Assessment of serum hepcidin levels in patients with non-ST elevation myocardial infarction

Burak Altun, Mehzat Altun, Gürkan Acar[1], Metin Kılınç[2], Hakan Taşolar[3], Ahmet Küçük[4], Ahmet Temiz, Emine Gazi, Bahadır Kırılmaz*

Department of Cardiology, Faculty of Medicine and *Department of Public Health Administration, Ministry of Health, Çanakkale Onsekiz Mart University; Çanakkale-*Turkey*
Departments of [1]Cardiology and [2]Biochemistry, Faculty of Medicine, Kahramanmaraş Sütçü İmam University; Kahramanmaraş-*Turkey*
[3]Department of Cardiology, Adıyaman University Training and Research Hospital; Adıyaman-*Turkey*
[4]Department of Anesthesiology, Faculty of Medicine, Harran University; Şanlıurfa-*Turkey*

Abstract

Objective: Hepcidin is an acute-phase reactant produced in the liver displaying intrinsic antimicrobial activity. There are few studies about hepcidin considered to be acute and chronic inflammatory marker in acute coronary syndromes patients. We investigated in our study whether the level of hepcidin has increased in the acute phase of non-ST elevation myocardial infarction patients (NSTEMI) known as acute inflammatory aggravation of chronic atherosclerotic process.

Methods: Seventy patients with NSTEMI and twenty healthy people were recruited as controls in this observational cross-sectional study. Serum hepcidin levels were determined by ELISA, and troponin levels were measured by standard laboratory methods. Levels of hepcidin and troponin were measured at admission and 6 hours later. Mean values of continuous variables were compared between groups using the Student t-test or Mann-Whitney U test, according to whether normally distributed or not, as tested by the Kolmogorov-Smirnov test. Serum troponin and hepcidin levels measured at admission and after 6th hours were compared using paired t-test.

Results: Hepcidin level was similar between NSTEMI and controls at admission (24.55±32.13, 23.67±33.62 ng/mL, p>0.05, respectively). Also, serum hepcidin levels did not change significantly from baseline in blood samples taken after 6 hour from admission in NSTEMI patients (24.55±32.13 ng/mL, 29.75±31.48 ng/mL, p=0.62, respectively). However, serum troponin levels were increased significantly compared to baseline (0.29±3.56, 2.92±7.2 ng/mL, p<0.01).

Conclusion: Our findings suggest that hepcidin could not be use as a marker of myocardial necrosis in acute phase such as troponin in patients with NSTEMI. *(Anadolu Kardiyol Derg 2014; 14: 515-8)*

Key words: hepcidin, myocardial infarction, inflammation

Introduction

Inflammatory markers have been shown to play a significant role in the pathogenesis and progression of atherosclerosis (1). Hepcidin, liver-expressed antimicrobial peptide, is an acute-phase reactant protein produced in liver displaying intrinsic antimicrobial activity (2). Nicolas et al. (3) found that gene encoding of hepcidin is regulated in response to anemia, hypoxia, and inflammation. Proinflammatory cytokines, such as IL-6, are required for induction of hepcidin and hypoferremia during inflammation (4).

There are few studies about hepcidin considered to be acute and chronic inflammatory marker in acute coronary syndromes (ACS) patients in the literature. Furthermore, discrepancy results were reported in those studies investigating serum hepcidin levels in this patient group (5-7).

Previously, there are no studies made with hepcidin in terms of usage for diagnostic purposes in patients with non-ST elevation myocardial infarction patients (NSTEMI). In light of this information, we investigated in our study whether the level of hepcidin has increased in the early period of NSTEMI known as acute inflammatory aggravation of chronic atherosclerotic process.

Methods

Study design

Seventy patients (29 females, 41 males; mean age 58.21±11.1 years) diagnosed with NSTEMI and 20 age-and gender-matched healthy volunteers (7 females, 13 males; mean age 60.75±9.05 years) as control group were included in our observational cross-sectional study between January and May 2010 in Adıyaman State Hospital. Patients were diagnosed as NSTEMI

Address for Correspondence: Dr. Hakan Taşolar, Adıyaman Üniversitesi Eğitim ve Araştırma Hastanesi, Kardiyoloji Kliniği, Adıyaman-*Türkiye*
Phone: +90 416 216 10 15-1387 Fax: +90 416 214 25 25 E-mail: hakantasolar@gmail.com
Accepted Date: 24.07.2013 **Available Online Date:** 02.01.2014

DOI:10.5152/akd.2014.4947

in case of symptoms of unstable coronary artery disease with objective signs of myocardial ischemia and elevated biochemical markers of myocardial necrosis according to European Society of Cardiology guideline (8). To detect an effect size of 0.10 at alpha error of 0.05 and statistical power of 0.80, 43 participants are required for our study. Considering the estimated consent and dropout rate of 70%, a total of 100 subjects are needed.

