J Biochem Tech (2020) Special Issue (1): 149-153
ISSN: 0974-2328

Comparison of Copper and Zinc Concentration in Noncardioembolic Ischemic Stroke Patients with Non-Stroke Patients

Javad Abolhasani, Parivash Rafiee*, Mojtaba Sehat

Received: 18 October 2019 / Received in revised form: 20 February 2020, Accepted: 27 February 2020, Published online: 25 March 2020

Abstract

Introduction: Stroke (brain attack) is a sudden disruption of brain function that occurs as a result of vascular events. There are many risk factors in relation with stroke, some of which are unavoidable, such as age and sex, but some of them, such as high blood pressure, diabetes, hyperlipidemia, cigarettes and alcohol can be prevented or are treatable. The role of parameters such as copper and zinc in the pathogenesis of this disease is unknown. This study was carried out aimed to compare the concentration of copper and zinc in patients with ischemic noncardioembolic stroke with **non-stroke patients**. **Materials and Methods:** Our case-control study was carried out on 61 patients with noncardioembolic ischemic stroke and 61 healthy individuals referred to Kashan Health Center. Serum levels of copper, zinc, ceruloplasmin and homocysteine were measured in peripheral blood samples and urinary copper levels of all participants were measured. Finally, the data were collected and analyzed using statistical tests. **Results:** According to our findings, serum copper levels in patients (109.4 µg / dl) were significantly higher than normal subjects (97.35 µg / dl). (P = 0.02) Also, the copper urine level in patients (7.02 µg / dl) was significantly higher than healthy subjects (4.0 µg / dl). (P = 0.002) Serum zinc levels in patients (dl) were higher than normal subjects, but no significant difference was observed serum zinc level between the two groups (P = 0.162). **Conclusion:** Our results suggest that evaluation of serum copper level in noncardioembolic ischemic stroke patients may be useful for the prognosis and diagnosis of stroke. In this regard, it should be noted that more studies are needed to clarify the role of copper and zinc in patients with acute ischemic stroke.

Keywords: Copper, Zinc, noncardioembolic ischemic Stroke, Ceruloplasmin, Homocysteine.

Introduction

A stroke is a brain focal defect that occurs suddenly and in result of vascular lesions (Guven et al., 2011). It is the third common cause of death in the United States and is the second common cause of death in the developing countries after myocardial infarction and is the most common disorder in the neurology which causes disability (Guven et al., 2011; Sorganvi et al., 2014; Sharmin et al., 2015). Major risk factors for stroke are high blood pressure, heart disease, diabetes, high blood lipids, and smoking (de la Ossa et al., 2010; Yang et al., 2016). Some reports in Iran show that the incidence of stroke was 50 per 100,000 population in during two years (Iskra et al., 2005). With respect to the high prevalence of this disease, factors that affect the pathogenesis of this disease are important. In recent years, the role of Zinc as an effective factor in the pathogenesis of brain stroke has been studied. It has been reported that some patients with acute stroke have a low serum zinc level (Jeffrey and Starkman, 2011; Muir, 2000). The zinc element is an integral part of the metalloenzymes of the human body and interferes with synthesis and stabilization of proteins and DNA and RNA and plays a structural role in the ribosome and cell membrane (Shuaib et al., 2004; Liu et al., 2010). This element is essential in the brain for maturation and cellular function (Solomon et al., 2014; Tapiero, Townsend and Tew, 2003). Almost 10% of the total zinc element of the brain (possibly ionized zinc) exists in synaptic vesicles. In animals and possibly in humans, the adequate proportion of zinc is essential for brain function and the prevention of diseases of the nervous system (Köksaldi, Hacişevki and Torun, 2008). However, elements such as zinc and copper are involved in the pathogenesis

Javad Abolhasani
Department of Neurology, School of Medicine, Kashan University of Medical Sciences, Kashan, Iran.

Parivash Rafiee*
Resident of neurology, Kashan University of Medical Sciences, Kashan, IR Iran.

Mojtaba Sehat
Department of Community Medicine, Kashan University of Medical Sciences, Kashan, IR Iran.

