

## **Serum Iron and Free Hemoglobin Concentrations in Patients with Acute Ischemic Stroke**

**Faezeh Ghadiani<sup>1</sup>, Durdi Qujeq<sup>1,2</sup>, Alijan Ahmadi Ahangar<sup>3\*</sup>**

<sup>1</sup>Department of Clinical Biochemistry, Babol University of Medical Sciences, Babol, Iran.

<sup>2</sup>Cellular and Molecular Biology Research Center (CMBRC), Health Research Institute, Babol University of Medical Sciences, Babol, Iran.

<sup>3</sup>Department of Neurology, Ayatollah Rouhhani Hospital, Babol University of Medical Sciences, Babol, Iran.

\*Corresponding Author: email address: [ahmadihangaralijan@yahoo.com](mailto:ahmadihangaralijan@yahoo.com) (A. Ahmadi Ahangar)

### **ABSTRACT**

A number of evidences suggest that during ischemic stroke, serum iron and hemoglobin (Hb) levels are changed. Yet, there are few reports in the literature related to this issue and resolution of this mechanism requires further experiments. The aim of the present study was to investigate the potential role of serum iron and hemoglobin levels as a biomarker in diagnosis of acute ischemic stroke. The sample size was 60 ischemic stroke patients who were admitted to the Rouhhani hospital in Babol, with 60 healthy volunteers selected as control group. Clinical evaluation consisted of complete medical history and physical examination and neuro-imaging's studies. Sampling strategy was based on clinical characteristics, including age, gender, and history of diseases. Laboratory measurements were performed in the department of clinical biochemistry. Serum iron and plasma hemoglobin levels were measured by standard kit of iron and hemoglobin ELISA Kit, as of the manufactures' manual. Data were analyzed through statistical software SPSS version 22. The mean level of serum iron and hemoglobin in patients with acute ischemic stroke were higher than those in control group ( $P < 0.05$ ). However, there was no relation between these biomarkers and age and gender of subjects ( $P > 0.05$ ). Our results reinforce the possibility of serum iron and hemoglobin as biomarker in diagnosis of ischemic stroke patients.

**Keywords:** Acute ischemic stroke; iron; hemoglobin

### **INTRODUCTION**

It has been previously reported that stroke is a serious neurological disease as the main leading cause of death and disability throughout the world. Recent evidence suggests that pathophysiology of stroke is highly complex and involves inflammatory pathways, oxidative damages, ionic imbalance and apoptosis. Many studies shows that brain damage is related to acidosis and that enhanced acidosis is detrimental because it accelerates delocalization of protein-bound iron[1]. Much evidence has been accumulated in recent years, indicating that it can be as an important element for the development of the nervous system. Preliminary studies revealed that alteplase has been used widely throughout the world for the treatment of acute ischemic stroke [2] There are some reports in the literature

showing that disruption of iron homeostasis after ischemic stroke causes neuronal damage [3] It is well recognized that hemoglobin contributes to the pro-inflammatory nature of HDL in atherosclerosis [4] It is widely known that intravascular hemolysis releases large amounts of free hemoglobin. There are some reports in the literature showing that serum-free hemoglobin concentrations in healthy individuals are related to haptoglobin type. It is well known that hemoglobinemia should be considered as a novel mechanism of human disease. There has been an increasing amount of reports on the fact that oxidative metabolism during ischemic stroke together with high iron content in the brain synergies to increase the oxidative damage. There is evidence that serum free hemoglobin is

associated with stroke [5-8] It has been well established that the increase of serum hemoglobin in ischemic stroke leads to inflammation and coagulation. Previous work has shown that hemoglobinemia should be considered as a novel mechanism of disease and the serum ferritin level may reflect the iron storage of the body [7,9]. However, with the exception of a few publications, little information is available on the role of serum iron and hemoglobin in acute ischemic stroke; further work seems essential to explain the action. The aim of the present study was to investigate the potential role of serum iron and hemoglobin as biomarkers in diagnosis of acute ischemic stroke.