Healthy control group was consisted of individuals who had no clinical or laboratory signs of atherosclerotic disease or any other chronic disease. Exclusion criteria for both groups were included anemia, chronic inflammatory diseases, chronic kidney disease, acute or chronic hepatic dysfunction, chronic lung disease, chronic respiratory insufficiency, usage of any medication (e.g., anti-inflammatory or immunosuppressive agents (steroids etc.), known to affect iron metabolism, statins, non-steroidal anti-inflammatory drugs), systemic hypertension, congestive heart failure, systemic rheumatic diseases and systemic connective tissue disease.

The study was conducted according to the guidelines of the Declaration of Helsinki and was approved by the local Ethical Committee. Informed consents were obtained from all participants.

Study variables

Venous blood samples were collected at the admission and after 6th hour of admission for patients group. Lipids, fasting plasma glucose and hemoglobin were measured by routine biochemical methods. C-reactive protein (CRP) were measured by nephelometric method.

Troponin I levels were measured by Alere Triage Cardiac Panel (Alere San Diego, Inc., San Diego, CA, USA) within 15 min with the Alere Triage MeterPro device (Alere San Diego, Inc, CA, USA). Measurable ranges of Troponin I were 0.05-30 ng/mL. The analytical sensitivity of Troponin I was 0.05 ng/mL, given by the manufacturer.

Hepcidin serum samples were stored at -80°C for 3 months. Stored samples were utilized for the measurement of hepcidin levels later. Tests were performed with Hepcidin Prohormone ELISA (Solid Phase Enzyme-Linked Immunosorbent Assay) kits manufactured by USCN Life Science (USCN life Science, Inc., Wuhan, China) with a code number E91979Hu. Measurable ranges of Hepcidin were 0-1000 ng/mL. The analytical sensitivity of hepcidin was found to be <3.95 ng/mL, given by the manufacturer.

Statistical analysis

All analyses were performed using SPSS package (SPSS for Windows 17.0, Chicago, IL). All continuous variables were expressed as mean±standard deviation; categoric variables were defined as percentages. Categoric data were compared with the chi-square test. Mean values of continuous variables were compared between groups using the Student t-test or Mann-Whitney U test, according to whether normally distributed or not, as tested by the Kolmogorov-Smirnov test. Serum troponin and hepcidin levels measured at admission and after 6th hours were compared using paired t-test. P value <0.05 was considered as significant.

Results

Clinical characteristics and laboratory findings for both groups are presented in Table 1. Age, sex, systolic and diastolic blood pressure, fasting plasma glucose, HDL-cholesterol, LDL-cholesterol and smoking status are similar between the groups. Serum triglyceride levels were slightly and CRP levels were significantly higher in patients group rather than controls (p=0.03, p<0.001 respectively).

In blood samples taken instantly at admission hepcidin level was similar between NSTEMI and controls (24.55±32.13 ng/mL, 23.67±33.62 ng/mL, p>0.05, respectively). Also, serum hepcidin levels did not change significantly from baseline in blood samples taken after 6 hour from admission (24.55±32.13, 29.75±31.48 ng/mL, p=0.62, respectively). However, serum troponin levels were increased significantly compared to baseline (0.29±3.56, 2.92±7.2 ng/mL, p<0.01) (Table 2).

Discussion

Main findings of our study are as follows: (1) serum levels of hepcidin were similar at admision between NSTEMI patients and control group, (2) while serum troponin levels were significantly increased, hepcidin levels did not significantly change six hour later compared to baseline in NSTEMI patients. Considering these results, we suggest that hepcidin could not be use as a marker of myocardial necrosis in acute phase such as troponin in patients with NSTEMI, since increasing in the level of troponin was not observed in hepcidin levels 6 hours later from admission.

