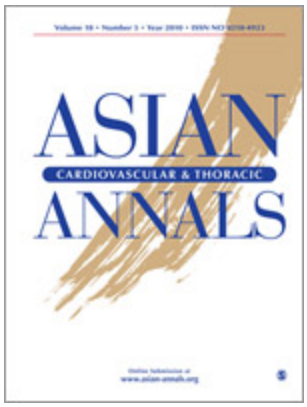


# ASSOCIATION OF HIGH MOBILITY GROUP BOX 1 PROTEIN WITH CORONARY ARTERY DISEASE

| | |
|---|---|
| Journal: | *Asian Cardiovascular and Thoracic Annals* |
| Manuscript ID | AAN-18-0465 |
| Manuscript Type: | Original Manuscript |
| Keywords: | Keywords: Coronary artery disease, HMGB1, Cytokine, Risk factors |
| Abstract: | Background: Recently, the role of inflammation in coronary artery disease and the association of inflammatory biomarkers with adverse outcomes have been investigated in many studies. We investigated the role of HMGB1 in the pathogenesis of coronary artery disease which is known to involve chronic inflammatory processes and the potential value of HMGB1as a therapeutic target.<br>Methods: 55 patients who presented to Cardiovascular Surgery Clinic and subsequently underwent coronary artery bypass surgery for coronary artery disease and 50 healthy subjects presenting to the cardiology outpatient clinic without any cardiovascular problem were included in the study.<br>Results: A total of 55 patients and 50 healthy subjects were included in the study. Mean age was 61.47±9.38 years for patients and 58.20±10.15 years for controls. There was no statistically significant difference between groups with respect to age and gender. Family history of coronary artery disease, aspirin use, hypertension and type 2 diabetes were significantly more prevalent in the patient group versus control group ($p<0.05$). A statistically significant difference was found between patients and healthy controls with respect to HMGB1 level ($p=0.001$).<br>Conclusions: Serum HMGB1 level was significantly increased in patients with coronary artery disease in comparison to healthy subjects ($p=0.001$). No associations were found between HMGB1 level and certain risk factors of coronary artery disease. These results suggest that serum HMGB1 level may be an independent risk factor in coronary artery disease. |
| | |

**Table 1. Baseline demographic and clinical characteristics of coronary artery disease patients and controls**

| | Coronary artery disease (n=55) | Controls (n=50) | p |
|---|---|---|---|
| Gender<br>Female/Male | 16/39<br>30 %/71 % | 17/33<br>34 % / 66 % | 0.58* |
| BMI (kg/m²)<br>mean± standard deviation | 29,14±4,1 | 26,46±3,57 | 0.001 |
| Age (years)<br>mean± standard deviation | 61,47±9,38 | 58,20±10,15 | 0.9 |
| Family History (%) | 47.3 | 16 | 0.01* |
| Smoking (%) | 46 | 32 | 0.15* |
| Hypertension (%) | 56 | 8 | 0.001* |
| Diabetes Mellitus (%) | 64 | 12 | 0.001* |
| Aspirin use (80-100 mg) | 90 | 10 | 0.001* |

n= number of individuals. Student's t test was used for statistical analysis.*The Chi-square test was used for statistical analysis. BMI, body mass index. p <0.05 was considered statistically significant.

**Table 2. Baseline laboratory parameters of coronary artery disease patients and controls**

| | Coronary artery disease (n=55) | Controls (n=50) | p |
|---|---|---|---|
| TC (mmol/L) Median (min-max) | 212 (17-300) | 193 (122-260) | <0.001 |
| HDL (mmol/L) Mean± standard deviation (SD) | 30 | 49,14±12,10 | <0.001* |
| LDL (mmol/L) Mean± SD | 151,2 ±52,22 | 115,56±34,6 | <0.001* |
| BUN, Median (min-max) | 17 ( 9-34) | 13(7-22) | 0.001 |
| Creatinine (mg/dL) Median (min-max) | 0.9 (0.5-4.5) | 1.12(0.6-99) | 0.007 |
| TG (mmol/L) Median (min-max) | 210(114-448) | 154(46-394) | <0.001 |
| SGOT, Median (min-max) | 22 (10-81) | 18(11-61) | 0.04 |
| SGPT, Median (min-max) | 23 (5-69) | 21(6-100) | 0.9 |
| HMGB1 (ng/ml) Median (min-max) | 20.50 (10.4-33.7) | 12.60 (3.3-1378.1) | 0.001 |

n= number of individuals. Mann-Whitney U test was used for statistical analysis. *Student's t test was used for statistical analysis.* BMI, body mass index; TC, total cholesterol; TG, triglycerides; HDL , high-density lipoprotein cholesterol; LDL, low-density lipoprotein-cholesterol; SGOT, serum glutamic oxaloacetic transaminase; SGPT, serum glutamic pyruvic transaminase. HMGB1, high mobility group box 1 protein; BUN, blood urea nitrogen, p <0.05 was considered statistically significant.