## MATERIALS AND METHODS

### *Subjects*

The present study is performed through case-control, using sampling strategy and sampling frame. This study was conducted according to the guidelines of the declaration of Helsinki, and all procedures involving human patients were approved by our university ethics committee (4-57-2013.5.7). The study protocol was reviewed and approved by the ethical committee of Babol University of Medical Sciences. Written informed consent was obtained from all patients after a full explanation of the purpose and nature of all procedures was provided. After all participants had signed the informed consent, serum iron and hemoglobin level were measured. Ischemic stroke was defined by focal neurological signs in a vascular territory of brain [4]. In this study, [n=60, 4 subjects were excluded], 56 (21 men and 35 women) acute ischemic stroke patients with mean age of 64 years old were admitted in emergency department of Babol Rouhhani Hospital. Acute ischemic stroke was diagnosed by a specialist, based on the clinical variables such as patient's history, physical examination and neuro-imaging studies. For the correct standard group, [n=60, 6 subjects were excluded], 54 (20 men and 34 women) persons were selected as a control group with mean age of 62 years old. The inclusion criteria was defined as acute ischemic stroke which should not last more than 24 hours after the onset of symptoms. Exclusion Criteria included: hemolysis sample for measuring iron

and hemoglobin. a previous diagnosis of kidney, Liver, and heart disease. Moreover, subjects with blood and digestive diseases were excluded. Clinical characteristics, including age, gender, and history of diseases were obtained from their records. Whether patients smoke and duration of smoking was also asked and recorded from their relatives. According to inclusion and exclusion criteria in group 1, 56 acute ischemic stroke patients and in group 2, 54 healthy subjects were included and 10 subjects were excluded. Written consent was received from all patients. Blood samples (3 ml) were taken from these patients; serum was then separated and kept at  $-80^{\circ}\text{C}$ , in the department of biochemistry.

### *Measurements*

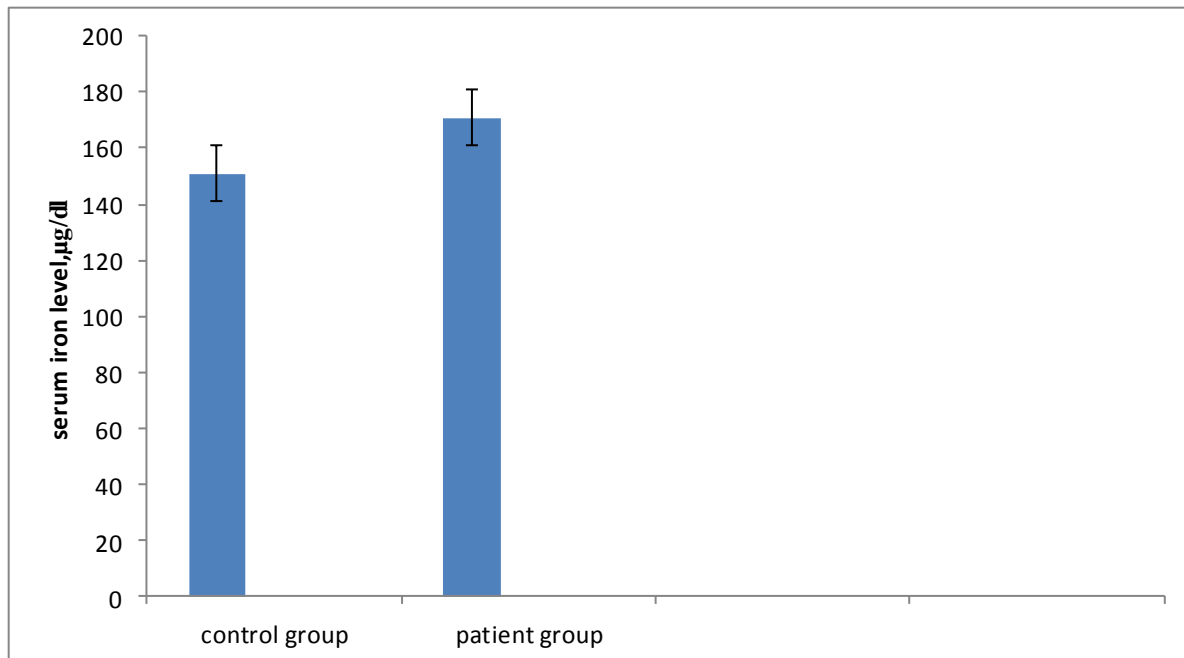
Iron level was determined by the using standard kit at 600 nm, according to the manufacturers instruction (Bioassay Technology Laboratory Company). Quantification of plasma hemoglobin levels in all samples were performed through using human hemoglobin ELISA kit at 450 nm (Bioassay Technology Laboratory Company).

