Hepcidin is not a marker of chronic inflammation in atherosclerosis

Hepcidin aterosklerozda kronik inflamasyonun bir göstergesi değildir

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ABSTRACT

Objective: To investigate the relationship between atherosclerosis, an inflammatory disease and hepcidin which is reported as an indicator of inflammation

Methods: A total of 75 subjects between 40 and 70 years of age were included in the study. The patient group consisted of 40 stable patients who had previously experienced an atherosclerotic event (18 women, 22 men; mean age 56.4±7.1 years). There were two control groups. The first control group consisted of 19 healthy subjects (11 women, 8 men; mean age 52.6± 7.4 years), while the second group included 16 patients (11 women, 5 men; mean age 56.5±9.3 years) with rheumatoid arthritis and anemia (diseased control group). Hepcidin measurement was performed using Hepcidin Prohormone ELISA (Solid Phase Enzyme-Linked Immunosorbent Assay) test kit.

Results: Mean serum hepcidin levels were 243.2±48.8 ng/ml, 374.5±86.4 ng/ml, and 234±59.9 ng/ml in the patient group, in diseased controls, and in healthy controls, respectively. Hepcidin levels were higher in diseased controls compared to the patient group and healthy controls (p=0.001). There were no significant differences between the patient group and healthy controls.

Conclusion: These findings did not support the hypothesis that hepcidin levels could be increased in atherosclerotic cardiovascular diseases as a marker of chronic inflammation. *(Anadolu Kardiyol Derg 2006; 6: 239-42)*

Key words: Hepcidin, atherosclerosis, inflammatory marker

ÖZET

Amaç: Kronik inflamatuvar bir hastalık olan ateroskleroz ile inflamatuvar bir belirteç olduğu bildirilen hepcidin arasındaki ilişkiyi araştırmak.

Yöntemler: Çalışmaya 40 ve 70 yaş arası toplam 75 olgu alındı. Hasta grubu aterosklerotik bir olay geçirmiş ve stabil olan 40 hastadan (18 kadın, 22 erkek; ortalama yaş 56.4±7.1 yıl) oluştu. İki kontrol grubu alındı. Birinci kontrol grubu 19 kişiden oluşan sağlıklı kontrol grubu idi (11 kadın, 8 erkek, ortalama yaş: 52.6± 7.4 yıl). İkinci kontrol grubu hastalıklı kontrol grubu idi. Burada romatoid artrit ve anemisi olan 16 hasta (11 kadın, 5 erkek, ortalama yaş: 56.5±9.3 yıl) vardı. Hepcidin ölçümü Hepcidin Prohormone ELISA (Solid Phase Enzyme-Linked Immunosorbent Assay) test kit'i kullanılarak yapıldı.

Bulgular: Ortalama serum hepcidin düzeyleri hasta, hastalıklı kontrol ve sağlıklı kontrol gruplarında sırasıyla 243.2±48.8 ng/ml, 374.5±86.4 ng/ml ve 234±59.9 ng/ml idi. Hastalıklı kontrol grubunun hepcidin düzeyleri hasta grubu ve sağlıklı kontrol grubuna göre yüksekti (p= 0.001). Hasta grubu ve sağlıklı kontrol grupları arasında anlamlı fark yoktu.

Sonuç: Bu bulgular kronik inflamasyonun bir göstergesi olarak hepcidin düzeylerinin, aterosklerotik kardiyovasküler hastalıklarda yüksek bulunabileceği hipotezini desteklemedi. *(Anadolu Kardiyol Derg 2006; 6: 239-42)*

Anahtar kelimeler: Hepcidin, ateroskleroz, inflamatuvar belirteç

Introduction

Inflammatory markers have been shown to play a significant role in every stage of atherosclerosis, which is regarded as a chronic inflammatory condition (1). Iron metabolism is impaired in chronic inflammatory diseases, and it is probably abnormal in the atherosclerotic process as well (2,3). Hepcidin is a 25-amino acid polypeptide produced by hepatocytes that has been shown to play a key role in iron metabolism, and it is an important mediator in anemia associated with chronic inflammatory diseases (4,5). Hepcidin is thought to regulate two key steps in iron metabolism, namely digestive iron absorption in enterocytes and iron recycling in macrophages (6). Hepcidin levels are low in iron metabolism diseases such as juvenile hemochromatosis, HFE-1 genetic hemochromatosis, and in conditions associated with increased iron requirements, while they are elevated in chronic inflammatory conditions. Pro-inflammatory cytokines such as interleukin-6 (IL-6) are thought to account for the elevated hepcidin levels in inflammatory processes (7,8).

