Circulating Heat Shock Protein 27 Is a Novel Marker of Subclinical Atherosclerosis in Type 2 Diabetes

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Abstract

Background: Heat shock protein 27 (HSP27) has been proposed as a vital protective factor in atherosclerosis. The objective of this study was to evaluate the association between circulating HSP27 and intima-media thickness (IMT) of common carotid artery in patients with type 2 diabetes and to determine if HSP27 is an independent marker for subclinical atherosclerosis in diabetes.

Methods: This was a cross-sectional community-based study consisting of 186 Chinese subjects with the median duration of diabetes of 8.2 years who underwent carotid IMT measurement by ultrasound. Serum HSP27 levels were assessed by ELISA method. *Results:* Serum HSP27 levels were significantly higher in IMT (+, ⏵1.0 mm) group than IMT (-, ≤1.0 mm) group, with the median value of 8.80 ng/mL (5.62-12.25) and 6.93 ng/mL (4.23-9.60) respectively (P=0.006). Spearman's rank correlation analysis demonstrated that the concentrations of circulating HSP27 were positively correlated with carotid IMT (r=0.198, P=0.007) and blood urea nitrogen (r=0.170, P⏵0.05). Furthermore, in the logistic model, serum HSP27 levels were independent predictors for carotid IMT in type 2 diabetic patients after adjustment for sex, smoking status, onset age of diabetes, duration of diabetes, BMI, SBP, FBG, HbA1c, TG, HDL-C, LDL-C, fasting insulin, fasting C peptide and HOMA-IR (OR=1.084, P⏵0.05).

Conclusions: Circulating HSP27, positively correlated with carotid IMT, is an independent predictor for early atherosclerotic changes in diabetes and serves as a novel marker for subclinical atherosclerosis in type 2 diabetes.

Background

Atherosclerotic cardiovascular diseases are the dominating causes of increasing mortality of diabetes patients. Increasing carotid intima-media thickness (IMT), closely associated with cardiovascular diseases, is generally accepted as a surrogate marker for atherosclerosis [1−3]. Oxidative stress, inflammation, dyslipidemia are possible factors involved in the onset and progression of arthrosclerosis.

Heat Shock Protein 27 (HSP27), also termed HSPB1 [4], is a ubiquitously expressed member of small heat shock protein family [5]. When the body is exposed to thermal, oxidative or hypoxic stresses [6], the expression of HSP27 is increased [7] to protect against these unfavorable factors. The basic role of HSP27 was originally found to be a intracellular molecular chaperone to facilitate the correct folding of proteins [8]. In recent years, multiple pleiotropic functions of HSP27 have been observed, including protecting cells from apoptosis [9−11], modulating actin cytoskeleton through promoting actin polymerization [12−15] and regulating the development of lung cancer [16]. The extracellular impacts of HSP27 on cardiovascular diseases have also been described in the protection of atherosclerosis [17]. In vitro models have showed that phosphorylated HSP27 is a promotor of proliferation of human endothelial cells and smooth muscle cells [18], which suggested a potential role of HSP27 in circulation system. In vivo models have demonstrated a progressive decline of HSP27 expression in the aortas of atherosclerotic animals by comparison to their counterparts [19]. In apolipoprotein E null (ApoE$^{-/-}$) mice,

prone to atherosclerosis, overexpression of HSP27 contributed to the reduction of lesion formation and the stability of plaques [20–22].

Clinical researches have reported that HSP27 expression was higher in normal arterial wall than atherosclerotic plaques both in carotid and coronary artery [23–26]. Serum HSP27 levels, on the other hand, were dramatically decreased in carotid atherosclerotic patients compared with healthy controls [23]. Similar to this consequence, reduced HSP27 levels were found in unstable plaques versus stable ones [27]. Furthermore, low circulating HSP27 levels were associated with high risks of coronary artery diseases [20]. Contrary to the data above, a remarkable increase in HSP27 level was reported in patients with acute coronary syndrome (ACS) [28] compared to the control group. HSP27 was also found higher in coronary heart diseases (CHD) compared with controls [29].

