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Elevated Serum Hepcidin and Ferritin Levels in Patients With Metabolic Syndrom in Macedonian Population

KEYWORDS: metabolic syndrome, hepcidin, ferritin

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ABSTRACT *The metabolic syndrome is a condition highly prevalent in western countries. The serum ferritin concentration reflects iron stores in the body. In the last fifteen years, hepcidin was established as the key iron-regulatory hormone, closely related with metabolic syndrom. Aim of this study is to find elevated serum hepcidin and ferritin levels in patients with metabolic syndrom in Macedonian population.The analysed group consisted of 240 subjects – 50% of them with metabolic syndrome and 50% control group. Hepcidin levels were measured with ELISA kit (DRG Hepcidin-25 bioactive, Marburg). Metabolic syndrome subjects have significantly higher serum levels of both ferritin and hepcidin as compared to subjects in control group.The concentration of serum hepcidine and ferritin are associated with gender, males had higher levels compared to females for both groups-control and group with metabolic syndrom.*

Introduction

The metabolic syndrome (MetS) is a condition highly prevalent in western countries, involving near one fourth of the adult population (1). Although definitions vary, the essential features of MetS are represented by the deadly quartet of hyperglycemia, dyslipidemia, hypertension, and obesity (2), leading to a substantial cardiovascular risk, but also to risk of hepatic diseases, namely nonalcoholic fatty liver disease.

Iron is a ubiquitous metal of vital importance to the normal physiologic processes of many organisms (3). The first evidence linking iron to MetS was the observation that patients with hereditary hemochromatosis were at higher risk of developing type II diabetes (4,5). Hemochromatosis is an inherited disorder commonly associated with European ancestry. Patients with type 2 diabetes have a high frequency of the C282Y mutation of the hemochromatosis gene (6). The prevalence of diabetes (23%) and impaired glucose tolerance (IGT) (30%) increased in hemochromatosis compared with matched control subjects (0% diabetes and 14% IGT) (5).

The serum ferritin concentration reflects iron stores in the body (7). Ferritin is a large hollow, symmetrical protein, usually comprised of mixtures of two kinds of paired homologous but not identical subunits (heavy and light; H and L; about 20 kDa) expressed from separate genes, for a total 24 subunits and a molecular weight of about 480 kDa (8-10).

In the last fifteen years, hepcidin was established as the key iron-regulatory hormone (11). Human hepcidin is predominantly produced by hepatocytes as a 25 amino acid peptide (2789.4 Da) (12,13). At the molecular level, hepcidin acts by binding and inactivating its cell membrane receptor ferroportin, the only known cellular iron exporter. Ferroportin is particularly expressed by cells critical for iron homeostasis, like absorbing duodenal enterocytes, reticuloendothelial macrophages (involved in iron storage and recycling), and hepatocytes (involved in iron storage and endocrine regulation) (14). Given its central role in iron homeostasis, Martinelli et al. (15) in 2012 for the first time reported increased serum hepcidin levels in subjects with the MetS.

Aim of this study is to find elevate serum hepcidin and ferritin levels in patients with metabolic syndrom in Macedonian population.

Material & Methods,

The study included 240 subjects - 60 males with MetS and 60 males as control group; 60 females with MetS and 60 females as control group. The present study only analyzed data on adults, aged ≥19 years old. 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- cholesterol; 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 as followed: 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.

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Ethics approvals for the study protocol and analysis of the data were obtained from the institutional review board of PHO dr. Trifun Panovski Clinical Hospital. Written informed consent was obtained from all subjects.

Clinical and laboratory measurements

Study data included a medical history, a physical examination, information provided by a questionnaire, anthropometric measurements, and laboratory measurements. The medical and drug prescription history were assessed by the examining physicians. All of the participants were asked to respond to a health-related behavior questionnaire, which included the topics of alcohol consumption, smoking, and exercise.In addition, the participants were asked about the frequency per week of physical activities they engaged in that lasts long enough to produce perspiration such as jogging, bicycling, and swimming (≥1 time/ week).

Blood samples were collected after 12 h of fasting and drawn from an antecubital vein. Serum levels of enzymes, lipid profile, CRP, fasting serum glucose, urea, creatinine, iron, ferritin wer measured by automated chemistry analyzer (Biosystems, Spain). Hepcidin levels were measured with ELISA kit (DRG Hepcidin-25 bioactive ELISA, Marburg).