Our objective was to investigate the possible role of hepcidin, a proposed inflammatory marker, in the atherosclerotic processes. For this purpose, hepcidin levels in patients with at-

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herosclerotic cardiovascular diseases were compared with those in healthy subjects and in a group of patients with rheumatoid arthritis, which may be associated with increased levels of hepcidin due to the frequent co-existence of anemia in this condition.

Methods

A total of 75 subjects between 40 and 70 years of age attending Outpatient Clinic of the Department of Internal Medicine, Göztepe Training and Research hospital (Istanbul, Turkey) were included in the study. Informed consent from the patients and local ethics committee approval (date and no. of approval: 02 February 2005/20) were obtained before the study procedures were commenced. The study was conducted in accordance with the Declaration of Helsinki.

Inclusion criteria: The patient group consisted of stable patients with a documented history of an atherosclerotic event (coronary artery disease confirmed by coronary angiography or previous coronary revascularization). Healthy control group consisted of subjects with no clinical or laboratory signs of atherosclerotic cardiovascular disease or any other chronic disease. The diseased control group consisted of patients with rheumatoid arthritis and anemia of chronic disease.

Exclusion criteria: Anemia, use of diet or medications known to interfere with iron metabolism, use of anti-inflammatory or immunosuppressive agents (steroids etc.), and patients with chronic inflammatory diseases were excluded from the patient and healthy control groups. Patients with severely impaired liver or kidney function, or a history of active bleeding or blood transfusion within 3 months before study entry, or current acute infections were also excluded from patient, diseased control, and healthy control groups.

Diagnosis of anemia was based on the WHO (World Health Organization) criteria (9). Demographic data were recorded and detailed physical examination was performed in patients meeting the inclusion/exclusion criteria who gave consent for participation. Also a 12-lead electrocardiography was performed. Blood pressure was measured from both arms after at least 10 minutes of rest and while the patient was sitting, and Korotkoff Phase I and IV sounds were used for the measurements. A second measurement was performed in the arm with a higher blood pressure value. Measurements were performed at least 3 minutes apart, and the average systolic and diastolic blood pressure values were calculated.

Venous blood samples were collected after 12 hours of overnight fasting and the sera were separated by centrifugation at 2500 rpm. Fasting plasma glucose, total cholesterol and triglycerides were measured by enzymatic methods, and high-density lipoprotein (HDL) cholesterol was measured by direct HDL method with an Olympus AU 5223 auto analyzer device. Low-density lipoprotein (LDL) cholesterol was calculated by Friedewald formula [10]. Full blood counts were performed in a Beckman Coulter ACT DIFF, and serum iron and total iron binding capacity were assessed by colorimetric methods in a Cromaline Photometer. Serum ferritin levels were measured by Electrochemiluminescence (ECLIA) method by a Roche Elecsys 2010 device.

Serum samples were stored at -20 °C for a short period of time. Tests were performed with Hepcidin Prohormone ELISA (Solid Phase Enzyme-Linked Immunosorbent Assay) kits manufactured by DRG International Inc. (USA), with a code number of EIA-4015 (11,12). The antibody used was prepared against Hepcidin - (28-47). The analytic sensitivity level of the test was 3.95 ng/ml (n:21, 2SD 0.055). The Intra-assay variation coefficient (CV%) was between 4.07-4.69 (n=12) and 4.82-9.76 (n=23) for three separate concentrations.

SPSS 10.0 for Windows was used for the statistical analyses. In addition to descriptive statistical methods (mean, standard deviation), One-way Anova test was used for the quantitative data. Tukey HSD and post hoc Bonferroni tests were used for pairwise comparisons, while qualitative data were compared with Chi-square test. The results were evaluated at a significance level of 0.05 with 95% confidence intervals.