Hepcidin is mainly produced in the liver, and increases in response to inflammation and its expression is regulated by anemia, hypoxia and inflammation (3). The relationship with expression of mRNA and protein of hepcidin in the liver and heart have been previously reported (9). It is previously shown in human hepatocyte culture (2) that hepcidin is induced by IL-6, but not IL-1 or TNF-α, indicating that hepcidin induction by inflammation is a type II acute-phase response. In a study, investigating the relationship of serum hepcidin and acute myocardial infarction (AMI), it has been found that the levels of serum hepcidin were not correlated with the serum levels of markers for inflammation, IL-6 and CRP. They also speculated that the serum hepcidin was transiently elevated in response to acute cardiac ischemia and measurement of serum hepcidin might helpful for diagnosis of AMI (5). Hartford et al. (10) examined the inflammatory response in ACS and to assess the markers of inflammation such as IL-6, CRP, phospsolipase A2 and ICAM-1. They emphasized that there are many different inflammatory processes in patients with ACS and all markers do not

Table 1. Clinical and laboratory characteristics of subjects

	NSTEMI (n=70)	Healthy controls (n=20)	*P**
Age, years	58.21±11.1	60.75±9.05	NS
Gender, M/F	70 (41/29)	20 (13/7)	NS
SBP, mm Hg	139.1±24.5	127.2±17.6	NS
DBP, mm Hg	87.5±11.9	82.24±9.62	NS
Trigliseride, mg/dL	162.3±64.6	122±35.6	0.03
LDL-cholesterol, mg/dL	116.3±39.2	115.6±32.1	NS
HDL-cholesterol, mg/dL	45.2±13.2	46.1±13.4	NS
Glucose, mg/dL	102.2±19.4	97.2±11.5	NS
Hemoglobin, g/dL	11.9±2.1	12.2±2.0	NS
Smoking status, n†	14 (%20)	5 (%25)	NS
CRP, mg/L	3.61±1.27	0.95±0.72	P<0.001
Hepcidin on admission, ng/mL	24.55±32.13	23.67±33.62	NS
Troponin I on admission, ng/mL	0.29±3.56	-	
Peak CK, IU/L	303.2 ±85.4	-	
Peak CKMB, IU/L	51.2±13.0	-	

Values are expressed as mean ± standard deviation and number (percentage)
*Student's t-test or Mann - Whitney U test for continuous variables
†Chi-square test for categoric variables
CK - creatine kinase, CKMB - creatine kinase MB isoenzyme, CRP - C-reactive protein, DBP - diastolic blood pressure, HDL - high density lipoprotein, LDL - low density lipoprotein, NS - not significant, SBP - Systolic blood pressure

respond to the same extent to all stimuli. In our study, although increased CRP levels were increased in patients group, similar to previous studies, we did not observed increased levels of hepsidin at admission and 6 hours later. As stated previously, the reason of this may be that the inflammation occurring in ACS have many mechanisms.

In recent years, the role of hepcidin as a marker of inflammation and myocardial necrosis has been investigated in coronary artery disease and contradictory results have been achieved. Oğuz et al. (7) reported that hepcidin levels did not increased in stable patients who had previously experienced atherosclerotic event as a marker of chronic inflammation. Conversely, Suzuki et al. (5) showed that the serum hepcidin level was transiently elevated in response to acute cardiac ischemia in patients with AMI. In an experimental study, Isoda et al. (6) demonstrated that hepcidin was strongly induced in cardiomyocytes under myocarditis and myocardial infarction. To the our knowledge, there are not any studies that compared serum hepcidin levels with healthy controls and investigated changes in serum hepcidin levels in the course of myocardial infarction in patients with NSTEMI. When we looked at our results, the levels of serum hepcidin were similar between NSTEMI patients and controls. Also, there was no significant increase in serum hepcidin levels in blood samples taken at the sixth hour after admission to hospital. Therefore, our results suggest that hepcidin may not play an important role in the diagnosis of NSTEMI and could not be used as a marker of myocardial necrosis in the acute stage of myocardial infarction.