The data are presented as mean± standard deviation (SD). The results were done with the SPSS version 13.

Results

The analysed group consisted of 240 subjects – 120 patients with MetS and 120 subjects in control group.

The values of the age in control group are present in Table 1.

Control group	**Descriptive Statistics –** age			p-value
Control group	N	mean ± SD	min – max	
Total	120	41,65 ± 12,3	18 – 60	
Male	60	39,73 ± 12,25	18 – 60	t = 1,7 p = 0,089 NS
Female	60	43,57 ± 12,2	23 – 60	

As we can see in Tab.1 there is no significant statistical difference between age of male and female participants in control group.

Table 2. Present mean values ±SD, median, rang of serum concentrations of: iron, ferritin, trensferin and hepcidine in control group.

Variable	Control group			p-value
	Total N = 120	Male n = 60	Female n = 60	
Iron (µmol/l) mean±SD, median, rang	13,97 ± 6,0 13,1 3,7 – 32,4	15,03 ± 5,77 13,85 4,5 – 32,4	12,91 ± 6,1 12,2 3,7 – 27,6	Z = 2,08 p = 0,037*
Ferritin (ng/ml) mean±SD, median, rang	94,59 ± 65,9 82,0 9,0 – 309,0	120,2 ± 70,67 116,0 9 – 309	69,01 ± 49,36 56,0 10 – 257	Z = 4,12 p = 0,00004**
Transferin (mg/dl) mean±SD, median, rang	256,6 ± 52,54 249,0 134,0 – 416,0	279,9 ± 56,28 276,5 185 – 416	232,86 ± 35,44 238 134 – 335	t = 5,45 p = 0,000**
Hepcidin (ng/mL) mean±SD, median, rang	9,25 ± 6,45 6,77 1,23 – 36,46	12,34 ± 7,37 10,97 3 – 36,5	6,16 ± 3,2 5,6 1,23 – 14,75	Z = 5,38 p = 0,000**

***p<0,05 **p<0,01**

In Tab. 2 we can see that there is a significant statistical difference between males and females serum concentrations of iron, ferritin, transferine and hepcidine in participants included in control group. We can see that concentration of iron, ferritin, transferine and hepcidine is higher in males control group in a correlation with female participants in control group.

The values of the age in subjects with MetS are present in Table 3.

	Descriptive Statistics – age			p-value
	N	mean ± SD	min – max	
Total	120	52,87 ± 7,42	32 – 60	
Male	60	51,03 ± 7,94	35 – 60	t = -2.78 p=0,006**
Female	60	54,7 ± 6,43	32 – 60	NS

****p<0,01**

In Tab. 3 we can see that there is not statistical significant difeference between age of patients with MetS.

Table 4. Present serum concentrations of iron, ferritin, trensferin and hepcidine in subjects with the MetS.

Variable	Patients with MetS			p-value
	Total N = 120	Male n = 60	Female n = 60	
Iron (µmol/l) mean±SD, median, rang	15,79 ± 5,28 14,95 5,7 – 28,8	17,23 ± 5,22 16,3 7,6 – 28,8	14,36 ± 4,99 13,5 5,7 – 24,4	t = 3,07 p = 0,0026**
Ferritin (ng/ml) mean±SD, median, rang	158,47 ± 118,75 129,0 11,0 – 668,0	197,9 ± 142,57 149,5 34 – 668	118,98 ± 70,31 111,5 11 - 456	Z = 4,04 p = 0,00005**
Transferin (mg/dl) mean±SD, median, rang	255,51 ± 52,77 244,0 172,0 – 582,0	264,4 ± 61,47 249 172 – 582	246,62 ± 40,96 239 175 - 405	Z = 1,89 p = 0,058 NS
Hepcidin (ng/mL) mean±SD, median, rang	18,38 ± 15,24 14,29 2,47 – 85,98	25,54 ± 18,33 20,75 2,47 – 85,98	11,23 ± 5,3 10,81 2,93 – 24,05	Z = 5,54 p = 0,000**

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**p<0,01

In Tab. 4 we can see that there is a significant statistical difference between males and females serum concentrations of iron, ferritin, and hepcidine in participants included in control group. We can see that serum concentrations of iron, ferritine, and hepcidine is higher in males control group in a correlation with female participants.