**Table 3. Pearson correlations of HMGB1 level with cardiovascular risk factors and p values**

| HMGB1 | | |
|---|---|---|
| n:105 | r | p |
| Age | 0.003 | 0.98 |
| BMI | -0.081 | 0.41 |
| TC | 0.117 | 0.24 |
| TG | 0.084 | 0.4 |
| HDL | 0.112 | 0.25 |
| LDL | 0.001 | 0.1 |

n= number of individuals. HMGB1, high mobility group box 1 protein; BMI, body mass index; TC, total cholesterol; TG, triglycerides; HDL, high-density lipoprotein cholesterol; LDL, low-density lipoprotein cholesterol; $p < 0.05$ was considered statistically significant.

**Table 4: Association between HMGB1 levels and cardiovascular risk factors**

| | HMGB1 | | |
|---|---|---|---|
| | Female | Male | p |
| Gender<br>Median (min-max) | n:33<br>18 (3,3-1378,1 | n:72<br>16,15 (6,75-32,01) | 0.4 |
| | (-) | (+) | |
| Smoking<br>Median (min-max) | n:64<br>16.9 (3.3-33.6) | n:41<br>16,11 (7,7-1378,1) | 0.9 |

n= number of individuals. Mann-Whitney U test was used for statistical analysis, $p < 0.05$ indicates statistical significance.

**ABSTRACT**

**Background:** Recently, the role of inflammation in coronary artery disease and the association of inflammatory biomarkers with adverse outcomes have been investigated in many studies. We investigated the role of HMGB1 in the pathogenesis of coronary artery disease which is known to involve chronic inflammatory processes and the potential value of HMGB1as a therapeutic target.

**Methods:** 55 patients who presented to Cardiovascular Surgery Clinic and subsequently underwent coronary artery bypass surgery for coronary artery disease and 50 healthy subjects presenting to the cardiology outpatient clinic without any cardiovascular problem were included in the study.

**Results:** A total of 55 patients and 50 healthy subjects were included in the study. Mean age was 61.47±9.38 years for patients and 58.20±10.15 years for controls. There was no statistically significant difference between groups with respect to age and gender. Family history of coronary artery disease, aspirin use, hypertension and type 2 diabetes were significantly more prevalent in the patient group versus control group ($p<0.05$). A statistically significant difference was found between patients and healthy controls with respect to HMGB1 level ($p= 0.001$).

**Conclusions:** Serum HMGB1 level was significantly increased in patients with coronary artery disease in comparison to healthy subjects ($p=0.001$). No associations were found between HMGB1 level and certain risk factors of coronary artery disease. These results suggest that serum HMGB1 level may be an independent risk factor in coronary artery disease.

**Keywords:** Coronary artery disease, HMGB1, Cytokine, Risk factors

## INTRODUCTION

Inflammation plays a key role in atherosclerotic coronary artery disease (1). Inflammatory cytokines promote the production of reactive oxygen species (ROS) which are involved in cardiovascular calcification (2).

Some studies have demonstrated that High Mobility Group Box 1 (HMGB1) protein is a novel proinflammatory cytokine associated with cardiovascular disease (3).

