition [1, 13]. In addition, hair samples can be easily collected and conveniently stored [14]. Thus In this study, we examined the status of zinc and copper in hair of students with lower IQ who have no history of genetic disease and hereditary as compared to that of ordinary students.

2. Methods

2.1. Hair Collection

This is a case-control study. A total of 60 hair samples were collected from the female students of the age group 9-13, not including colored or treated hair. The experimental group consisted of 30 students with a low IQ and no history of genetic diseases studying in Rahmat special school in Urmia, Iran. The control group included 30 students with a regular IQ level (ordinary students) from different state school in Urmia, Iran. All the students' consent forms were signed by legal representatives to authorize sample collection. The length of hair samples cut was 1-2 cm. Stainless steel scissors were used to cut the hair samples from the nape of the neck close to the occipital region. The samples were kept in plastic bags.

2.2. Sample Preparation

The samples were cut into smaller pieces in the laboratory with a sterile surgical scalpel, to enhance the washing procedure carried out as recommended by Varrica [15], following the sequence acetone-water-water-acetone. More specifically, hair samples were immersed in 20 mL of acetone (Merck, Germany) and 20 mL demineralized water (Tanso, Binazir Karan, Iran) and stirred, each time, in an ultrasonic bath for 15 minutes. The washed samples were individually placed in glass beakers and dried for 24 hours at 40°C in a drying oven, and then weighed. A total of 3 mL of HNO₃ (Suprapur, Merck, Germany) was added to about 150 mg of washed hair sample and digested for 24 hours in teflon vessels, at room temperature. Digestion was then completed by adding 500 µL of H₂O₂ (Suprapur, Merck, Germany) for an additional 24 hours. After digestion, the solutions were diluted by the addition of demineralized water to reach a volume of 25 mL. Stock standard solutions of zinc and copper (1000 mg/L) (Sigma-Aldrich) were used for preparing of the standard solutions with the demineralized water.

2.3. Measurement

Trace element determinations were carried out with an atomic absorption spectrophotometer (PG990, United Kingdom) at the Department of biochemistry, faculty of medicine, Urmia University of medical sciences. All of the processed samples and reference materials were analyzed in duplicate.

2.4. Statistical Analysis

The data was analyzed by a statistics specialist with SPSS 15. The statistical method used was 'two independent sample test of significance' and the P Value of 0.05 and less was considered as significant.

3. Results

In this study, the concentrations of zinc and copper were measured for both the experimental and control groups. The level of zinc was 141.70 ± 88.56 (Mean \pm SD) µg/g in the samples of the experimental group and 198 ± 90.90 µg/g in that of the control group as shown in Table 1. The copper level measured for the experimental group was 11.90 ± 3.97 µg/g while it was 13.23 ± 3.97 µg/g for the control group as shown in Table 1. The analysis of the data obtained, intriguingly, showed that the concentration of zinc was significantly different between the hair samples of the lower IQ students and the regular students ($P = 0.018$). It also was found that in terms of the copper levels, there was no significant difference between the hair samples of the two groups ($P = 0.18$) as shown in the Table 1.

4. Discussion

The current study examines the lower IQ students having no particular diseases studying in the special schools in terms of the levels of zinc and copper. Our results showed that the level of zinc in these students is lower than that of the ordinary students. Zinc is a trace element important for neurogenesis, neuronal growth, synaptogenesis, and neurotransmission [6]. A deficiency of zinc may not only lead in poor cognitive performance, but also cause some alteration in attention, activity, neuropsychological behavior and motor development [16, 17]. A class of glutamergic neurons store zinc in specific synaptic vesicles which is released as a neuro-modulator in an activity-dependent manner [18]. Synaptic plasticity indicates the presence of high zinc concentration at synapses which is critical for learning and memory [19]. The roles of zinc in central nervous system include: a, the involvement of zinc - dependent enzymes in brain growth; b, the participation of zinc-finger proteins in brain structure and neurotransmission;