Results

A total of 75 subjects were included in the study. The patient group consisted of 40 patients (18 women, 22 men; mean age 56.4±7.1 years). The first control group consisted of 19 healthy subjects (11 women, 8 men; mean age 52.6± 7.4 years), and the second diseased control group included 16 patients (11 women, 5 men; mean age 56.5±9.3 years).

Demographic characteristics: The groups were comparable with respect to mean age, gender distribution, number of diabetic patients, cigarette smoking, and alcohol consumption ($p > 0.05$). There were more hypertensives in the patient group, compared to healthy controls and diseased controls ($p=0.022$). There were no subjects with hypertension, diabetes mellitus or alcohol use among the healthy control cases (Table 1).

Medications: In the patient group, 24 (60%) of subjects were receiving antihypertensive agents, 16(40%) were receiving lipid lowering drugs, and 25(62.5%), 2(5%) and 1(2.5%) cases were receiving antiaggregants, oral antidiabetics and oral antidiabetics plus insulin, respectively. Cases in the diseased control group were receiving the following drugs: 4 cases (25%) antihypertensive agents, 1(6.2%) oral antidiabetics, 12(75%) non-steroid anti-inflammatory drug, 14 (87.5%) corticosteroid, 14(87.5%) immunosuppressive agent, 5(31.2%) folic acid and 2(12.5%) alendronat. In the healthy control group, 2(10.5%) cases were receiving temporary non-steroid anti-inflammatory treatment.

Anthropometric and biochemical data: The groups were similar with respect to systolic and diastolic BP, fasting plasma glucose, HDL cholesterol, and LDL cholesterol ($p > 0.05$). Total cholesterol was higher in the patient group compared to healthy control group ($p=0.038$), while there were no differences with regard to this parameter between the patient group and diseased control group, and between diseased control group and healthy control group ($p > 0.05$). Triglycerides were higher in the patient group compared to diseased control and healthy control groups ($p=0.030$); diseased controls and healthy controls were comparable with that respect ($p > 0.05$). Hemoglobin, hematocrit, serum iron, and total iron binding capacity were lower in the diseased control group compared to patient and healthy control groups ($p=0.001$ for all), and they were comparable between the patient group and healthy control group ($p > 0.05$). Hepcidin levels were higher in the diseased control group compared to the patient group and healthy control group ($p=0.001$), while it was similar between patient and healthy control groups ($p > 0.05$) (Table 2).

Discussion

In this study, we found no significant association between the presence of atherosclerotic disease and hepcidin levels.

Atherosclerosis is a chronic inflammatory condition associated with increased levels of inflammatory markers such as IL-6, tumor necrosis factor-α (TNF-α), soluble intercellular adhesion molecule-I, P-selectin and C-reactive protein (CRP) (13). Among these markers, most interest has been focused on CRP. There is growing evidence that serum CRP concentration is an important risk factor for cardiovascular diseases and does have prognostic significance in patients with coronary artery disease (14). Turkoglu et al (15) found that high level CRP is an independent strong marker of CAD in middle-aged patients with stable angina and positive treadmill exercise test.

Chronic inflammatory diseases such as rheumatoid arthritis are associated with varying degrees of anemia (16). Anemia of chronic disease can also be seen in some other mild inflammatory conditions, though less severe (17). Hepcidin has recently gained increased attention as an important marker of iron metabolism. Fleming and Sly (18) suggested that increased hepcidin expression may be responsible for some of the findings com-

Table 1. Demographic characteristics of patients

	Patient Group (n=40)	Diseased Control Group (n=16)	Healthy Control Group (n=19)	p
Mean age, years	56.4±7.1	56.5±9.3	52.6±7.4	NS
male	54.6±7.2	54.6±10.8	53.3±8.7	NS
female	58.7±6.4	57.5±9.0	52±6.6	NS
Sex, n (%)				
male	22 (55)	5 (31.3)	8 (42.1)	NS
female	18 (45)	11 (68.8)	11 (57.9)	
Habits, n (%)				
Smoking	3 (7.5)	2(12.5)	2(11.1)	NS
Alcohol	1 (2.5)	-	-	NS
Co-morbid conditions, n (%)				
Hypertension	26(65)	5 (31.3)	-	0.022
Diabetes Mellitus	3 (7.5)	1 (6.3)	-	NS