HMGB is a group of non-histone DNA-binding nuclear proteins which have diverse intracellular functions. HMGB1 is a 30 kDA protein bound to chromatin and released by eukaryotic cells. It is a single polypeptide chain of 215 amino acids in length and is organized into two DNA binding regions (termed box1 and box2) and a C terminal (4). The members of the HMGB protein superfamily include HMGB1, HMGB2 and HMGB3. While HMGB1is ubiquitously expressed and continues to be expressed in adults, the expression of HMGB2 and HMGB3 is more restricted and takes place only in a number of cells at certain developmental stages of life (5). HMGB1 acts as a chromatin binder. HMGB1 is capable of interacting with DNA and modifies interactions between DNA and a number of transcription factors including p53 and steroid hormone receptors by binding nonspecifically to the minor groove of DNA. It has roles in DNA repair, transcription, differentiation, extracellular signaling and somatic recombination (6). Apart from its nuclear functions, HMGB1acts as an extracellular signaling molecule when passively secreted from necrotic cells or actively secreted from inflammatory cells (7). HMGB1 can interact with receptors including RAGE (receptor for advanced glycation endproducts), TLR-2 (Toll-like receptor-2), TLR-4 and TLR-9. RAGE receptor mediates intracellular actions of extracellular HMGB1 (8). HMGB1 has been found to act as an extracellular signaling molecule in inflammation, cell differentiation, cell migration and tumor metastasis (6,9). HMGB1 has also been shown to function as an early proinflammatory mediator during states of cellular stress or damage, particularly hypoxia and necrosis (10). It was reported that HMGB1 is also a critical mediator in vascular diseases such as atherosclerosis, myocardial ischemia/reperfusion injury, heart failure and peripheral artery diseases (11). Hu et al. showed that the severity of coronary artery stenosis is correlated with serum HMGB1 levels in patients with coronary artery disease (3). Moreover, several studies have shown that high plasma HMGB1 levels may predict unfavorable clinical outcomes in heart failure such as in-hospital mortality in patients with ST-segment elevation myocardial infarction after revascularization therapy (12).

A separate study reported a positive correlation between the severity of stenosis and HMGB1 levels in coronary artery disease (3).

In light of these data, we aimed to determine serum HMGB1 levels in patients with coronary artery disease (CAD) and matched healthy subjects and investigate whether serum HMGB1 levels are associated with established risk factors for CAD.

## MATERİAL/PATİENTS AND METHODS

For this study, we enrolled 55 patients who presented to Cardiovascular Surgery Clinic of Gaziantep Özel Medikal Park Hospital between 01.06.2018 and 01.011.2018 and subsequently underwent coronary artery bypass surgery based on their SYNTAX Score (an angiographic grading tool to determine the complexity of coronary artery disease) after coronary angiography and 50 healthy subjects presenting to the cardiology outpatient clinic without any cardiovascular problem. Known risk factors for CAD including smoking, hypertension, high-density lipoprotein cholesterol (HDL cholesterol) below 40 mg/dl, total cholesterol greater than 200 mg/dl, low-density lipoprotein (LDL) cholesterol ("bad cholesterol") greater than 130 mg/dl, type 2 diabetes and obesity were recorded. Patients with any chronic inflammatory disease, psychiatric illness, malignant disease or neurogenic disease were excluded. 5 cc venous blood sample was obtained from each participant under sterile conditions and sera were separated and then stored at -80ºC until the time of HMGB1 analysis by ELISA method. It was obtained approval from the ethics committee of SANKO University clinical researches before the study (08/2017).

### HMGB1 measurement

HMGB1 analysis was performed by ELISA methodology using Biotek® ELx808 (Winooski, Vermont, USA) device located in the Biochemistry Laboratory of Gaziantep University. All concentration/absorption curves and analytical calculations were conducted using the software installed on the Biotek® ELx808 device. A commercially available testing kit (RelAssayDiagnostics® Mega Tıp Ltd., Turkey) was used for HMGB1 quantification in the samples.

For HMGB1 measurement, a blank well having a value of 0 ng/ml was used and absorbance values (optical density=OD) from all wells were obtained at a wavelength of 450 nm. Measurements were taken 10 minutes after adding ELISA stop solution. Regression equation was then constructed for the standard curve based on concentration and OD values.

### Statistical analyses

Data were presented as mean ± standard deviation or percentage. Data from all samples were analyzed by the Graph Pad Instat (v.3.05, Graph Pad Software Inc., San Diego, CA) statistical software. Student's unpaired t,

Mann-Whitney U, chi-square tests and correlation analysis were used to compare patients' data with control group data. A p value less than 0.05 was considered statistically significant.

**RESULTS**

A total of 55 patients including 16 (30%) females and 39 (71%) males and 50 healthy subjects including 17 (34%) females and 33 (66%) males. Mean age was 61.47±9.38 years for patients and 58.20±10.15 years for controls. There was no statistically significant difference between groups with respect to age and gender. Family history of CAD, aspirin use, hypertension and type 2 diabetes were significantly more prevalent in the patient group versus control group ($p<0.05$). Baseline clinical and demographic characteristics of patient and control groups are presented in Table 1.