Table 2. Comparison of serum hepcidin and troponin levels on admission and 6th hours in patients with non ST elevated myocardial infarction

	On admission	After 6th hours	*P**
Hepcidin, ng/mL	24.55±32.13	29.75±31.48	0.62
Troponin I, ng/mL	0.29±3.56	2.92±7.2	<0.01

*Student's t-test or Mann-Whitney U test for continuous variables

Study limitations

This was an observational cross-sectional study, and patients could not be followed prospectively for adverse cardiac events. We did not find an association between hepcidin levels and NSTEMI patients. However, all NSTEMI patients may not have severe atherosclerosis. If our study had been done in patient populations like arteriosclerosis obliterans in which atherosclerosis was seen more severe, maybe we could achieve different results. Another limitation of our study was that the levels of hepcidin were measured only admission and 6th hours, and not measured at 12th and 24th hours.

Conclusion

The levels of serum hepcidin were not different between NSTEMI patients and healthy controls. Also, serum hepcidin levels did not significantly change in the first 6 hours of NSTEMI. Since there are conflicting results reported in studies that investigate hepcidin levels in patients with acute myocardial infarction, our results needs to be supported by further studies.

Acknowledgement: This work was supported by Kahramanmaraş Sütçü İmam University Scientific Research Projects Management.

Conflict of interest: None declared.

Peer-review: Externally peer-reviewed.

Authorship contributions: Concept - B.A., M.A., G.A., M.K., A.K.; Design - B.A., M.A., G.A., M.K., A.K., H.T.; Supervision - B.A., M.A., G.A., M.K., A.K., H.T.; Resource - B.A., M.A., G.A., M.K., A.K., A.T., H.T.; Materials - B.A., M.A., G.A., M.K., A.K., A.T.; Data collection&/or Processing - B.A., M.A., G.A., M.K., A.K., A.T., E.G., B.K., H.T.; Analysis &/or interpretation - B.A., M.A., E.G., B.K., H.T.; Literature search - B.A., M.A., E.G., B.K., H.T.; Writing - B.A, M.A., G.A.; Critical review - B.A., M.A., G.A., M.K., A.K.; A.T., E.G., B.K., H.T.

References

1. Ross R. Atherosclerosis: an inflammatory disease. N Engl J Med 1999; 340: 115-26. [CrossRef]
2. 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. [CrossRef]

3. Nicolas G, Chauvet C, Viatte L, Danan JL, Bigard X, Devaux I, et al. The gene encoding the iron regulatory peptide hepcidin is regulated by anemia, hypoxia, and inflammation. J Clin Invest 2002; 110: 1037-44. [CrossRef]
4. 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. [CrossRef]
5. Suzuki H, Toba K, Kato K, Ozawa T, Tomosugi N, Higuchi M, et al. Serum hepcidin is elevated during the acute phase of myocardiyal infarction. Tohoku J Exp Med 2009; 218: 93-8. [CrossRef]
6. Isoda M, Hanawa H, Watanabe R, Yoshida T, Toba K, Yoshida K, et al. Expression of the peptide hormone hepcidin increases in cardiomyocytes under myocarditis and myocardial infarction. J Nutr Biochem 2010; 21: 749-56. [CrossRef]
7. Oğuz A, Uzunlulu M, Hekim N. Hepcidin is not a marker of chronic inflammation in atherosclerosis. Anadolu Kardiyol Derg 2006; 6: 239-42.
8. Hamm CW, Bassand JP, Agewall S, Bax J, Boersma E, Bueno H, et al. ESC Committee for Practice Guidelines. ESC Guidelines for the management of acute coronary syndromes in patients presenting without persistent ST-segment elevation: The Task Force for the management of acute coronary syndromes (ACS) in patients presenting without persistent ST-segment elevation of the European Society of Cardiology (ESC). Eur Heart J 2011; 32: 2999-3054. [CrossRef]
9. Simonis G, Mueller K, Schwarz P, Wiedemann S, Adler G, Strasser RH, et al. The iron-regulatory peptide hepcidin is upregulated in the ischemic and in the remote myocardium after myocardial infarction. Peptides 2010; 31: 1786-90. [CrossRef]
10. Hartford M, Wiklund O, Mattsson Hultén L, Perers E, Person A, Herlitz J, et al. CRP, interleukin-6, secretory phospholipase A2 group IIA, and intercellular adhesion molecule-1 during the early phase of acute coronary syndromes and long-term follow-up. Int J Cardiol 2006; 108: 55-62. [CrossRef]