### *Statistical analysis*

Descriptive statistics and analysis were performed in SPSS 20.0 for Windows. All values are expressed as mean  $\pm$  standard error. Probability values  $P < 0.05$  were considered as statistically significant. Correlation values were evaluated by Spearman's correlation test. independence sample t-test was also used.

## RESULTS

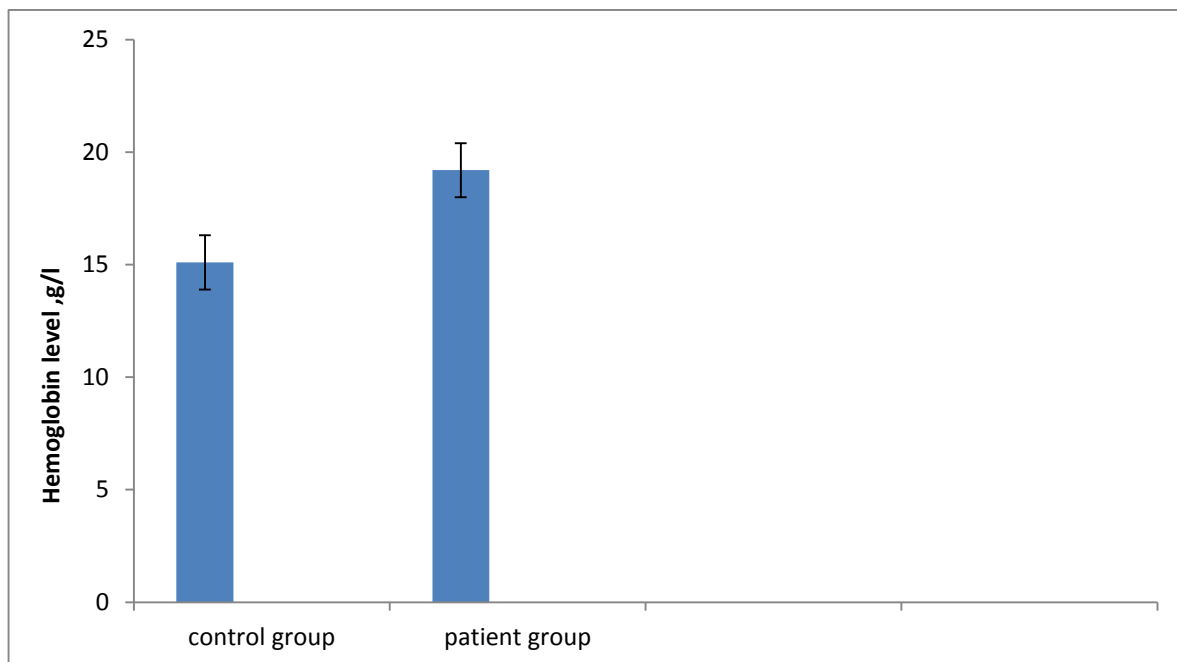
In this study, 56 patients and 54 persons as control group were examined. The two groups were matched for the demographical variables like sex, education and age. Variables have normal distribution and we used parametric method for analysis. The serum iron level for patients and control group is shown in figure. There is significant increase in iron level of patients' group in comparison with control group ( $p=0.009$ ). Age and sex did not show any significant correlation with iron (for age  $r_s=0.05$ ,  $P=0.58$ ), (for gender  $r_s=0.117$ ,  $P=0.203$ ). No significant correlation was found between hemoglobin and iron level ( $r_s=0.021$ ,  $P=0.818$ ).



