Original Research Article

Evaluation of Serum Magnesium, Iron, Copper and Zinc Levels in Ischemic and Hemorrhagic Stroke Patients and Healthy Controls

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ABSTRACT

Introduction: Many studies have shown a relationship between serum level of trace elements and risk of stroke, but the exact mechanism of this relationship is not clear. The purpose of this study was to measure changes of serum magnesium, iron, copper, and zinc levels in ischemic and hemorrhagic stroke patients to evaluate their potential diagnostic utility.**Materials and methods:** Overall, 53 healthy individuals (30 men, 32 women) and 53 ischemic and hemorrhagic stroke patients (30 men, 23 women) who were admitted to the Rouhani Hospital in Babol (Iran) were enrolled in the study within 24 hours after stroke onset. Diagnosis was made based on medical history and physical examination by a neurologist. After blood sampling, serum copper was assessed by atomic absorption spectrophotometry, and zinc, magnesium and iron levels were assessed by spectrophotometry. Data analysis was performed in SPSS (version 21) using independent sample t-test and chi-square test. **Results:** Serum concentrations of copper was significantly higher in the patients (58.8 ±14.7 mg/dL) compared with the controls (45.7 ±10.0 mg/dL). Serum concentrations of zinc was significantly higher in the patients (113.2 ±17.3 mg/dL) compared with the controls (95.60 ±12.80 mg/dl). Moreover, serum concentrations of iron was significantly higher in the patients (148.5 ±30.4 mg/dL) compared with the controls (74.22 ±33.3 mg/dL). However, the patients (1.4 ±0.8 mEq/L) had significantly lower level of magnesium level compared to the controls (2.1 ±0.3 mEq/L). **Conclusions:** Our results suggest that evaluation of serum magnesium, iron, copper and zinc levels in ischemic and hemorrhagic stroke patients may be useful for the prediction and diagnosis of stroke status.

KEYWORDS: Stork, magnesium, zinc, iron, copper

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INTRODUCTION

It is well known that ischemic stroke occurs because of obstruction within a blood vessel supplying blood to the brain, but hemorrhagic stroke occurs when a weakened blood vessel ruptures. Stroke is also one of the main causes of morbidity and mortality with large economic losses [1,2]. Evidence suggests that trace elements can help protect neurons and prevent neuronal injury. It also improves blood flow to ischemic region, boosts energy and inhibits hippocampal neurons necrosis [2]. Inflammation is one of the main post-stroke symptoms [3]. Quality of life of stoke patients is also reduced significantly [4].

Zinc is a necessary cofactor for many enzymes and has an important role in synaptic transmission. Evidence suggests that zinc deficiency can lead to ischemic injury [5]. Copper has a critical role in the proper functioning of enzymes. Copper has important role in iron metabolism and can cause cytotoxic effects by partaking in reactions that leads to production of reactive oxygen species [6]. Iron has several vital functions in the body. A preliminary study revealed that high iron levels increase the

risk of cerebrovascular attack. The effects of trace elements and heavy metals on patients with acute hemorrhagic stroke has been studied [7]. Rapid identification of stroke patients in the emergency department is an essential part of therapy. In this regard, detection of biomarkers for the prediction and diagnosis of stroke is of great importance. Our previous investigations on stroke patients include evaluation of serum levels of calcium, chloride, potassium and sodium [8], investigation of the association of GABA and L-arginine levels and some stroke risk factors [9], determination of serum iron and free hemoglobin concentrations [10], and evaluation of serum uric acid, glucose and nitrite-nitrate levels [11]. The aim of the present study was to assess the potential diagnostic utility of serum magnesium, iron, copper, and zinc level in stroke.

