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## RESEARCH ARTICLE

# SERUM HEPCIDIN AND INTERLEUKIN-6 LEVELS IN ISCHEMIC STROKE

**[1]Petrova, J., [2,*]Manolov, V., [3]Vasilev, V., [4]Tzatchev, K. and [5]Marinov, B.**

[1]Department of Neurology, Medical University, Sofia, Bulgaria
[2]Department of Medical Genetics, Medical University, Sofia, Bulgaria
[3]Department of Clinical Laboratory and Clinical Immunology, Medical University, Sofia, Bulgaria
[4]University Hospital "Maichin Dom", Sofia, Bulgaria

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**ABSTRACT**

**Aim:** Increased hypoxia inducible factors leads to an oxidative stress, which plays an important role in neuronal injuries caused by cerebral ischemia. The free iron in human organism increases significantly during ischemia and is responsible for oxidative damage in the brain. We aimed to evaluate serum hepcidin levels in patients with ischemic stroke and connect expected levels to interleukin-6 concentration.
**Data:** We quantify serum hepcidin levels using ELISA assay in 43 patients with acute ischemic stroke. The samples were taken in the "Aleksandrovska" hospital, Department of Neurology for a period 2013 – 2014 year. We measure serum iron levels (AAS), IL-6 (CLIA) and ferritin (ECLIA) concentration. Results were compared to a control group from previous studies.
**Results:** We found significant elevated serum hepcidin levels in patients with acute ischemic stroke 79.8 ± 10.9 μg/L compared to the control group 22.4 ± 3.9 μg/L ($P < 0.001$). Serum IL-6 levels showed significant differences between the groups: control group: 2.6 ± 1.1 ng/L to patients with ischemic stroke 19.9 ± 2.1 ng/L ($P < 0.001$).
**Conclusions:** Our results indicate that serum iron and hepcidin levels are a part of etiology of cerebral ischemia.



## INTRODUCTION

The hypoxia inducible factor-1 (HIF) rises erythropoietin, downregulates hepcidin, and induces ferroportin. In human volunteers exposed to high-altitude hypoxia for a few days, ferroportin messenger ribonucleic acid synthesis increases more than 6-fold (Robach *et al.,* 2007). Hepcidin has been proposed as a risk factor for atherosclerosis (Sullivan, 2007), and there is recent evidence supporting this possibility (Valenti, 2009). Low hepcidin production combined with increased ferroportin synthesis strongly favors release of iron from reticuloendothelial storage sites and thus supports a shift of endogenous iron from storage to new red blood cell hemoglobin. Oxidative stress plays an important role in neuronal injuries caused by cerebral ischemia. It is known that free iron increases significantly during ischemia and is responsible for oxidative damage in the brain. Many mechanisms are involved in ischemia-induced brain injuries, such as oxidative stress (Aki *et al.,* 2009), increased intracellular calcium concentration (Tacchini *et al.,* 2008), inflammation (Amantea *et al.,* 2010), and elevated excitatory amino acids ( Jia *et al.,* 2009). Iron, the most abundant trace metal in the brain, is also believed to play a critical role in neuronal injuries caused by oxidative stress in ischemia, although the exact mechanism is not understood. Increased levels of free iron and ferritin have been observed in ischemic brain (Viatte *et al.,* 2005). Elevated hypoxia inducible factor 1 (HIF-1) expression causes high secretion of hepcidin (Nicolas *et al.,* 2002). Elevated serum hepcidin levels lead to internalization and degradation of the only known intracellular iron exporter ferroportin (Nemeth *et al.,* 2004). Increased hepcidin levels causes iron retention in macrophages, hepatocytes and duodenal enterocytes (Zhang *et al.,* 2009). New studies suggest that ferroportin is also expressed in the brain and might play a role in iron export from nerve cells (Moos *et al.,* 2007). In the brain, iron homeostasis depends on both iron uptake by the cells and iron export from the cell.

***Corresponding author: Manolov, V.***
*Department of Medical Genetics, Medical University, Sofia, Bulgaria.*

## MATERIALS AND METHODS

For a period of one year and a half we determined serum hepcidin levels using ELISA assay in 43 patients with ischemic stroke, (average age 57.4 ± 3.9), diagnosed in "Aleksandrovska" hospital, Dept. of Neurology. Their results were compared to age matched 39 controls. We measure serum iron levels, ferritin and IL-6. Pearson's coefficient and

Student's t-test were used for evaluation of correlation and statistical significance. We quantify hepcidin levels using verified ELISA method (Manolov *et al.,* 2014). For iron quantification we used AAS (Perkin Elmer) and for serum ferritin levels – ECLIA method (Roche Diagnostics). IL-6 was measured by ELISA method. Patients were signing the informed consent according to the Declaration of Helsinki (Directive 2001/20 / EC).