Table 5. Present statistical analyzes of correlation between 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
Iron mean±SD, median	15,03 ± 5,77 13,85	12,91 ± 6,1 12,2	7,23 ± 5,22 16,3	14,36 ± 4,99 13,5
Difference males Control group vs MetS group t = 2,18 p=0,03* females Control group vs MetS group t = 1,4 p=0,16 NS				
Ferritin mean±SD, median	120,2 ± 70,67 116,0	69,01 ± 49,36 56,0	197,9 ± 142,57 149,5	118,98 ± 70,31 111,5
Difference males Control group vs MetS group Z = 3,2 p=0,01** females Control group vs MetS group Z = 4,8 p=0,000002**				
Transferin mean±SD, median	279,9 ± 56,28 276,5	232,86 ± 35,44 238	264,4 ± 61,47 249	246,62 ± 40,96 239
Difference males Control group vs MetS group t = 1,4 p=0,15 NS females Control group vs MetS group t = 1,96 p=0,053 NS				
Hepcidin mean±SD, median	12,34 ± 7,37 10,97	6,16 ± 3,2 5,6	25,54 ± 18,33 20,75	11,23 ± 5,3 10,81
Difference males Control group vs MetS group t = 5,18 p=0,000001** females Control group vs MetS group t = 6,3 p=0,000**				

***p < 0,05 **p < 0,01**

In table 5 we can see that there is a statistical significant difference betwin males control group and MetS group (t = 2,18 p=0,03 for p<0,05). There is present difference betwin ferritin levels for males control group and MetS group (Z = 3,2 p=0,01for p<0,01) and for females control group and MetS group (Z = 4,8 p=0,000002 for p<0,01). Also there is present difference betwin hepcidin levels for males control group and MetS group (t = 5,18 p=0,000001for p<0,01) and for females control group and MetS group (t = 6,3 p=0,000 for p<0,01).

Discussion

In the recent years, a bulk of evidence, particularly from epidemiological studies (16-19) have established a link between iron metabolism and insulin resistant states, including type 2 diabetes mellitus and the MetS (20,21). Increased body iron stores predicted the development of MetS and diabetes in healthy individuals of European ancestry (22) and recently in healthy East Asians (23). Increasing evidence has shown that body iron excess is associated with one or more MetS components (16, 24-27).

In our study we found that the males hepcidine and ferritine levels are higher compared to females in bouth groups – control group and MS group. MetS subjects heve significantly higher serum levels of both ferritin and hepcidin as compared to subjects in control group, which is similar to previous studies of Martinelli. Martinelli et al. establish for the first time at population level that subjects with MetS have increased serum levels of hepcidin. In subjects of both sexes hepcidin increased linearly with increasing number of the five classical MetS features. These data indicate that the fundamental iron regulatory feedback is preserved in MetS, i.e. that hepcidin tends to progressively increase in response to a moderate increase of iron stores, likely in the attempt to counterbalance it by limiting intestinal iron absorptionIn view of the rapidly growing evidence for pleiotropic effects of hepcidin, this may have relevant implications for the MetS pathophysiology (15).

Statistical analysis showed that males had statistically higher hepcidin and ferritin levels than women. That means that concentration of serum hepcidine and ferritin levels are associated with gender what is comparable to the scarce data reported by a few other groups (28).

Galesloot TE et al (29) find variation in hepcidin concentration over age differed between men and women. Men showed a stable hepcidin concentration, although a non-significant trend for an age-related increase in serum hepcidin was previously reported based on 65 men. In women, serum hepcidin concentration was substantially higher for postmenopausal than for premenopausal women.

Elevated serum ferritin concentrations have recently been implicated in the pathogenesis of many chronic inflammatory diseases including the MetS (30). Cross-sectional studies have shown associations of elevated serum ferritin concentration with MetS (16, 26, 27).

Conclusion:

MetS subjects had significantly higher serum levels of both ferritin and hepcidin as compared to subjects without MetS. The concentration of serum hepcidine and ferritin levels are associated with gender. In our study we found that the males hepcidine and ferritine levels are higher compared to females in bouth groups – control group and MS group. Elevated serum ferritin concentration is implicated in the pathogenesis of MetS. We show associations of elevated serum ferritin and hepcidin concentration with MetS.

Conflict of interest

The authors state that no conflict of interest exists. The authors have not received any funding or benefits from industry to conduct this study.