MATERIALS AND METHODS
Study population
From August 2012 to July 2014, consecutive patients with ischemic and hemorrhagic stroke (n=53, 32 women) who were admitted to the Rouhani Hospital in Babol (Iran) were enrolled within 24 hours after stroke onset. In addition, 53 healthy individuals (30 men, 32 women) were included in the study as controls. The subjects were matched in terms of age and gender. Mean age of patients and controls was 67 years. On admission, a neurologist made the diagnosis for ischemic and hemorrhagic stroke based on medical history, clinical examination and neuroimaging using the National Institutes of Health Stroke Scale (NIHSS). Informed consent was obtained from all subjects prior to participation in the study. Inclusion criteria were accurate diagnosis of stork. Exclusion criteria were presence of vascular disease or risk factors of stroke, consumption of food supplements or drugs that could affect electrolytes concentration, and history of kidney disease. A day after the diagnosis, blood samples (5 ml) were collected from patients. The study protocol received

approval from the ethics committee of Babol University of Medical Sciences.

Biochemistry analysis
Serum levels of zinc, iron and magnesium were analyzed in a biochemistry laboratory using a spectrophotometer (model: M501, serial number: nol0904105, Camspec Co.) [12,13]. Zinc specifically forms a stable colored complex with chromogen (5-Br-PAPS). Xylitol blue was used for measuring serum magnesium levels. Ferene/end point method was used for determination of iron level. In acidic solution, transferrin bound iron is released as Fe^{3+} and then converts to Fe^{2+} by reducing substances. This ion creates a purple colored complex with ferene. Serum level of copper was measured by an atomic absorption spectrophotometer fitted with deuterium background correction and copper hallow-cathode lamp as the radiation source [14]. In addition, argon was used as the purge gas for the spectrophotometry experiment. Stock standard copper solutions were purchased from Merck (Germany) at concentration of 1000 mg/l (PPM) in a 0.5 mole HNO_3 (ultra-pure) solution. Serial dilutions (5, 10, 20, 40 and 50 PPB) were prepared using deionized water. Then, we injected 10 μl of each solution into the graphite tube.

Statistical analysis
Data analysis was done in SPSS software (version 21). Results were expressed as mean ± standard deviation (SD). Moreover, mean values in the two groups were compared using independent sample t-test and chi-square test. P-values less than 0.05 were considered as statistically significant.

RESULTS
Concentration of copper, iron and zinc was significantly higher in the patients compared to the controls. However, magnesium level was significantly lower in the patient group compared to the control group (Table 1). Table 2 demonstrates the serum concentrations of magnesium, iron, zinc and copper in the study groups based on gender.

Table 1. Serum concentrations of magnesium, iron, zinc and copper in the patients and controls

Trace element Groups	Copper (µg/dL)		Zinc (µg/dL)		Iron (µg/dL)		Magnesium (mEq/L)	
	Mean ± SD	Medium (Min-Max)	Mean ± SD	Medium (Min-Max)	Mean ± SD	Medium (Min-Max)	Mean ± SD	Medium (Min-Max)
Stroke patients	58.8±14.7	59.2(20-100)	113.2±17.3	114(75-173)	148.5±30.4	159(69-199)	1. 4±0.8	1.4(2-3.6)
Controls	45.7±10.0	41(33-68)	95.60±12.80	94(73-173)	74.22±33.3	73(30-171)	2.1±0.3	2.0(1.1-3)
P-value	< 0.001		< 0.001		< 0.001		< 0.001	

Table 2. Serum concentrations of magnesium, iron, zinc and copper in the patients and controls based on gender

Gender	Element	Stroke patients (Mean ± SD)	Controls (Mean ± SD)	Total (Mean ± SD)	P-value
Male	Magnesium (mEq/L)	1.45±0.74	3.92±0.39	1.81±0.69	<0.001
	Iron (µg/dL)	143.33±35.33	88.56±25.70	121.78±45.44	<0.001
	Zinc (µg/dL)	112.70±19.92	96.53±12.13	104.61±18.27	<0.001
	Copper	59.00±8.36	46.45±10.80	52.73±11.48	<0.001
Female	Magnesium (mEq/L)	1.46±0.89	63.13±37.41	1.79±0.74	0.002
	Iron (µg/dL)	125.43±21.36	88.13±37.41	103.45±50.56	<0.001
	Zinc (µg/dL)	113.95±13.60	94.39±13.80	104.17±16.81	<0.001
	Copper (µg/dL)	58.53±20.47	44.80±9.17	51.67±17.15	0.006