Table 1. The Levels of Zinc and Copper Concentration in Hair of Control and Patients Groups (N = 30)^a

| Element in hair, $\mu\text{g/g}$ | Control Group | Patient Group | Levene's Test for Equality of Variances | | T-Test for Equality of Means | | |
|----------------------------------|------------------|--------------------|---|------|------------------------------|----|--------------------|
| | | | F | Sig. | T | df | Sig. (2-tailed) |
| Zn | 198 \pm 90.90 | 141.70 \pm 88.56 | 0.13 | 0.72 | -2.43 | 58 | 0.018 ^b |
| Cu | 13.23 \pm 3.97 | 11.90 \pm 3.97 | 0.26 | 0.60 | -1.33 | 58 | 0.18 |

^aValues are expressed as mean \pm SD.^bA significant difference ($P < 0.05$) between patient and control groups.

c, the involvement of zinc - dependent neurotransmitters in brain memory function; d, the involvement of zinc in the precursor production of neurotransmitters; e, the role of metallothionein-III as a protein that binds zinc in neurons [20]. It is also responsible for the movement of zinc from the cytoplasm into synaptic vesicles [16]. Children with ADHD (attention deficit hyperactivity disorder) have been shown to have lower levels of zinc and copper in both hair and serum as compared to the normal children in control groups [21] so zinc deficiency has an important role in the pathogenesis of ADHD [22]. On the other hand there was a statistically significant decrease of serum Zn in men with MCI (mild cognitive impairment) compared to normal controls [23]. A significant decrease in the hair concentration of Zn in schizophrenic patients than that of its control group was observed [24]. The amount of zinc in children with autism spectrum disorders (ASDs) has also proved to be decreased [25].

Both copper deficiency and copper excess both disrupt neural function. Copper is of great importance for the normal development and function of the brain, so that the value of copper increases in the serum of children with autism spectrum disorders (ASDs) [25]. Being a structural component and/or a cofactor of several enzymes, copper is involved in many physiological pathways in the brain. The occurrence of pathological anxiety and depression seems to involve a dysregulation in the NE system. Norepinephrine or norepinephrine is the principal sympathetic neurotransmitter and an important modulator of mood and attention [26] and copper involves the participation of enzyme in synthesizes NE [27, 28]. Rats with a deficiency in copper during development and then repleted with cu for several months showed altered auditory startle response and impaired coordination behavior [27]. On the other hand copper is involved in the activity of cytochrome c oxidase and it also facilitates brain's use of oxygen which consumes a large fraction of the total amount of oxygen [29]. Therefore copper deficit may predispose the brain even more susceptible to oxidative stress since defective cytochrome c oxidase activity may result in increased superoxide production by the respiratory chain and/or impaired activity of the copper-dependent superoxide dis-

mutases (SOD) may weaken the antioxidative defense [28]. A deficiency of copper has been proved to be related to microglial activation in the cerebral cortex and thalamus. Proinflammatory molecules such as radical oxygen species and cytokines which are secreted by activated microglia can ultimately damage oligodendrocytes and growth retardation [30]. Also myelination is a copper dependent process in CNS [29] and myelin is lost in the neuropathies of copper deficiency [31].

The increase of copper seems to be effective in the cognitive function. Non-ceruloplasmin bound copper (NCC) is slightly bound to peptides, albumin, amino acids and, due to its low molecular weight, is able to cross the brain-blood barrier [32] so that the increase of plasma copper levels lowers cognitive function [5]. It has been found that high dietary intake of copper may be associated with accelerated cognitive decline [33] as well as the plasma copper levels in Alzheimer's patients is higher than that of healthy people [34] and copper-chelating agents slowed cognitive decline in Alzheimer's patients [35]. It is thought that a brain damage may occur due to the interaction of copper with copper with amyloid- β ($A\beta$) peptide which can cause a b amyloid (b-amyloid) conversion to rouse form, generating H_2O_2 and impacts on cognition [5, 32] but in this study there was no significant changes in copper levels in patients, as though there is no correlation between serum copper level and Alzheimer's patients in Iran [36].

4.1. Conclusion

The present study concludes that impaired learning could be due to a zinc deficiency, therefore, a nutrition improved with zinc supplements may help the enhancement and development of learning in students with lower IQ, though a normal brain function requires the maintenance of brain cu homeostasis, as well.

Acknowledgments

This research was funded by West Azerbaijan University of Medical Sciences and Health Services under Grant No. 1033. We would also like to thank Mr. Sattar Sadeghi for his assistance in working with Atomic Absorption Spectrophotometer.