## RESULTS

We found significant elevated serum hepcidin levels in patients with acute ischemic stroke 79.8.1 ± 10.9 μg/L compared to the control group 22.4 ± 3.9 μg/L ($P < 0.001$) (Figure 1). Serum IL-6 levels showed significant differences between the groups: control group: 2.6 ± 1.1 ng/L to patients with ischemic stroke 19.9 ± 2.1 ng/L ($P < 0.001$) (Figure 2). Serum hepcidin levels were established in the previous study (Manolov *et al.,* 2014). Serum iron levels were increased in patients with ischemic stroke: 39.8 ± 4.7 μmol/L to 20.5 ± 5.4 μmol/L ($P < 0.001$).

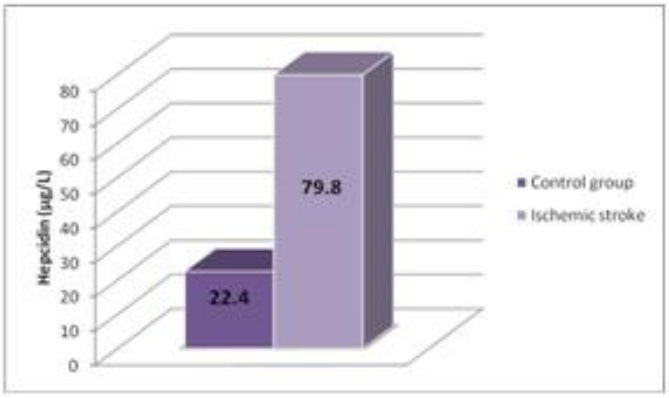


**Figure 1. Measured serum hepcidin levels**

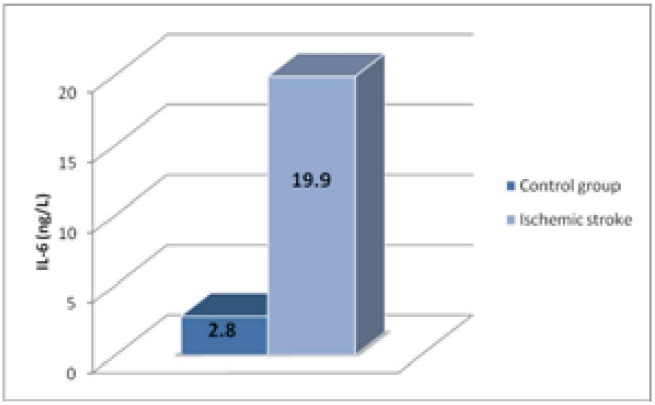


**Figure 2. Serum IL-6 concentrations in included groups**

We found a significant correlation between serum iron levels and hepcidin concentration in both groups: patients with ischemic stroke ($r = 0.620$, $P < 0.001$) and in control group ($r = 0.423$, $P < 0.001$).

## DISCUSSION

Functional studies have demonstrated that hepcidin is the central regulator of systemic iron homeostasis by regulating ferroportin, the only protein known to release iron from cells (Zhang and 2009). Ischemia-reperfusion increases hepcidin expression and down-regulates ferroportin expression in the cerebral cortex and the hippocampus (Ding *et al.,* 2001). It is known that inflammation up-regulates hepcidin expression and that cytokines are a major mediator of the inflammatory response. HIF-1 is one of the factors activated in early ischemia that can induce vascular endothelial growth factor, erythropoietin, etc. (Mabjeesh *et al.,* 2003). Previous studies have shown that iron chelators, can reduce injury caused by cardiac ischemia and reperfusion (Tang *et al.,* 2008) and cerebral ischemia (Hamrick *et al.,* 2005). In our study we found high serum hepcidin levels, elevated IL-6, iron and ferritin concentrations in patients with ischemic stroke.

### Conclusion

Recently, there are two pathways that contribute to iron overload in ischemic brain tissues as outlined. A) Ischemia increases the expression of cytokines that up-regulates hepcidin by the JAK/STAT3 pathway, which causes iron accumulation. And B) Ischemia up-regulates the HIF-1α level, which leads to iron accumulation in the ischemic tissues. Our results indicate that serum hepcidin plays an important role for iron overload in cerebral ischemia.

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