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REFERENCE

1.Ford, E.S., Giles, W.H.&Dietz, W.H. (2002).Prevalence of the metabolic syndrome among US adults: findings from the third National Health and Nutrition Examination Survey. JAMA, 287: 356–359. doi:10.1001/jama.287.3.356. | 2.Alberti, K.G., Eckel, R.H., Grundy, S.M., Zimmet, P.Z., Cleeman, J.I., et al.(2009). Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. Circulation,120:1640–1645. doi: 10.1161/CIRCULATIONAHA.109.192644. | 3.Heeney, M.M.& Andrews, N.C. (2004).Iron homeostasis and inherited iron overload disorders: an overview. Hematology Oncology Clinics North America, 18:1379–1403. | doi: http://dx.doi.org/10.1016/j.hoc.2004.06.018 | 4.Conte, D., Manachino, D., Colli, A., Gual,A., Aimo, G., Andreoletti, M. et al.(1998).Prevalence of genetic hemochromatosis in a cohort of Italian patients with diabetes mellitus. Annals ofinternalmedicine, 128:370-3.doi:10.7326/0003-4819-128-5-199803010-00005 | 5.McClain, D.A., Abraham, D., Rogers, J., Brady, R., Gault, P., Ajioka, R. et al. (2006).High prevalence of abnormal glucose homeostasis secondary to decreased insulin secretion in individuals with hereditaryhaemochromatosis. Diabetologia, 49:1661-9. | 6.Kwan, T., Leber, B., Ahuja, S., Carter, R., Gerstein, H.C. (1998).Patients with type 2 diabetes have a high frequency of the C282Y mutation of the hemochromatosis gene. Clin Invest Med, 21:251-7. | 7.Cook, J.D., Flowers, C.H.& Skikne, B.S.(2003). The quantitative assessment of body iron. Blood, 101:3359–3364.doi: http://dx.doi.org/10.1182/blood-2002-10-3071 | 8.Clegg, G.A., Fitton, J.E., Harrison, P.M., Treffry, A. (1980). Ferritin: Molecular structure and iron storage mechanisms. Progress in Biophysics and Molecular Biology 36(2-3):56-86. doi: 10.1016/0079-6107(81)90004-3 | 9.Harrison, P.M., Hoy, T.G., Macara, I.G., Hoare, R.J.(1974). Ferritin iron uptake and release. Structure-function relationships. Biochemical Journal 143:445-51.PMCID: PMC1168401 | 10.Linder M.C., Kakavandi H.R., Miller P., Nagel G.N. (1989).Dissociation of ferritins. Archivesof Biochemistry and Biophysics, 269:485–496. | 11.Ganz, T.(2011).Hepcidin and iron regulation, 10 years later. Blood, 117: 4425–4433. doi: 10.1182/blood-2011-01-258467 | 12.Krause, A., Neitz, S., Magert, H.J., Schulz, A., Forssmann, W.G., Schulz-Knappe, P., Adermann, K.(2000). LEAP-1, a novel highly disulfide-bonded human peptide, exhibits antimicrobial activity.Federation of European Biochemical Societies Letter, 480:147–50. doi:10.1016/S0014-5793(00)01920-7 | 13.Pigeon, C.,Ilyin, G., Courselaud, B.,Leroyer, P.,Turlin, B.,Brissot, P.,Loreal, O.(2001). A new mouse liver-specific gene, encoding a protein homologous to human antimicrobial peptide hepcidin, is overexpressed during iron overload. The Journal of Biological Chemistry,, 276:7811–9. doi: 10.1074/jbc.M008923200 | 14.Nemeth, E., Tuttle, M.S., Powelson, J., Vaughn, M.B., Donovan, A., et al. (2004).Hepcidin regulates cellular iron efflux by binding to ferroportin and inducing its internalization. Science, 306:2090–2093. doi: 10.1126/science.1104742 | 15.Martinelli, N., Traglia, M., Campostrini, N., Biino, G., Corbella, M., et al. (2012). Increased serum hepcidin levels in subjects with the metabolic syndrome: a population study. Plos one, 7: 29. doi: 10.1371/journal.pone.0048250 | 16.Bozzini, C., Girelli, D., Olivieri, O., Martinelli, N., Bassi, A., et al. (2005). Prevalence of body iron excess in the metabolic syndrome. Diabetes Care,28: 2061–2063. doi: 10.2337/diacare.28.8.2061 | 17.Sheu, W.H., Chen, Y.T., Lee, W.J., Wang, C.W., Lin, L.Y. (2003).A relationship between serum ferritin and the insulin resistance syndrome is present in non-diabetic women but not in non-diabetic men. Clinical Endocrinology, 58(3):380-5. doi: 10.1046/j.1365-2265.2003.01729.x | 18.Jiang, R., Manson, J.E., Meigs, J.B., Ma, J., Rifai, N., et al.