Footnotes

Authors' Contribution: All authors participated in this study and read the manuscript. Khadije Meghrazi designed study, developed the original idea and prepared manuscript. Mojtaba Soleymani Sabet cooperated in child sampling and edited the manuscript. Fateme Kheradmand cooperated in developing of the study protocol and in preparing of manuscript. Masoomeh Akhgar, data analysis.

Conflicts of Interests: The authors have no conflicts of interest to declare.

Funding/Support: Urmia University of Medical Sciences.

References

- Golasik M, Przybyłowicz A, Wozniak A, Herman M, Gawecki W, Gólusinski W, et al. Essential metals profile of the hair and nails of patients with laryngeal cancer. *J Trace Elem Med Biol*. 2015;**31**:67–73. doi: [10.1016/j.jtemb.2015.03.001](https://doi.org/10.1016/j.jtemb.2015.03.001). [PubMed: 26004894].
- Senofonte O, Violante N, D'Ilio S, Caimi S, Peri A, Caroli S. Hair analysis and the early detection of imbalances in trace elements for members of expeditions in Antarctica. *Microchem J*. 2001;**69**(3):231–8. doi: [10.1016/S0026-265X\(01\)00091-1](https://doi.org/10.1016/S0026-265X(01)00091-1).
- Ogboko B. Trace element indices in hair and saliva of school children. *J Basic Appl Sci Res*. 2011;**1**(3):169–77.
- Dauncey MJ. New insights into nutrition and cognitive neuroscience. *Proc Nutr Soc*. 2009;**68**(4):408–15. doi: [10.1017/S0029665109990188](https://doi.org/10.1017/S0029665109990188). [PubMed: 19698201].
- Gao S, Jin Y, Unverzagt FW, Ma F, Hall KS, Murrell JR, et al. Trace element levels and cognitive function in rural elderly Chinese. *J Gerontol A Biol Sci Med Sci*. 2008;**63**(6):635–41. doi: [10.1093/gerona/63.6.635](https://doi.org/10.1093/gerona/63.6.635). [PubMed: 18559640].
- Maret W, Sandstead HH. Zinc requirements and the risks and benefits of zinc supplementation. *J Trace Elem Med Biol*. 2006;**20**(1):3–18. doi: [10.1016/j.jtemb.2006.01.006](https://doi.org/10.1016/j.jtemb.2006.01.006). [PubMed: 16632171].
- Maylor EA, Simpson EE, Secker DL, Meunier N, Andriollo-Sanchez M, Polito A, et al. Effects of zinc supplementation on cognitive function in healthy middle-aged and older adults: the ZENITH study. *Br J Nutr*. 2006;**96**(4):752–60. [PubMed: 17010236].
- 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. [PubMed: 23118818].
- Strachan S. Trace elements. *Curr Anaesth Crit Care*. 2010;**21**(1):44–8. doi: [10.1016/j.cacc.2009.08.004](https://doi.org/10.1016/j.cacc.2009.08.004).
- Socha K, Karpinska E, Kochanowicz J, Soroczynska J, Jakoniuk M, Wilkiel M, et al. Dietary habits; concentration of copper, zinc, and Cu-to-Zn ratio in serum and ability status of patients with relapsing-remitting multiple sclerosis. *Nutrition*. 2017;**39–40**:76–81. doi: [10.1016/j.nut.2017.03.009](https://doi.org/10.1016/j.nut.2017.03.009). [PubMed: 28606574].
- Ulvi H, Yigiter R, Yoldas T, Dolu Y, Var A, Mungen B. Magnesium, zinc and copper contents in hair and their serum concentrations in patients with epilepsy. *East J Med*. 2002;**7**:31–5.
- Tokdemir M, Polat SA, Acik Y, Gursu F, Cikim G, Deniz O. Blood zinc and copper concentrations in criminal and noncriminal schizophrenic men. *Arch Androl*. 2003;**49**(5):365–8. doi: [10.1080/01485010390219746](https://doi.org/10.1080/01485010390219746). [PubMed: 12893514].
- Chojnacka K, Gorecka H, Chojnacki A, Gorecki H. Inter-element interactions in human hair. *Environ Toxicol Pharmacol*. 2005;**20**(2):368–74. doi: [10.1016/j.etap.2005.03.004](https://doi.org/10.1016/j.etap.2005.03.004). [PubMed: 21783613].
- Raposo JC, Navarro P, Sarmiento A, Arribas E, Irazola M, Alonso RM. Analytical proposal for trace element determination in human hair. Application to the Biscay province population, northern Spain. *Microchem J*. 2014;**116**:125–34. doi: [10.1016/j.microc.2014.04.012](https://doi.org/10.1016/j.microc.2014.04.012).
- Varrica D, Tamburo E, Milia N, Vallascas E, Cortimiglia V, De Giudici G, et al. Metals and metalloids in hair samples of children living near the abandoned mine sites of Sulcis-Inglesiente (Sardinia, Italy). *Environ Res*. 2014;**134**:366–74. doi: [10.1016/j.envres.2014.08.013](https://doi.org/10.1016/j.envres.2014.08.013). [PubMed: 25212264].