NS- Nonsignificant

Table 2. Comparisons of the physical examination and laboratory findings of the groups

	Patient Group (n=40)	Diseased Control Group (n=16)	Healthy Control Group (n=19)	p
Blood pressure, mmHg				
Systolic	139.1±24.4	138.7±28.3	126.9±17.4	NS
Diastolic	87.3±12.2	83.0±11.6	81.7±11.0	NS
Fasting plasma glucose, mg/dl	104.7±19.4	96.4±11.9	96.6±10.5	NS
Total cholesterol, mg/dl	194.8±47.7*	188.5±35.9	163.1±36.2	0.038
Triglycerides, mg/dl	162.3±64.6§†	121.7±82.6	122±35.7	0.030
HDL cholesterol, mg/dl	45.6±13.1	46.7±13.4	39.6±6.0	NS
LDL cholesterol, mg/dl	116.3±39.0	117.9±31.4	98.8±32.7	NS
Hemoglobin, g/dl	13.6±1.0†	10.9±0.5‡	14.2±1.4	0.001
Hematocrit, %	39.7±10.5†	31.8±2.7‡	41.1±4.2	0.001
Iron, µg/dl	68.4±20.5†	34.9±13.2‡	71±19.1	0.001
TIBC, µg/dl	341.3±38.7†	268.7±34.4‡	334.2±42.3	0.001
Ferritin, ng/ml	72.5±49.2	94.8±43.5	89.7±66.3	NS
Hepcidin, ng/ml	243.2±48.8†	374.5±86.4‡	234±59.9	0.001

Tukey HSD test, data are represented as Mean±SD

post hoc Bonferroni test: *p=0.022 vs. healthy control, §p<0.0001 vs. healthy control, †p<0.0001 vs. diseased control group, ‡p<0.0001 vs. healthy control

HDL- high density lipoprotein, LDL- low density lipoprotein, NS- nonsignificant, SD- Standard Deviation, TIBC- total iron binding capacity monly seen in anemia of chronic disease such as decreased iron, increased iron in the cells of the reticuloendothelial system, and decreased intestinal absorption of iron. Nemeth et al. (19) found increased urinary excretion of hepcidin in patients with inflammation anemia compared to healthy individuals, patients with iron deficiency, and well-controlled hereditary hemochromatosis. In that study, a correlation between increased urinary hepcidin and serum ferritin levels was also observed. It has been shown that hepcidin expression is stimulated by lipopolysaccharides and IL-6, and inhibited by TNF-α (20). In knock-out mice, Nemeth et al. (19) observed neither an increase in hepcidin expression nor a decrease in serum iron upon turpentine injection in contrast to wild type; and in cultured hepatocytes stimulated by bacterial lipopolysaccharides, a complete blockade of the acute increase in hepcidin mRNA was observed by the addition of IL-6 neutralizing antibodies. In that study, IL-6 infusion resulted in urinary excretion of hepcidin and significant decrease in serum iron in healthy human volunteers.

Studies failed to show an association between anemia of chronic disease and atherosclerosis, even though atherosclerosis is regarded as a subclinical inflammation. However, it is possible that the functional impairment in iron metabolism may precede the clinical manifestation of anemia, which is the hypothesis tested in our study. In rheumatoid arthritis patients with anemia of chronic disease, hepcidin levels were higher compared to the patient group and healthy controls. This finding suggests that hepcidin may be a reliable marker in anemia of chronic disease. However, no significant differences with regard to hepcidin levels were observed between patients with atherosclerotic cardiovascular disease and healthy controls.

A drawback of our study is that urinary excretion of hepcidin was not evaluated and only serum levels were measured. It is possible that serum hepcidin levels increase only in significant inflammatory processes, such as those in patients with rheumatoid arthritis, and that urinary excretion may be detected in a chronic, subclinical low-grade inflammatory process, such as atherosclerosis.