***Email:** Parivash_rafiee@yahoo.com

of diseases with neuropathological components such as Alzheimer's disease, Parkinson's disease, other degenerative diseases, and stroke. The basis of the pathological remaining of the intermediary elements is under investigation. Various mechanisms have been proposed in this regard. Some studies have reported that although these materials are not harmful, changes in the level of these can contribute to injury (Ovbiagele et al., 2008). Other studies have declared that ischemic injury, such as stroke, can lead to excessive release of zinc and copper from the neuron, such as neurotoxicity disorder (Guven et al., 2011). Some researchers have declared that zinc element may be an independent risk factor for stroke. As a result, this can be considered and prevented the risk of stroke (de la Ossa et al., 2010). Considering that the pathology of these elements is still under investigation and the findings in stroke patients are conflicting and considering that so far in Iran there has been few statistical studies on the concentration of copper and zinc in stroke patients, this study was conducted with the purpose of comparing the concentration of copper and zinc in Ischemic stroke patients known as non-cardioembolic stroke with non-stroke patients.

Material and Methods

This study is a case-control study was carried out on 61 noncardioembolic ischemic stroke patients admitted to Shahid Beheshti Hospital of Kashan (Iran) in August 2017 to July 2018, as the case group and 61 healthy subjects or patients with neurological headache, discopathies and degenerative diseases admitted to Shahid Beheshti Hospital which had the conditions for the exclusion criteria of the case group in the same period, as the control group. The subjects were matched in terms of age and gender. The inclusion criteria for the case group included the over 50 years of age, and the approval of the disease by the physician and the inclusion criteria for entering the control group included subjects with no stroke. And the exclusion criteria included patients who had liver and kidney diseases, malignancy, autoimmune disease, hypothyroidism, and Wilson, as well as the use of supplements The noncardioembolic ischemic stroke was diagnosed by the neurologist based on medical history, clinical examination and neuroimaging (CT scan or MRI) using the National Institutes of Health Stroke Scale (NIHSS). We study of risk factors such as high blood pressure, diabetes and drug history, as well as Serum concentration of copper, soroloplasmin, homocysteine, zinc and copper in the urine were performed. Relevant tests are performed in the laboratory of Shahid Beheshti Hospital in Kashan during 24 hours the beginning of the signs. Serum copper was assessed by atomic absorption spectrophotometry, and zinc levels were assessed by spectrophotometry. Then, the relationship of these measurements between case and control groups is calculated by using statistical methods, chi-Square test, independent t test and multivariate regression models. For quantitative variables, central and variation indicators were presented. For qualitative variables, absolute and relative frequency and frequency distribution tables are provided. The role of intervening variable is done through logistic regression analysis. A significant level of less than 5% is considered and SPSS version 21 software will be used for analysis.

Results

In this study, 61 patients with stroke were considered as case group and 61 non stroke patients were considered as control group. Table 1 shows detailed demographic information for each of these groups. According to the results, a significant difference in gender and BMI was observed between the two groups. The value of the P-value for each of the variables is listed in Table 1.

Table 1- The demographic characteristics of the participants in the study

Variable		Case Group	Control Group	P-Value
Age	Mean ± SD	74.8 ± 12.18	74.75 ± 12.01	0.983
Gender	Male	28 (45.9%)	40 (65.6%)	0.029
	Female	33 (54.1%)	21 (34.4%)	
Vocation	House worker	22 (36.1%)	11 (18%)	0.110
	Employee	3 (4.9%)	7 (11.5%)	
	Handicraft profession	33 (54.1%)	36 (59%)	
	Intellectual career	1 (1.6%)	1 (1.6%)	
	Tradesman	2 (3.3%)	6 (9.8%)	
BMI	Mean ± SD	26.30 ± 4.46	24.71 ± 4.35	0.049
Smoking	Negative	51 (83.6%)	55 (90.2%)	0.283
	Positive	10 (16.4%)	6 (9.8%)	
Addiction	Negative	56 (91.8%)	60 (98.4%)	0.207
	Positive	5 (8.2%)	1 (1.6%)	
Family history of stroke	Negative	59 (96.72%)	60 (98.4%)	0.90
	Positive	2 (3.28%)	1 (1.6%)	

Underlying disease	Negative	7 (11.5%)	24 (39.3%)	<0.001
	Positive	54 (88.5%)	37 (60.7%)	

The laboratory findings of the patients were examined, which are listed in Table 2 in detail. The levels of serum copper, urine copper, zinc, homocysteine and ceruloplasmin have been reported in table 3. According to the results, a significant difference was observed between hemoglobin and hematocrit levels between the two case and control groups. A significant difference was observed in serum copper, copper, zinc, and homocysteine levels.

