Research Paper Medical Science

# Hepcidin levels in patients with metabolic syndrom and healthy subjects in R. Macedonia

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**ABSTRACT**

In the last ten years, hepcidin has emerged as the key iron-regulatory hormone. Given its central role in iron homeostasis, hepcidin represents an appealing candidate to be investigated in subjects with metabolic syndrom features, but until now methodological difficulties have hampered large epidemiological studies. The aim of this studie is to investigate the relationships between hepcidin and metabolic syndrom. This study was carried out from January 2014 to August 2014 at the Department of medical biochemistry and Diabetes Center of Public Health Organization Clinical hospital d-r Trifun Panovski in Bitola (R.Macedonia). The total number of patients with metabolic syndrom was 120, and the remaining 120 patients we have been chosen healthy people, blood donors. Hepcidin was determined by ELISA kit (DRG Hepcidin-25 bioactive ELISA, Marburg). We found the concentration of hepcidin in males and females with metabolic syndrom had statistically higher than control group.

**KEYWORDS** hepcidin, metabolic syndrome, healthy subjects

**Introduction**

The metabolic syndrom (MetS) is a condition highly prevalent in western countries, involving near one fourth of the adult population [1]. The components of the MetS are: abdominal obesity given as waist circumference for men >102 cm (>40 in) and for women >88 cm (>35 in); triglycerides ≥150 mg/dL; HDL cholesterol for men <40 mg/dL and for women <50 mg/dL; blood pressure ≥130/≥85 mm Hg; fasting glucose ≥110 mg/dL. This components are defined by the third report of the U.S. National Heart, Lung and Blood Institute's Expert Panel on Detection, Evaluation and Treatment of High Blood Cholesterol in Adults (ATP III) (U.S. National Cholesterol Education Program Expert Panel on Detection, Evaluation and Treatment of High Blood Cholesterol in Adults, 2001) [2].

In 1997, Moirand et al. first reported the presence of histologically proven liver iron overload in overweight subjects with abnormal glucose metabolism and dyslipidemia [3].

This condition, later designated as dysmetabolic iron overload syndrome (DIOS) [4], is now known to occur in about one third of subjects with NAFLD and represents the most severe counterpart of the so-called dysmetabolic hyperferritinemia (DHF) [5]. Nevertheless, the complex pathophysiological links between iron and metabolic derangements remain poorly understood [5].

In the last ten years, hepcidin has emerged as the key iron-regulatory hormone [6]. Hepcidin was initially isolated from plasma ultrafiltrate [7] and named liver-expressed antimicrobial peptide (LEAP-1). Around the same time, it was isolated from human urine and named hepcidin after its hepatic origin and bactericidal effect in vitro [8].

The development of severe iron overload [9] by knocking out the gene in mice suggested that hepcidin is involved in iron metabolism, whereas this key role in regulation was underlined by the discovery of hepcidin mutations in patients [10]. Hepcidin was found to be regulated by inflammation, iron stores [11], hypoxia and anemia [12].

The human hepcidin gene (HAMP; OMIM 606464), located on chromosome 19q13.1, encodes a precursor protein of 84 amino acids (aa). During its export from the cytoplasm, this full-length preprohepcidin undergoes enzymatic cleavage, resulting in the export of a 64 aa pro-hepcidin peptide into the ER lumen [13]. Next, the 39 aa pro-region peptide is probably posttranslationally removed by a furin-like proprotein convertase7 resulting in mature bioactive hepcidin-25 (25 aa form). In human urine, Park et al. also identified hepcidin-22 and hepcidin-20, which are N-terminally truncated isoforms of hepcidin-25. It was confirmed that in addition to hepcidin-25, the 20 aa isoform is detectable in both human urine and serum, while the 22 aa isoform can only be detected in urine [14]. These results support the hypothesis that the 22 aa peptide is merely an urinary degradation product of hepcidin-25 [15].

Given its central role in iron homeostasis, hepcidin represents an appealing candidate to be investigated in subjects with MetS features, but until now methodological difficulties [16] have hampered large epidemiological studies. The aim of this studie is to investigate the relationships between hepcidin and MetS.