- Takeda A, Tamano H, Ogawa T, Takada S, Ando M, Oku N, et al. Significance of serum glucocorticoid and chelatable zinc in depression and cognition in zinc deficiency. *Behav Brain Res*. 2012;**226**(1):259–64. doi: [10.1016/j.bbr.2011.09.026](https://doi.org/10.1016/j.bbr.2011.09.026). [PubMed: 21946308].
- Contestabile A, Pena-Altamira E, Virgili M, Monti B. Zinc supplementation in rats impairs hippocampal-dependent memory consolidation and dampens post-traumatic recollection of stressful event. *Eur Neuropsychopharmacol*. 2016;**26**(6):1070–82. doi: [10.1016/j.euroneuro.2015.12.041](https://doi.org/10.1016/j.euroneuro.2015.12.041). [PubMed: 26774280].
- Frederickson CJ, Koh JY, Bush AI. The neurobiology of zinc in health and disease. *Nat Rev Neurosci*. 2005;**6**(6):449–62. doi: [10.1038/nrn1671](https://doi.org/10.1038/nrn1671). [PubMed: 15891778].
- Sindreu C, Storm DR. Modulation of neuronal signal transduction and memory formation by synaptic zinc. *Front Behav Neurosci*. 2011;**5**:68. doi: [10.3389/fnbeh.2011.00068](https://doi.org/10.3389/fnbeh.2011.00068). [PubMed: 22084630].
- Salgueiro MJ, Zubillaga MB, Lysionek AE, Caro RA, Weill R, Boccio JR. The role of zinc in the growth and development of children. *Nutrition*. 2002;**18**(6):510–9. doi: [10.1016/S0899-9007\(01\)00812-7](https://doi.org/10.1016/S0899-9007(01)00812-7). [PubMed: 12044825].
- Elbaz F, Zahra S, Hanafy H. Magnesium, zinc and copper estimation in children with attention deficit hyperactivity disorder (ADHD). *Egypt J Med Hum Genet*. 2017;**18**(2):153–63. doi: [10.1016/j.ejmhg.2016.04.009](https://doi.org/10.1016/j.ejmhg.2016.04.009).
- Arnold LE, DiSilvestro RA. Zinc in attention-deficit/hyperactivity disorder. *J Child Adolesc Psychopharmacol*. 2005;**15**(4):619–27. doi: [10.1089/cap.2005.15.619](https://doi.org/10.1089/cap.2005.15.619). [PubMed: 16190793].
- Dong J, Robertson JD, Markesbery WR, Lovell MA. Serum zinc in the progression of Alzheimer's disease. *J Alzheimers Dis*. 2008;**15**(3):443–50. doi: [10.3233/JAD-2008-15310](https://doi.org/10.3233/JAD-2008-15310). [PubMed: 18997297].
- Rahman A, Azad MA, Hossain I, Qusar MM, Bari W, Begum F, et al. Zinc, manganese, calcium, copper, and cadmium level in scalp hair samples of schizophrenic patients. *Biol Trace Elem Res*. 2009;**127**(2):102–8. doi: [10.1007/s12011-008-8230-8](https://doi.org/10.1007/s12011-008-8230-8). [PubMed: 18810332].
- Faber S, Zinn GM, Kern J, Kingston HM. The plasma zinc/serum copper ratio as a biomarker in children with autism spectrum disorders. *Biomarkers*. 2009;**14**(3):171–80. doi: [10.1080/13547500902783747](https://doi.org/10.1080/13547500902783747). [PubMed: 19280374].
- Goddard AW, Ball SG, Martinez J, Robinson MJ, Yang CR, Russell JM, et al. Current perspectives of the roles of the central norepinephrine system in anxiety and depression. *Depress Anxiety*. 2010;**27**(4):339–50. doi: [10.1002/da.20642](https://doi.org/10.1002/da.20642). [PubMed: 19960531].
- Pyatskowitz JW, Prohaska JR. Rodent brain and heart catecholamine levels are altered by different models of copper deficiency. *Comp Biochem Physiol C Toxicol Pharmacol*. 2007;**145**(2):275–81. doi: [10.1016/j.cbpc.2006.12.013](https://doi.org/10.1016/j.cbpc.2006.12.013). [PubMed: 17287146].
- Scheiber IF, Mercer JF, Dringen R. Metabolism and functions of copper in brain. *Prog Neurobiol*. 2014;**116**:33–57. doi: [10.1016/j.pneurobio.2014.01.002](https://doi.org/10.1016/j.pneurobio.2014.01.002). [PubMed: 24440710].
- Klevay LM. Myelin and traumatic brain injury: the copper deficiency hypothesis. *Med Hypotheses*. 2013;**81**(6):995–8. doi: [10.1016/j.mehy.2013.09.011](https://doi.org/10.1016/j.mehy.2013.09.011). [PubMed: 24120700].
- Benetti F, Ventura M, Salmini B, Ceola S, Carbonera D, Mammi S, et al. Cuprizone neurotoxicity, copper deficiency and neurodegeneration. *Neurotoxicology*. 2010;**31**(5):509–17. doi: [10.1016/j.neuro.2010.05.008](https://doi.org/10.1016/j.neuro.2010.05.008). [PubMed: 20685220].
- Jaiser SR, Winston GP. Copper deficiency myelopathy. *J Neurol*. 2010;**257**(6):869–81. doi: [10.1007/s00415-010-551-x](https://doi.org/10.1007/s00415-010-551-x). [PubMed: 20232210].