**Figure 1.** Mean serum iron concentration in patient and control groups.

The mean plasma hemoglobin level for patients and control group are shown in figure. There is significant increase in hemoglobin level of patients' group compared to control group ( $P=0.022$ ). Age and sex did not show any

significant correlation with hemoglobin (for age  $r_s=-0.07$ ,  $P=0.448$ ), (for gender  $r_s=0.059$ ,  $P=0.52$ ). No significant correlation was found between hemoglobin and iron ( $r_s=0.021$ ,  $P=0.818$ ).



**Figure 2.** Mean plasma Hb concentration in patients and control group

To determine the ability of each indicator to differentiate between healthy subjects and

patients, AUC in serum case for iron and hemoglobin were reported (figure 3 and table 1).

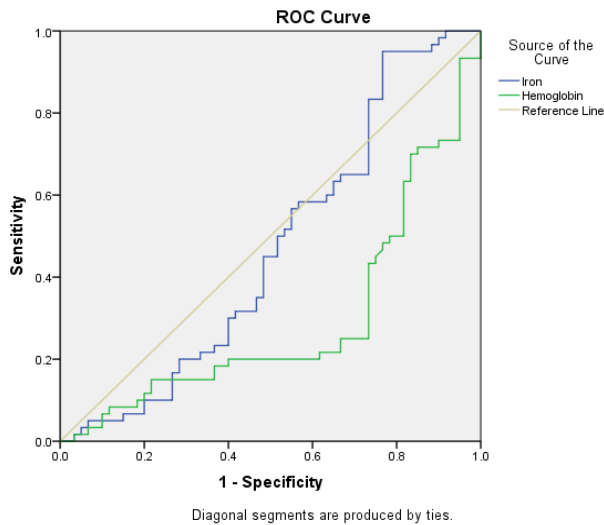


Figure 3. Markers ROC in serum case in comparison with serum control

Table 1. Area under the curve in serum case for iron

Test results variable(s)	Area	Standard Error	Asymptotic sig.	Asymptotic 95% confidence Interval	
				Lower Bound	Upper Bound
Iron	0.468	0.054	0.546	0.362	0.574
Hemoglobin	0.292	0.049	0.000	0.197	0.388

**DISCUSSION**

Results of this study demonstrated that serum iron levels were increased in ischemic stroke patients compared to the control group. Our results were in agreement with what other researchers reported previously [10]. Likewise, findings of this study demonstrated that plasma hemoglobin levels were increased compared with the control group. Elevated plasma Hb level might be due to severity of desecration of RBCs, decreased intravascular Hb-scavenger level and organ dysfunction in stroke patients. The action mechanism remains unclear and further studies are needed to clarify this mechanism. Further basic and clinical experimental investigations seem essential to explore the role of Hb in stroke patients. Our study on stroke is in progress to elucidate its mode of action. In terms of future applications, we will examine this problem in more details as we go forward. Our results are in perfect harmony with those reported previously [11] These results strongly suggest that our findings

could provide new possibility of serum iron and plasma hemoglobin as biomarker in diagnosis of ischemic stroke patients. The strong point of

present study is the introduction of the possibility of serum iron and hemoglobin levels as biomarker in early diagnosis of ischemic stroke patients. On the other hand, decreased iron and hemoglobin level cause decrease in oxygen content of blood and oxygen to the brain and ultimately causing damage to the brain cells [12]. Because of the influence of iron stores, contamination and hemolysis, ferritin measurement is more appropriate than serum iron in brain injury [13]. The previous results from our laboratory confirmed the possibility of serum iron and hemoglobin levels as the potential biomarkers in diagnosis of acute ischemic stroke. Yet, beside all these positive results and suggestions, many more experiments are needed to address this issue. To that end, deeper investigations are being carried out in our laboratory .

**CONCLUSION**

What makes this study creative is introducing the possibility of serum iron and hemoglobin as biomarker in early diagnosis of ischemic stroke patients. Serum iron and hemoglobin levels can be used as an indicator of ischemic stroke. It is

promising that serum iron and hemoglobin will play an important diagnostic role in ischemic stroke disease.