Although these studies have implicated certain roles of HSP27 in atherosclerotic plaques or CHD, little is known about the direct relationship between serum HSP27 concentration and subclinical arthrosclerosis in the context of type 2 diabetes. In the present study, we examined whether circulating HSP27 levels were associated with carotid IMT in patients with type 2 diabetes and determined if HSP27 can serve as a potential predictor for the early stage of atherosclerosis in diabetes.

Methods

Participants and Study Design

The trial was designed as a cross-sectional study. A total of 186 Chinese patients living in downtown Shanghai with type 2 diabetes were recruited from a community-based research [30]. According to their values of carotid IMT detected by color ultrasound, diabetic patients were stratified into the IMT (-; ≤1.0 mm) group (n = 110) and IMT (+; ⬚1.0 mm) group (n = 86) respectively. After the nature of the study was explained, all the participants gave their written informed consent. The research was approved by the Institutional Review Broad of Huashan Hospital, Fudan University School of Medicine.

Anthropometric Parameters and Biochemical Indexes

Detailed history of subjects was taken using questionnaires. Physical examination and anthropometric measurements of all recruited participants were performed by trained physicians. BMI was calculated using the formula: weight (kg)/square meter of height (m^2). Waist circumference (WC) was performed undressed at the umbilical level in a standing position. Blood pressure measurement was obtained using a standard manual mercury sphygmomanometer at a steady state on the upper arm. Venous blood samples were collected from antecubital veins of each subject after an overnight fast between 7:00 and 8:00 AM. Fasting blood glucose (FBG), serum insulin, total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), blood urea nitrogen (BUN), serum creatinine (Scr) and HSP27 were determined using standard methods in a certified laboratory with Hitachi 7080 analyzer. Glycated hemoglobin A1c (HbA1c) was assessed by high-pressure liquid chromatography (HLC-723G7; Tosoh, Shanghai, China). The homeostasis model assessment of

insulin resistance (HOMA-IR) was calculated based on the formula of Matthews at al [31]. The MDRD Study equation was used for the calculation of estimated glomerular filtration rate (eGFR) [32].

Diabetes mellitus was defined as having ≥1 of the following criteria: Fasting plasma glucose ≥ 7.0 mmol/L; Plasma glucose ≥ 11.1 mmol/L two hours after a 75 gram oral glucose load as in a glucose tolerance test (OGTT); Symptoms of high blood sugar and casual plasma glucose ≥ 11.1 mmol/L.

Measurement of Serum HSP27 Concentration

Serum HSP27 levels were measured in duplicate with a commercial enzyme-linked immunosorbent assay kit (QIA119, Calbiochem, San Diego, California) according to the manufacturer's instructions. The intra- and inter-assay coefficients of variation of the ELISA kit were 6.2 % and 8.3%, respectively.

Measurement of Carotid IMT

According to the European Mannheim carotid IMT consensus, the intima-media thickness of the common carotid arteries of all the subjects was measured by using an Acuson Sequoia 512 (Siemens Medical Solutions USA, Mountain View, CA, USA). While the patients were in a supine position, the procedure was performed by an experienced ultra-sonographer who was unaware of the subjects' demographic and clinical characteristics. Three sites of the arteris including the bilateral distal common carotid arteries, carotid bulbs, and proximal internal carotid arteries were included. Different scanning angles (anterior, lateral, posterior) were used to identify the thickest IMT in each wall. Both left and right carotid IMTs were assessed and three measurements were performed of each subject. The mean values of the maximum IMT in both left and right sides of common carotid arteries were described as carotid artery IMT.