Table 2. The laboratory findings of the patients in the case and control group

Variable		Case Group	Control Group	P-Value
Hematology factors	Hemoglobin	12.35 ±1.91	13.20±2.03	0.020
	Hematocrit	36.57 ± 5.20	38.85±5.03	0.0160
	WBC	7.69 ± 2.43	7.95±3.99	0.666
	Platelet	225.34 ± 72.42	229.27±104.56	0.811
Lipid Profile	TG	140.28 ±131.55	132.48±79.32	0.784
	Cholesterol	168.69 ± 42.30	166.08±46.32	0.803
	LDL	86.07 ± 38.92	76.96±29.62	0.318
	HDL	44.38 ±13.16	43.83±14.04	0.868
Chemical factors	Sodium (Na)	139.12 ±2.82	139.54+4.82	0.564
	Potassium (K)	4.26 ±0.42	4.18±0.50	0.357
	Calcium (Ca)	9.30 ±0.51	9.28±0.73	0.879
	Phosphorus (P)	4.08 ±3.48	3.51±0.80	0.375
	Magnesium (Mg)	1.97 ±0.74	1.96±0.43	0.905
	BUN	18.07 ±6.36	16.80±6.17	0.267
	Creatinine	1.16 ±0.28	1.12±0.27	0.524

Table 3- Serum concentrations of zinc, copper, Ceruloplasmin and Homocycteine in the patients and controls

Groups / Variables	Mean ± SD		P-vaue
	Case Group	Control Group	
Serum copper (µg/dL)	109.41 ± 24.63	97.35±20.83	0.004
Urine copper (µg/dL)	7.02±5.16	4.07±3.94	<0.001
Zinc (Zn) (µg/dL)	74.61±22.93	65.76±17.75	0.019
Ceruloplasmin (mg/dl)	43.6±9.19	42.91±7.43	0.614
Homocycteine (µmol/L)	21.17±11.01	17.81±7.21	0.048

After the elimination of the intervening effect of age, sex, BMI, Hb and homocysteine, there was still a significant relationship between serum copper levels and stroke. ($p = 0.026$). The difference in serum copper in two groups (healthy and patient) after elimination of the effect of age, sex, body mass, Hb and homocysteine was reduced only by 2.2 units, and this difference was significant in the two groups. After the elimination of the intervening effect of age, sex, BMI, Hb and homocysteine, there was still a significant relationship between urine copper levels and stroke. ($p = 0.002$). The difference in serum copper in two groups (control and case) after elimination of the effect of age, sex, body mass, Hb and homocysteine was reduced only by 0.12 units, and this difference was significant in the two groups.

After the elimination of the effect of age, sex, body mass, Hb and homocysteine there was not significant relationship between zinc levels and stroke in the two groups. ($p = 0.162$)

Discussion

The results of this study show that the serum and urine level of copper significantly higher in stroke patients compared to non-stroke individuals. Also, according to our results, the serum zinc level in the patient group was higher than control, but there was no significant difference. The change in the status of chemical elements such as copper and zinc has been observed in many cases of cerebrovascular disease. Previous study in Iran suggested that evaluation of serum copper and zinc levels in ischemic and hemorrhagic

stroke patients (Moemeni et al., 2018). Bagheri et al. similarly concluded that with increasing serum copper levels, the severity of atherosclerosis in atherosclerotic patients increases (Bagheri et al., 2015).

Klimenko et al in another study examined the level of copper and zinc in 20 patients with TIA and concluded that TIA in women was associated with a decrease in serum copper concentration. This finding is inconsistent with our findings. The difference between the sample size and the time difference between the symptoms of the patients is the most significant difference in this study with this study, which can also justifies this finding. In this study, the serum levels of zinc in men increase (Klimenko et al., 2016).

Kodali et al in a study reported increased plasma copper concentrations in stroke patients (Kodali et al., 2012). This view was supported by the study of Lai and Altamura and showed that the levels of total copper and copper bound to small proteins in the serum of ischemic stroke patients increased (Lai et al., 2016; Altamura et al., 2009). These articles predict evidences of copper dyshemoestasis disorder as soon as ischemic injury in patients, which is consistent with our findings. The difference is that, in the our study, the level of free copper was not measured and is considered as limitations of our study

Atherosclerosis was the most common cause of ischemic stroke. The relationship between copper and atherosclerosis, especially coronary artery disease, has been proven in several previous studies. For example, based on Grammer et al.'s analysis, an increase in serum copper and ceruloplasmin concentration was associated with an increase atherosclerotic coronary artery disease in angiography and mortality due to vascular diseases (Grammer et al., 2014).