#### **Limiting the findings**

We couldn't discuss on why we did not see any relationship between demographical variable like sex and age with serum iron and hemoglobin level changes.

#### **Conflict of interest**

The authors declare that they have no conflict of interest.

#### **ACKNOWLEDGMENTS**

We would like to thank the department of neurology of Rouhhani Hospital. This work was supported by grant No. 923548.

*"The authors declare no conflict of interest"*

#### **Authors' Contributions**

Dr Durdi Qujeq designed the study, performed the data analysis, prepared the manuscript, approved the final version and supervised the study. Dr Ahmadi Ahangar performed the physical examination of patients, selected the candidates and interpreted the results. Ms Ghadyani collected the data and performed the biochemical analysis.

#### **Footnotes**

Funding/support : This research was supported by the faculty of Medicine, Babol University of Medical Sciences, Babol, Iran

#### **REFERENCES**

- 1.SIESJo, BO K. Mechanisms of ischemic brain damage. *Critical Care Medicine*. 1988;16: 954-963.
- 2.Yayan, Josef.Effects of alteplase in the treatment of acute ischemic stroke. *International Journal of General Medicine*. 2012 ; 5: 743-744.
- 3.Selim, Magdy H Ratan, Rajiv R. The role of iron neurotoxicity in ischemic stroke. *Ageing Research Reviews* 2004;3: 345-353.
- 4.Watanabe, Junji Chou, Katherine J Liao, James C Miao, Yunan Meng, Hsiang-Hui Ge, Helen Grijalva, Victor Hama, Susan Kozak, Kathy Buga, Georgette. Differential association of hemoglobin with pro-inflammatory high density

lipoproteins in atherogenic/hyperlipidemic mice-a novel biomarker of atherosclerosis. *Journal of Biological Chemistry*. 2007;282: 23698-23707.

5.Lezcano, Nelson E Odo, Nadine Kutlar, Abdullah Brambilla, Donald Adams, Robert J. Regular transfusion lowers plasma free hemoglobin in children with sickle-cell disease at risk for stroke. *Stroke*. 2006;37: 1424-1426.

6.NaN, Ouyang J, Taes YE, Delanghe JR.Serum free hemoglobin concentrations in healthy individuals are related to haptoglobin type. *Clin Chem* . 2005 ; 51 : 1754-1755

7.Rother, Russell P Bell, Leonard Hillmen, Peter Gladwin, Mark T. The clinical sequelae of intravascular hemolysis and extracellular plasma hemoglobin: a novel mechanism of human disease. *Jama*. 2005; 293: 1653-1662.

8.Armengou, A Davalos, A. A review of the state of research into the role of iron in stroke. *The Journal of Nutrition, Health & Aging*. 2002;6: 207-208

9.Addison, GM Beamish, MR Hales, CN Hodgkins, M Jacobs, a Llewelin, P. An immunoradiometric assay for ferritin in the serum of normal subjects and patients with iron deficiency and iron overload. *Journal of Clinical Pathology*. 1972 ;25: 326-329.

10.García-Yébenes I, Sobrado M, Moraga A, Zarruk JG, Romera VG, Pradillo JM, Perez de la Ossa N, Moro MA, Dávalos A, Lizasoain I.Iron overload, measured as serum ferritin, ncreases brain damage induced by focal ischemia and early reperfusion. *Neurochem Int*. 2012;61(8):1364-9

11.Huang, Poyin Lo, Li-Hua Chen, Yin-Chun Lin, Ruey-Tay Shiea, Jentaie Liu, Ching-Kuan. Serum free hemoglobin as a novel potential biomarker for acute ischemic stroke. *Journal of Neurology*. 2009 ;256: 625-631.

12.Mehta PJ, Chapman S, Trouth AJ, KurukumbiM.Acute ischemic stroke secondary to iron deficiency anemia: A case report. *Case Reports in Neurological Medicine* 2012:1-5.

13.Davalos A, Fernandez-Real JM, Ricart W, Soler S, Molins A, Planas E, Genis D..Iron-related damage in acute ischemic stroke. *Stroke*1994; 25:1543-1546.