In conclusion, although no significant increase in hepcidin levels was observed in patients with atherosclerosis, we believe that this association deserves further study, as atherosclerosis is a common and serious condition worldwide.

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References

1. Ross R. Atherosclerosis: an inflammatory disease. N Engl J Med 1999; 340: 115-26.
2. Andrews NC. Disorders of iron metabolism. N Engl J Med 1999; 341: 1986-95.
3. Shah SV, Alam MG. Role of iron in atherosclerosis. Am J Kidney Dis 2003; 41 (Suppl 1):S80-3.
4. Ganz T. Hepcidin, a key regulator of iron metabolism and mediator of anemia of inflammation. Blood 2003; 102: 783-8.
5. Park CH, Valore EV, Waring AJ, Ganz T. Hepcidin, a urinary antimicrobial peptide synthesized in the liver. J Biol Chem 2001; 276: 7806-10.
6. Deicher R, Hörl WH. Hepcidin: a molecular link between inflammation and anaemia. Nephrol Dial Transplant 2004; 19: 521-4.
7. Nicolas G, Chauvet C, Viatte L, Danan JL, Bigard X, Devaux I, et al. The gen encoding the iron regulatory peptide hepcidin is regulated by anemia, hypoxia, and inflammation. J Clin Invest 2002; 110: 1037-44.
8. Nemeth E, Rivera S, Gabayan V, Keller C, Taudorf S, Pedersen BK, et al. IL-6 mediates hypoferremia of inflammation by inducing the synthesis of the iron regulatory hormone hepcidin. J Clin Invest 2004; 113: 1271-6.
9. WHO Scientific Group. Nutritional anaemias. Geneva: World Health Organization; 1968,
10. Friedewald WT, Levy RI, Fredrickson DS. Estimation of the concentration of low-density lipoprotein in plasma, without use of the preparative ultracentrifuge. Clin Chem 1972; 18: 499-502.
11. Kulaksiz H, Gehrke SG, Janetzko A, Rost D, Bruckner T, Kallinowski B, et al. Pro-hepcidin: expression and cell-specific localization in the liver and its regulation in hereditary hemochromatosis, chronic renal insufficiency, and renal anemia. GUT 2004; 53: 735-43.
12. Krause A, Neitz S, Magert HJ, Schulz A, Forssmann WG, Schulz-Knappe P, et al. LEAP-1, a novel highly-disulfide bonded human peptide, exhibits antimicrobial activity. FEBS Lett 2000; 480: 147-50.
13. Blake GJ, Ridker PM. Novel clinical markers of vascular wall inflammation. Clin Res 2001; 89: 763-71.
14. Acarturk E. C-reactive protein and coronary artery disease. Anadolu Kardiyol Derg 2004; 4: 203-4.
15. Turkoglu EI, Gurgun C, Zoghi M, Turkoglu C. The relationship between serum C-reactive protein levels and coronary artery disease in patients with stable angina pectoris and positive exercise stress test. Anadolu Kardiyol Derg 2004; 4: 199-202.
16. Wilson A, Yu HT, Goodnough LT, Nissenson AR. Prevalence and outcomes of anemia in rheumatoid arthritis: a systematic review of the literature. Am J Med 2004; 116 (Suppl 7A): S50-7.
17. Weiss G, Goodnough LT. Anemia of chronic disease. N Engl J Med 2005; 352: 1011-23.
18. Fleming RE, Sly WS. Hepcidin: a putative iron-regulatory hormone relevant to hereditary hemochromatosis and the anemia of chronic disease. Proc Natl Acad Sci USA 2001; 98: 8160-2.
19. Nemeth E, Valore EV, Territo M, Schiller G, Lichtenstein A, Ganz T. Hepcidin, a putative mediator of anemia of inflammation, is a type II acute-phase protein. Blood 2003; 101: 2461-3.
20. Andrews NC. Anemia of inflammation: the cytokine-hepcidin link. J Clin Invest 2004; 113: 1251-3.