Table 2 shows comparison of laboratory findings between patient and control groups. Statistically significant differences were observed between patients and controls in serum LDL, serum creatinine and blood urea nitrogen (BUN) levels. A highly significant difference was found between serum HMGB1 levels of the two groups ($p= 0.001$) with serum HMGB1 levels of 20.50 (10.4-33.7) in the patient group and 12.60 (3.3-1378.1) in the control group (Table 2).

Associations between risk factors for coronary artery disease and serum HMGB1 were investigated and no association was found between CAD and age, gender, body mass index (BMI), total cholesterol (TC), triglycerides (TG), HDL or LDL ($p>0.05$; Tables 3 and 4).

**DISCUSSION**

Recent studies have identified HMGB1 as a novel proinflammatory mediator in cardiovascular diseases (13-14).

HMGB1 protein is a non-histone chromosomal nuclear protein that maintains the nucleosomal structure and stability and regulates gene transcription (8).

In their study, Scaffidi et al. found that HMGB1, which is present in all mammalian cells, is passively released by necrotic cells. They reported that HMGB1 released by necrotic or damaged cells triggers the expression of proinflammatory cytokines including tumor necrosis factor- α (TNF-α) , initiating the inflammatory cascade (9).

Kalinina et al. (2004) first detected increased expression of HMGB1 in atherosclerotic plaques recovered from human postmortem samples (15). In a 2006 study, Goldstein et al. demonstrated elevated serum HMGB1 levels in patients with cerebral or myocardial ischemia (13). Additionally, high levels of HMGB1have been found in

patients with severe sepsis and in the sublining layer of patients with rheumatoid arthritis; increased serum HMGB1concentrations were also detected in humans and animals with hemorrhagic shock (16).

Hu et al. (3) have shown markedly elevated serum HMGB1 levels in patients with stable angina pectoris (SAP) and unstable angina pectoris (USAP) and reported that serum HMGB1 levels were correlated with coronary artery stenosis in patients with angina pectoris, especially in SAP patients. The same authors suggested that increased serum HMGB1 level may be a novel marker to predict adverse clinical outcomes of atherosclerotic coronary artery disease and may be involved in the pathogenesis of atherosclerotic CAD.

Several studies have shown atherosclerosis has a multifactorial etiology and its pathophysiology involves a complex series of events, and inflammatory processes induced by hyperlipidemia contribute to both initiation and propagation of atherosclerosis (1,17).

Hyperlipidemia has been shown to promote expression of a number of proinflammatory cytokines including TNF-α, interleukin-6 (IL-6) and C-reactive protein (CRP) (18). One study reported reduced levels of serum HMGB1 in hyperlipidemic patients treated with atorvastatin (19). In the present study, patients and control subjects did not differ significantly with respect to age and gender (p=0.9, p=0.58). Serum HMGB1 levels were statistically significantly different between patients with coronary artery disease and healthy subjects (p=0.001) with significantly elevated serum HMGB1 levels among patients. Also, family history of CAD, diabetes mellitus and hypertension were found to be significantly more common in the patient group versus control group (p=0.001). BMI was also significantly greater in the patient group compared to control group (p=0.001), which is consistent with literature.

In 2015, Jianjun et al. examined the relation between risk factors of coronary artery disease and serum HMGB1 and found correlations with TC, LDL and BMI (20). On the other hand, Hu et al. suggested that increased HMGB1 level is an independent risk factor for coronary artery disease (3). Correlation analyses were conducted in this study to determine whether risk factors for coronary artery disease were associated with serum HMGB1levels and no significant correlations were observed with age, BMI, TC, TG, HDL and LDL concentrations (r=0.003,r=-0.081, r=0.117, r=0.084, r=0.112, r=0.001). In contrast to Jianjun et al.'s study which found a significant association between HMGB1 level and blood lipid concentrations, we did not detect any such significant associations. However, the absence of any correlations between CAD risk factors and HMGB1 level as observed in our study suggests that HMGB1 may be an independent risk factor for CAD.

Kohno et al. showed that HMGB1 may be used to predict potential adverse clinical outcomes after acute myocardial infarction (20). All of these studies indicate that HMGB1 is a critical proinflammatory cytokine which is predominantly involved in the pathogenesis of atherosclerotic coronary artery disease. Moreover, these findings suggest that HMGB1 level may be a novel predictor of negative clinical outcomes associated with atherosclerotic CAD.

Our study is limited by small sample size. Therefore, larger samples would be needed for future studies. Also, a limited number of risk factors were examined in the study sample in relation to atherosclerotic coronary artery disease. Thus, we believe that an increased number of variables examined in a larger sample would result in better delineation of CAD risk factors in relation to HMGB levels.