32. Babiloni C, Squitti R, Del Percio C, Cassetta E, Ventriglia MC, Ferreri F, et al. Free copper and resting temporal EEG rhythms correlate across healthy, mild cognitive impairment, and Alzheimer's disease subjects. *Clin Neurophysiol.* 2007;**118**(6):1244-60. doi: [10.1016/j.clinph.2007.03.016](https://doi.org/10.1016/j.clinph.2007.03.016). [PubMed: [17462944](https://pubmed.ncbi.nlm.nih.gov/17462944/)].
33. Morris MC, Evans DA, Tangney CC, Bienias JL, Schneider JA, Wilson RS, et al. Dietary copper and high saturated and trans fat intakes associated with cognitive decline. *Arch Neurol.* 2006;**63**(8):1085-8. doi: [10.1001/archneur.63.8.1085](https://doi.org/10.1001/archneur.63.8.1085). [PubMed: [16908733](https://pubmed.ncbi.nlm.nih.gov/16908733/)].
34. Agarwal R, Kushwaha SS, Tripathi CB, Singh N, Chhillar N. Serum copper in Alzheimer's disease and vascular dementia. *Indian J Clin Biochem.* 2008;**23**(4):369-74. doi: [10.1007/s12291-008-0081-8](https://doi.org/10.1007/s12291-008-0081-8). [PubMed: [23105789](https://pubmed.ncbi.nlm.nih.gov/23105789/)].
35. Squitti R, Rossini PM, Cassetta E, Moffa F, Pasqualetti P, Cortesi M, et al. d-penicillamine reduces serum oxidative stress in Alzheimer's disease patients. *Eur J Clin Invest.* 2002;**32**(1):51-9. [PubMed: [11851727](https://pubmed.ncbi.nlm.nih.gov/11851727/)].
36. Sadighi B, Shafa M, Shariati M. Comparison of serum copper and seruloplasmin level in Alzheimer patients and healthy control subjects in Kerman city in Iran. *J Neurol Sci.* 2005;**238**:301. doi: [10.1016/s0022-510x\(05\)81155-4](https://doi.org/10.1016/s0022-510x(05)81155-4).