DISCUSSION

Although trace elements such magnesium, iron, copper and zinc are essential for body functions, but they can cause toxicity at levels beyond necessary for their biological functions. In this study, we found that the serum concentration of magnesium is significantly lower in stroke patients compared to healthy individuals. This is in contrast with findings of a previous study [17]. Previous studies suggested that serum level of magnesium in stroke patients regulate vasomotor tonicity, blood pressure and peripheral blood flow. [17]. Conversely, another study demonstrated that magnesium intake reduce the risk of ischemic stroke [18]. In addition, another study revealed that magnesium supplementation can considerably improve performance of stroke patients [19] and facilitate their recovery [20]. Researchers also reported that high

magnesium hair accumulation is not associated with risk of ischemic stroke [15]. A strong association has been shown between magnesium intake and reduced risk of stroke [4]. However, a study stated that serum concentrations of magnesium do not reflect the exact level of total magnesium levels in the body [16]. The inconsistencies in the results of these studies could be attributed to the small sample size, different methodology, and most importantly patients.

Zinc deficiency has been thought to be involved in neuronal damage [6]. We found no significant difference in the zinc level between stroke patients and healthy individuals. The level of serum copper in the patients was significantly higher than in the healthy controls. In our study, the patients had higher levels of serum iron compared to the healthy controls. A relationship has been

reported between serum iron level and risk of stroke. Moreover, serum ferritin concentrations have a direct relationship with stroke in women [7]. A previous study indicated that an increase occurs in iron storage before stroke [21]. It is well demonstrated that free iron increases following ischemic stroke [22]. There is also a relationship between low zinc level and stroke[23]. Researchers have shown that zinc, manganese, copper and selenium levels alter in nerve tissues [24], indicating their possible involvement in stroke. Trace elements alterations in serum may have diagnostic and prognostic value for stroke. However, the mechanism of this phenomenon is not completely understood. It is recommended to design future studies with a larger sample size to further identify the association of trace elements and risk of stroke.

CONCLUSION
We showed that the concentrations of magnesium, zinc, iron and copper might be a suitable biomarker for assessing the status of stroke. However, further studies should be performed to confirm our results.

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REFERENCES
1. Carbonell T, Rama R. Iron, oxidative stress and early neurological deterioration in ischemic stroke. Current Medicinal Chemistry ,2007;14(8): 857-874.

2. Afshari D, Moradian N, Rezaei M.Evaluation of the intravenous magnesium sulfate effect in clinical improvement of patients with acute ischemic stroke. Clinical Neurology and Neurosurgery ,2013; 115(4): 400-404.

3. Saleh A, Schroeter M, Ringelstein A, Hartung HS, Siebler M, Mödder U, Jander S . Iron oxide particle-enhanced MRI suggests variability of brain inflammation at early stages after ischemic stroke. Stroke ,2007;38(10): 2733-2737.

4. Nie, Z. L., Wang, Z. M., Zhou, B., Tang, Z. P., & Wang, S. K. Magnesium intake and incidence of stroke: Meta-analysis of cohort studies. Nutrition, Metabolism and Cardiovascular Diseases.2013;23(3): 169-176.

5. Shuttleworth CW, and Weiss JH. Zinc: new clues to diverse roles in brain ischemia. Trends in Pharmacological Sciences ,2011;32(8): 480-486
.
6. Tapiero H, Townsend D, Tew K.Trace elements in human physiology and pathology. Copper. Biomedicine & Pharmacotherapy,2004; 20(9) :386-398.
7. Karadas S, Sayın R, Aslan M, Gonullu H, Kat C, Dursun R, Duran L, Gonullu E, Demir HSerum levels of trace elements and heavy metals in patients with acute hemorrhagic stroke. J Membr Biol. 2014;247(2):175-80.