**Methods**

This study was carried out from January 2014 to August 2014 at the Department of medical biochemistry and Diabetes Center of Public Health Organization Clinical hospital d-r Trifun Panovski in Bitola (R.Macedonia). All biochemical parameters were measured in a biochemistry laboratory at the Department of medical biochemistry of Public Health Organization Clinical hospital d-r Trifun Panovski in Bitola (R.Macedonia), just hepcidin was measured in Deparmtent of Serology in of Public Health Organization Center for Public Health in Bitola(R.Macedonia). The study was approved by the Ethics Committee of Health Organization Clinical hospital d-r Trifun Panovski, and all of the procedures were performed in accordance with ethical approval institutional guidelines. The study protocol followed the ethical guidelines of the most recent Declaration of Helsinki. Written consent was obtained from

the participants prior to the start of the study.

The total number of patients with MetS was 120, recruited from Diabetes Research Centre of Health Organization Clinical hospital d-r Trifun Panovski, Bitola, R.Macedonia. Individuals aged 18 years or older were eligible to participate in the study. In this analysis we included subjects with available complete data allowing their classification according to established criteria for MetS. In detail, the following features were considered: 1) abdominal obesity, defined as the presence of waist circumference ≥102 cm in men or ≥88 cm in women; 2) fasting plasma glucose ≥ 6.1 mmol/l or drug treatment for elevated blood glucose; 3) serum triglycerides ≥ 1.69 mmol/l or drug treatment for elevated triglycerides; 4) serum HDL cholesterol in men< 1.03 mmol/l and <1.29 mmol/l in women or drug treatment for low HDL-C; 5) blood pressure ≥130/85 mmHg or drug treatment for elevated blood pressure. Subjects were considered to have MetS when they had at least three of the above-mentioned five traits.

Exclusion criteria were history of: cirrhosis or chronic hepatitis B and C, clinical evidence of bleeding in the previous 6 months, anemia (hemoglobin <120 g / L), treatment with iron in the previous year, alcohol consumption - women with daily consumption of alcohol > 40 g / day and men with daily alcohol consumption > 60 g / day, donation of blood in the previous 6 months, haemochromatosis, concomitant infections, malignant disease, chronic diseases other than diabetes mellitus type 2, immunosuppressive therapy, acute infections or invasive procedures (operations, catheterization) in the previous 6 months, neurological, endocrine or other systemic diseases, cardiovascular incident in the previous 6 months and pregnancies.

The remaining 120 patients we have been chosen healthy people, blood donors from the Department of Transfusion Medicine of Health Organization Clinical hospital d-r Trifun Panovski, Bitola, R.Macedonia. Patient had light indoor clothes and were barefooted during the measurement of their height and weight. Their standing height was measured with stadiometer to the nearest 0.1 sm. Weight was measured using a digital weight scale eith a precision of 0.1 kg. Waist and hip were measured with the tape measure. Waist-to-hip ratio (WHR) was calculated by dividing the circumference of the waist by dividing of the hip. The blood samples were taken after overnight fast (12 hours). Blood pressure was measured using a mercury manometer. Hepcidin was determined by ELISA kit (DRG Hepcidin-25 bioactive ELISA, Marburg).

The data are presented as mean± standard deviation (SD) and $p \leq 0.05$ is considered statistically significant. The results were done with the SPSS version 16.

**Results**

All 240 participants were divided in 4 groups: males control group, females control group, males with MetS group and females with MetS group.

Participants were age 18 to 60 years, for males control group (mean 39,73 ± 12,25), females control group (mean 43,57 ± 12,2), males MetS group (mean 51,03 ± 7,94) and females MetS group (mean 54,7 ± 6,43). There is not statistical significant difference between age of patients.

The concentration of hepcidin in males control group was ranged from 3 ng/mL to 36 ng/mL (mean 12,34 ± 7,37) and in females control group was ranged from 1,235 ng/mL to 14,748 ng/mL (mean 6,163± 3,202). The concentration of hepcidin in males with MetS was ranged from 2,474 ng/mL to 85,98 ng/mL (mean 25,54 ± 18,33) and in females with MetS was ranged from 2,933 ng/mL to 24,055 ng/mL (mean 11,228± 5,302).