In our study the level of copper in urine and homocysteine with copper in the patients group was significantly higher than the control group and the difference between the two groups was significant. Meanwhile, there is a significant relationship between serum levels of homocysteine in the two groups.

According to the results of our study, zinc in the serum of patients was higher in comparison with the control group. In a study conducted in 2003 using a clinical model of stroke in mice, it was observed that zinc therapy increased infarct volume and also worsened the neurological deficiency. This supports the hypothesis that zinc leads to worsening of the prognosis of ischemic brain injury in the embolic stroke in mice (Shuaib et al., 2004). Sensi et al in another study in animal models suggested that zinc and homeostasis disorders- dependent pathways may cause brain damage in the stroke. These findings are inconsistent with the study of Munshi et al. in India, which zinc concentrations in patients with ischemic stroke significantly was lower than normal subjects. In this study, it has been suggested that zinc is an independent risk factor of stroke and can therefore be targeted by preventive interventions (Munshi et al., 2010). In justifying these results, it can be said that extracellular zinc stimulates of zinc-containing neurons to release stored substances which leads to create a toxic environment for other neurons. Also, increasing intracellular zinc can induce the production of activated cellular oxygen species and the loss of mitochondrial membrane potential, which these changes in turn reduce cellular energy and, consequently, ischemic cell death. Increasing extracellular and intracellular zinc in neuronal cells inhibits the exchange of biochemical, which leads to decrease pH and subsequently impair the function of the neurons and leads to increase ischemic cell death (Minami et al., 2002; Allen and Attwell, 2002; Dineley, Votyakova and Reynolds, 2003; Dineley, Brocard and Reynolds, 2002).

In our study, the results have shown increase homocysteine in the serum of ischemic stroke patients compared with the control group. When copper ion and homocysteine in vitro are in contact with endothelial cells in the culture medium, significant changes have been observed in atrogienic activity (Weiss, 2005).

Homocysteine alone can produce a direct toxic effect on endothelial cells. However, the effect of homocysteine and copper may accumulate and effect the interactions of atherosclerosis and stroke (Dong et al., 2013). The findings of the above study are consistent with the results of our study.

Conclusion

Patients had serum and urinary copper levels were significantly higher than the control group.
However, no significant difference was observed in the level of zinc in two groups in the study. We suggested copper can be considered as a risk factor for stroke or a disorder hemostasis which can lead to stroke. However, further studies need for confirm our results and pathological mechanisms caused by it will be created.

References

Allen NJ, & Attwell D. (2002). Modulation of ASIC channels in rat cerebellar Purkinje neurons by ischaemia ‐ related signals. The Journal of physiology. 543(2):521-9.

Altamura C, Squitti R, Pasqualetti P, Gaudino C, Palazzo P, & Tibuzzi F, et al. (2009). Ceruloplasmin/Transferrin system is related to clinical status in acute stroke. Stroke. 40(4):1282-8.

Bagheri B, Akbari N, Tabiban S, Habibi V, & Mokhberi V. (2015). Serum level of copper in patients with coronary artery disease. Nigerian medical journal: journal of the Nigeria Medical Association. 56(1):39.

de la Ossa NP, Sobrino T, Silva Y, Blanco M, Millán M, Gomis M, et al. (2010). Iron-related brain damage in patients with intracerebral hemorrhage. Stroke. 41(4):810-3.

Dineley K, Brocard J, & Reynolds I. (2002). Elevated intracellular zinc and altered proton homeostasis in forebrain neurons. Neuroscience. 114(2):439-49.

Dineley KE, Votyakova TV, & Reynolds IJ. (2003). Zinc inhibition of cellular energy production: implications for mitochondria and neurodegeneration. Journal of neurochemistry. 85(3):563-70.

Dong D, Wang B, Yin W, Ding X, Yu J, & Kang YJ. (2013). Disturbance of copper homeostasis is a mechanism for homocysteine-induced vascular endothelial cell injury. PloS one. 8(10):e76209.