**In conclusion,** HMGB1 levels were higher in patients with coronary artery disease compared to those in healthy controls. Our findings suggest that HMGB1 may be an independent risk factor for CAD, other than established risk factors. In light of our data, HMGB1 may be used as a diagnostic marker in coronary artery disease but further clinical studies are needed.

**Funding**

The authors declared that this study has received no financial support.

**Ethical approval:** All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

**Informed consent:**Informed consent was obtained from all individual participants included in the study.

**Acknowledgement:** Not applicable



**REFERENCES**

1. Hansson GK. Inflammation, atherosclerosis, and coronary artery disease. *N Engl J Med* 2005; 352:1685–1695

2. Agharazii M, St-Louis R, Gautier-Bastien A, et al. Inflammatory Cytokines and Reactive Oxygen Species as Mediators of Chronic Kidney Disease-Related Vascular Calcification. *Am J Hypertens* 2015;28:746-755.
3. Hu X, Jiang H, Bai Q, et al. Increased serum HMGB1 is related to the severity of coronary artery stenosis. *Clin Chim Acta* 2009;406:139–142.
4. Goodwin GH, Sanders C and Johns EW. A new group of chromatin-associated proteins with a high content of acidic and basic amino acids. *Eur J Biochem* 1973;38:14-19.
5. Stros M, Polanská E, Struncová S, et al. HMGB1 and HMGB2 proteins up-regulate cellular expression of human topoisomerase II alpha. *Nucleic Acids Res* 2009;37: 2070-86.
6. Müller S, Scaffidi P, Degryse B, et al. New EMBO members' review: the double life of HMGB1 chromatin protein: architectural factor and extracellular signal. *Embo J* 2001;20:4337-40.
7. Thomas JO and Travers AA. HMG1 and 2, and related 'architectural' DNA binding proteins. *Trends Biochem Sci* 2001;26:167-74.
8. Lotze MT and Tracey KJ. High-mobility group box 1 protein (HMGB1): nuclear weapon in the immune arsenal. *Nat Rev Immunol* 2005;5:331-42
9. Scaffidi P, Misteli T and Bianchi ME. Release of chromatin protein HMGB1 by necrotic cells triggers inflammation. *Nature* 2002;418:191-5.
10. Klune JR, Dhupar R, Cardinal J, et al. HMGB1: endogenous danger signaling. *Mol Med* 2008;14(7-8):476-84.
11. Cai J, Wen J and Bauer E. The Role of HMGB1 in Cardiovascular Biology: Danger Signals. *Antioxid Redox Signal* 2015;23(17):1351-69.
12. Andrassy M, VolzHC, Riedle N, et al. HMGB1 as a predictor of infarct transmurality and functional recovery in patients with myocardial infarction. *J InternMed* 2011;270(3):245-53.
13. Goldstein RS, Gallowitsch-Puerta M, Yang L, et al. Elevated high-mobility group box 1 levels in patients with cerebral and myocardial ischemia. *Shock* 2006;25:571–4.
14. Kohno T, Anzai T, Naito K, et al. Role of high-mobility group box 1 protein in postinfarction healing process and left ventricular remodelling. *Cardiovasc Res* 2009;81:565–73.
15. Kalinina N, Agrotis A, Antropova Y,et al. Increased expression of the DNA binding cytokine HMGB1 in human atherosclerotic lesions. *Arterioscler Thromb Vasc Biol* 2004; 24: 2320-2325.
16. Wang H, Bloom O, Zhang M, et al. HMG-1 as a late mediator of endotoxin lethality in mice. *Science* 1999;285:248Y251,
17. Libby P, Ridker PM and Maseri A. Inflammation and atherosclerosis. *Circulation* 2002;105:1135–1143.

18. Siasos G, Tousoulis D, Oikonomou E, et al. Inflammatory markers in hyperlipidemia: from experimental models to clinical practice. *Curr Pharm Des* 2011;17:4132–4146.

19. Daoqun J, Yongbo W, Lınz H, et al. Atorvastatin reduces serum HMGB1 levels in patients with hyperlipidemia. *ExpTherMed* 2012; 4(6): 1124–1126.

20. Jianjun Yin, Daoqun Jin and Hong Wang. Serum glycated albumin is superior to hemoglobin A1c for correlating with HMGB1 in coronary artery disease with type 2 diabetic mellitus patients. *Int J Clin Exp Med* 2015; 8(4): 4821–4825.