Statistical analysis showed that males and females with MetS had statistically higher hepcidin levels than control group. Also, males hepcidine levels are higher compared to females in bouth groups – control group and MetS group.

**Table 1. Present statistical analyzes of correlation between hepcidin levels in two groups - control and MetS group**

| Variable | Contol groups N = 120 | | MetS groups N = 120 | |
|---|---|---|---|---|
| | males n= 60 | females n= 60 | males n=60 | females n=60 |
| Hepcidin mean±SD, (median- ng/mL) | 12,34 ± 7,37 (10,99) ng/mL | 6,16 ± 3,2 (5,6) ng/mL | 25,54 ± 18,33 (20,75) ng/mL | 11,23 ± 5,3 (10,81) ng/mL |
| Difference | males control group vs MetS group<br>females control group vs MetS group | | t = 5,18 p=0,000001**<br>t = 6,3 p=0,000** | |

The hepcidin concentrations in 4 groups: males control group, females control group, males with MetS, females with MetS are shown in Table 1.

**Discussion**

It has been demonstrated that the prevalence of MetS is increasing worldwide, largely the result of greater obesity and sedentary lifestyles. This is a problem because MetS increase the risk of diabetes, cardiovascular disease and mortality.

In the recent years, a bulk of evidence, particularly from epidemiological studies have established a link between iron metabolism and insulin resistant states, including type 2 diabetes mellitus and the MetS [5,17].

Some prospective studies have shown a positive association between elevated circulating ferritin concentrations and risk of type2 diabetes and MetS independent of obesity, inflammation, adipokines, and other risk factors [18]. View of iron overload disorders has radically changed with discovering of hepcidin [6], which has been demonstrated to be inappropriately low in genetic hemochromatosis [19].

Our results establish for the second time at population level that subjects with MetS have increased serum levels of hepcidin, the first one this was establish in 2013 year by Martinelli [20].

We found that concentration of serum hepcidine is associated with gender. Males hepcidine levels are higher than females levels.

We found a statistically higher hepcidin levels in both groups with MetS, compared to control groups, and males hepcidine levels are almost twice higher than females hepcidine levels in bout groups (control group and group with MetS ).

Recent experimental studies have found that leptin is able to stimulate hepatic hepcidin production [21], and a positive correlation has been found between serum levels of leptin and hepcidin in obese children [22].

Since the discovery of hepcidin 15 years ago, multiple studies have contributed insights into the regulation of hepcidin and its functional properties. The first reliable assays to quantify hepcidin in human body fluids have recently been developed, and proof-of-principle studies in human iron disorders highlight hepcidin as a promising novel tool in diagnostic medicine [23].

**Conclusions**

In summary, the rapid progress in the understanding of how hepcidin controls iron homeostasis and the intense research as

to how hepcidin levels can be altered promise new therapies in the future for diseases exacerbated by iron overload or iron depletion.

Our data support the crucial role of iron overload for metabolic diseases, even in a country with relatively high prevalence of iron deficiency.

In conclusion, hepcidin is a promising diagnostic tool but efforts must be undertaken to assess the relevance of specifically measuring hepcidin-25, to harmonize assay outcomes throughout the world, to define clinical decision limits, and to make assays available to clinical laboratories before hepcidin assays can be fully included in clinical practice.

**Competing interests**
None of the authors have professional, personal, or financial conflicts of interest to report.

**Authors' contributions**
All the authors collected information, designed and organized the structure of the contents and wrote the manuscript, reviewed literature, discussed and suggested the contents as well as edited the manuscript. Also, all the authors read and approved the final manuscript.

**Acknowledgements**
I would like to express my special appreciation and thanks to my advisor Professor Dr. Danica Labudovic have been a tremendous mentor for me. I would like to thank you for encouraging my research and for allowing me to grow as a research scientist. Your advice on both research as well as on my career have been priceless. Thanks to Pharmachem for ELISA kits that sponsored me And many thanks to my husband Sasho Ilkovski for support and technical help.

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