Grammer T, Kleber M, Silbernagel G, Pilz S, Scharnagl H, Lerchbaum E, et al. (2014). Copper, ceruloplasmin, and long-term cardiovascular and total mortality (the Ludwigshafen Risk and Cardiovascular Health Study). Free radical research. 48(6):706-15.

Guven H, Çilliler AE, Koker C, Sarikaya SA, & Comoglu SS. (2011). Association of serum calcium levels with clinical severity of acute ischemic stroke. ACTA Neurologica Belgica. 111(1):45.

Iskra M, Barałkiewicz D, Majewski W, & Pioruńska-Stolzmann M. (2005). Serum Magnesium, Copper and Zinc concentration changes in Lower limb ischemia and postoperative treatment. Magnesium Research. 18(4):261-7.

Jeffrey L, & Starkman S. (2011). Magnesium in clinical stroke. Magnesium in the Central Nervous System. 205.

Klimenko LL, Skalny AV, Turna AA, Tinkov AA, Budanova MN, Baskakov IS, et al. (2016). Serum trace element profiles, prolactin, and cortisol in transient ischemic attack patients. Biological trace element research. 172(1):93-100.

Kodali P, Chitta KR, Figueroa JAL, Caruso JA, & Adeoye O. (2012). Detection of metals and metalloproteins in the plasma of stroke patients by mass spectrometry methods. Metallomics. 4(10):1077-87.

Köksaldi E, Hacişevki A, & Torun M. (2008). The levels of trace elements and electrolytes in serum and cerebrospinal fluid of patients with acute stroke. Ankara Universitesi Eczacilik Fakultesi Dergisi. 37:111-21.

Lai M, Wang D, Lin Z, & Zhang Y. (2016). Small molecule copper and its relative metabolites in serum of cerebral ischemic stroke patients. Journal of Stroke and Cerebrovascular Diseases. 25(1):214-9.

Liu F, Akella P, Benashski SE, Xu Y, & McCullough LD. (2010). Expression of Na–K–Cl cotransporter and edema formation are age dependent after ischemic stroke. Experimental neurology. 224(2):356-61.

Minami A, Takeda A, Yamaide R, & Oku N. (2002). Relationship between zinc and neurotransmitters released into the amygdalar extracellular space. Brain research. 936(1):91-4.

Moemeni, Hojatollah, et al. (2018). Evaluation of serum Magnesium, Iron, Copper and Zinc levels in ischemic and hemorrhagic stroke patients and healthy controls. Journal of Clinical and Basic Research, 2 (1): 6-10.

Muir KW. (2000). Therapeutic potential of magnesium in the treatment of acute stroke. Journal of Stroke and Cerebrovascular Diseases. 9(6):257-67.

Munshi A, Babu S, Kaul S, Shafi G, Rajeshwar K, Alladi S, et al. (2010). Depletion of serum zinc in ischemic stroke patients. Methods and findings in experimental and clinical pharmacology. 32(6):433-6.

Ovbiagele B, Starkman S, Teal P, Lyden P, Kaste M, Davis SM, et al. (2008). Serum calcium as prognosticator in ischemic stroke. Stroke. 39(8):2231-6.

Sharmin N, Sultana N, Mazumder P, & Rahman T. (2015). Serum calcium and lipid profile in acute ischemic stroke. Bangladesh Journal of Medical Science. 14(3):286.

Shuaib A, Yang T, Salam A, & Wang CX. (2004). Effect of zinc in ischemic brain injury in an embolic model of stroke in rats. Neuroscience letters. 356(1):69-71.

Solomon EI, Heppner DE, Johnston EM, Ginsbach JW, Cirera J, Qayyum M, et al. (2014). Copper active sites in biology. Chemical reviews. 114(7):3659-853.

Sorganvi V, Kulkarni M, Udgiri R, Kadeli D, & Atharga S. (2014). Risk factors for ischemic stroke-a case control study. IJABR. 4(1):9-12.

Tapiero H, Townsend D, & Tew K. (2003). Trace elements in human physiology and pathology. Copper. Biomedicine & pharmacotherapy. 57(9):386-98.

Weiss N. (2005). Mechanisms of increased vascular oxidant stress in hyperhomocysteinemia and its impact on endothelial function. Current drug metabolism. 6(1):27-36.

Yang G, Hu R, Zhang C, Qian C, Luo Q-Q, Yung W-H, et al. (2016). A combination of serum iron, ferritin and transferrin predicts outcome in patients with intracerebral hemorrhage. Scientific reports. 6.