8.Moemeni H, Qujeq D, Ahmadi
Ahangar A, Hajian K, Parsian H. Evaluating the Serum Levels of Calcium, Chloride, Potassium and Sodium in the Stroke Patients .International Journal of Medical Laboratory 2016;3(2):104-110.

9. Hosinian M, Qujeq D, Ahmadi Ahangar A.The Relation Between GABA and L-Arginine Levels With Some Stroke Risk Factors in Acute Ischemic Stroke Patients. Int J Mol Cell Med, 2016;5(2):100-5.

10. Ghadiani F, Qujeq D, Ahmadi Ahangar A. Serum Iron and Free Hemoglobin Concentrations in Patients with Acute Ischemic Stroke. Journal of Paramedical Sciences (JPS) , 2016 7(3) : 24-28.

11.Hoseinian M, Qujeq D, AhmadiahangarA.Evaluation of Serum Uric Acid, Glucose and Nitrite-Nitrate Levels in Ischemic Stroke Patients .Journal of Clinical and Basic Research (JCBR) 2017; 1(4): 9-12.

12.Rajabali A, Qujeq D, Kashifardd M .Dtermination of Serum IRON, TIBC and Ferrtin of Patients with Hepatitis B and Fibrosis of the Liver, A Case-Control Study.J Ardabil Univ Med Sci, 2008; 8(1): 45-48.

13.Qujeq D, Sadogh M, Savadkohi S.Association between helicobacter pylori infection and serum iron profile. Caspian J Intern Med, 2011 ;2(3):266-9.

14.Mohammadi E, Qujeq D, Taheri H, Hajian-Tilaki K.Evaluation of serum trace element levels and superoxide dismutase activity in patients with inflammatory bowel disease: Translating basic research into clinical application. Biol Trace Elem Res, 2017;177(2):235-240.

15. Karaszewski B, Kozera G, Dorosz A, Łukasiak J, Szczyrba S, Łysiak-Szydłowska W, Nyka WM. High magnesium or potassium hair accumulation is not associated with ischemic stroke risk reduction: a pilot study. Clinical Neurology and Neurosurgery,2007;109(8): 676-679.

16. Cojocaru IM, et al.Changes of magnesium serum levels in patients with acute ischemic stroke and acute infections. Rom J Intern Med,2009;47: 169-171.

17. Bayir A, Ak A, Kara H, Sahin TK. Serum and cerebrospinal fluid magnesium levels, Glasgow Coma Scores, and in-hospital mortality in patients with acute stroke. Biological trace element research ,2009;130(1): 7-12.

18. Egger M, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. BMJ .1997;315(7109): 629-634.

19. Aslanyan S, Weir CJ, Muir KW, Lees KR; IMAGES Study Investigators. Magnesium for treatment of acute lacunar stroke syndromes further analysis of the IMAGES trial. Stroke .2007;38(4): 1269-1273.

20. Larsson SC , Virtamo J, Wolk APotassium, calcium, and magnesium intakes and risk of stroke in women. American Journal of Epidemiology ,2011; 174(1): 35-43.

21. Millerot E, Prigent-Tessier AS, Bertrand NM, Faure PJ, Mossiat CM, Giroud ME, Beley AG, Marie C. Serum ferritin in stroke: a marker of increased body iron stores or stroke severity? Journal of Cerebral Blood Flow & Metabolism ,2005;25(10): 1386-1393.

22. Millerot-Serrurot E, et al. Temporal changes in free iron levels after brain ischemia: Relevance to the timing of iron chelation therapy in stroke. Neurochemistry International, 2008;52(8): 1442-1448.

23. Munshi A, Babu S,Kauls S,Rajeshwar K, suvarna A, Jyothy A Depletion of serum zinc in ischemic stroke patients. Methods Find Exp Clin Pharmacol ,2010;32(6): 433-6.

24.Zangieva ZK, Torshin IIu, Gromova OA, Nikonov AA.Trace elements in the nervous tissue and ischemic stroke Zh Nevrol Psikhiatr Im S S Korsakova. 2013;113(3 Pt 2):30-6.