(2004). Body iron stores in relation to risk of type 2 diabetes in apparently healthy women. JAMA,291: 711–717. doi:10.1001/jama.291.6.711. | 19.Jehn, M.L., Guallar, E., Clark, J.M., Couper, D., Duncan, B.B., et al.(2007). A prospective study of plasma ferritin level and incident diabetes: the Atherosclerosis Risk in Communities (ARIC) Study. American Journal of Epidemiology,165:1047–1054.doi: 10.1093/aje/kwk093 | 20.Rajpathak, S.N., Crandall, J.P., Wylie-Rosett, J., Kabat, G.C., Rohan, T.E., et al.(2009). The role of iron in type 2 diabetes in humans. Biochimica et Biophysica Acta ,1790:671–681. doi:10.1016/j.bbagen.2008.04.005 | 21.Dongiovanni, P., Fracanzani, A.L., Fargion, S., Valenti, L.(2011). Iron in fatty liver and in the metabolic syndrome: a promising therapeutic target.Journal of Hepatology, 55:920–932. doi:10.1016/j.jhep.2011.05.008 | 22.Fernandez-Real, J.M., Lopez-Bermejo, A. & Ricart, W. (2005). Iron stores, blood donation, and insulin sensitivity and secretion. Clinical Chemistry,51:1201-5. doi: 10.1373/clinchem.2004.046847 | 23.Ryoo, J.H., Kim, M.G., Lee, D.W., Shin, J.Y. (2011). The relationship between serum ferritin and metabolic syndrome in healthy Korean men. Diabetes Metabolisam Research and Reviews, 27:597-603. doi: 10.1002/dmrr.1211. | 24.Sun, L., Franco, O.H., Hu, F.B., Cai, L., Yu, Z., et al. (2008). Ferritin Concentrations, Metabolic Syndrome, and Type 2 Diabetes in Middle- Aged and Elderly Chinese. Journal of Clinical Endocrinology &amp Metabolism, 93(12):4690-6. doi: 10.1210/jc.2008-1159 | 25.Choi, K.M., Lee, K.W., Kim, H.Y., Seo, J.A., Kim, S.G., et al.(2005). Association among serum ferritin, alanine aminotransferase levels, and metabolic syndrome in Korean postmenopausal women.Metabolism, 54(11):1510-4. doi: 10.1016/j.metabol.2005.05.01826.González, A.S., Guerrero, D.B., Soto, M.B., Díaz, S.P., Martinez-Olmos, M., et al.(2006). Metabolic syndrome, insulin resistance and the inflammation markers C-reactive protein and ferritin.European Journal of Clinical Nutrition, 60: 802-809. doi:10.1038/sj.ejcn.1602384; | 27.Jehn, M., Clark, J.M. & Guallar, E.(2004). Serum ferritin and risk of the metabolic syndrome in US adults. Diabetes Care, 27:2422–2428. doi: 10.2337/diacare.27.10.2422 | 28.Ganz, T., Olbina, G., Girelli, D., Nemeth, E., Westerman, M.(2008). Immunoassay for human serum hepcidin. Blood, 112(10):4292-4297.doi: http://dx.doi.org/10.1182/blood-2008-02-139915 | 29.Galesloot, T.E., Vermeulen, S.H., Geurts-Moespot, A.J., Klaver, S.M., Kroot, J.Jet al.(2011). Serum hepcidin: reference ranges and biochemical correlates in the general population. Blood, 117(25):e218-25. doi: 10.1182/blood-2011-02-337907.. | 30.Fernandez-Real, J.M., Penarroja, G., Castro, A., Garcia-Bragado, F., Hernandez-Aguado, I., Ricart, W.(2002). Blood letting in highferritin type 2 diabetes: effects on insulin sensitivity and beta-cell function. Diabetescare, 51:1000-4. doi: 10.2337/diacare.25.12.2249 |