Statistical Analysis

The quantitative data were evaluated using the Kolmogorov–Smirnov test to determine whether they followed a normal distribution. The parameters were taken as normally distributed if $P > 0.05$. Normally distributed data were reported as means and standard deviations, while variables with a skewed distribution were expressed as median (interquartile range). Categorical variables were presented as frequencies and percentages. Student's t-test, Mann-Whitney U test and Chi-squared test were used for comparisons between two groups. Spearman's correlation was used to evaluate the correlation between serum HSP27 concentration and other clinical indexes. Binary logistic regression analysis was applied to assess the determinants of carotid IMT after adjustment for confounding factors, showing odds ratios (ORs) and 95% confidence intervals (CI). All statistical analyses were conducted using the SPSS version 25 statistical package (Chicago, IL, USA) and Prism 8. Two-sided P value less than 0.05 was considered to be statistically significant.

Results

Baseline Characteristics of Patients

Basic characteristics of the 186 subjects according to carotid IMT (1.0 mm) are shown in Table 1. The median age of IMT (+) group was 70 (64.25-75), six years older than the IMT (-) group (P<0.001). Compared with IMT (-) individuals, the IMT (+) group exhibited higher onset age of diabetes (P<0.01) and had significantly higher levels of SBP (P<0.05), DBP (P<0.05), TC (P<0.05) and UACR (P<0.05). There was no statistical difference in sex, duration of diabetes, BMI, WC, WHR, smoking status, FBG, PBG, fasting insulin, fasting C peptide, 2h insulin, 2h C peptide, HOMA-IR, HbA1c, BUN, Scr, serum uric acid, eGFR, TG, LDL-C, HDL-C between the IMT (-) and IMT (+) group (all P>0.05, Table 1).

Serum HSP27 between the Two Groups

The median value of HSP27 in the subjects with type 2 diabetes was 7.85 ng/mL (IQR: 4.78-10.92, Figure 1). Among all the patients, the serum HSP27 in the IMT (+) group was 8.80 ng/mL (IQR: 5.62-12.25, Table 1), significantly higher than that in the IMT (-) subjects of 6.93ng/mL (IQR: 4.23-9.60) (P=0.006, Figure 2).

Association of Serum HSP27 with Carotid IMT

Logistic regression was used to find independent determinants of carotid IMT and to estimate odds ratios and 95% CI. Carotid IMT (≤1.0 mm or >1.0 mm) was introduced as dependent variable, while clinical characteristics including age, sex (male/female), smoking status (yes/no), onset age of diabetes, duration of diabetes, BMI, SBP, DBP, FBG, HbA1c, TC, TG, HDL-C, LDL-C, fasting insulin, fasting C peptide and HOMA-IR were defined as covariates. After adjusting for sex, smoking status, onset age of diabetes, duration of diabetes, BMI, SBP, FBG, HbA1c, TG, HDL-C, LDL-C, fasting insulin, fasting C peptide and HOMA-IR, the logistic regression model illustrated that age, DBP, TC and HSP27 were still independent risk predictors for carotid IMT in type 2 diabetes, with the adjusted odds ratios of 1.069 (P<0.001), 1.041 (P<0.05), 1.347 (P<0.05) and 1.084 (P<0.05), respectively (Table 2).

Correlations of Serum HSP27 Levels with Other Clinical Parameters

As presented in Table 3, serum HSP27 levels of the subjects correlated positively with BUN (r=0.170, P<0.05) and carotid IMT (r=0.198, P=0.007), whereas the relationships between HSP27 and other clinical parameters were not found significant.

Discussion

The major findings of this study were that median HSP27 concentrations in IMT (+) group were significantly higher than those in IMT (-) group and serum HSP27 levels were positively correlated with carotid IMT (r = 0.198, P = 0.007). Our finding that serum HSP27 concentrations were positively associated with BUN but not with other clinical parameters is in alignment with the previous study, which showed that HSP27 was related to serum creatinine [33]. Furthermore, we identified that HSP27 acted as an independent predictor for subclinical atherosclerosis in type 2 diabetic patients, even after adjusting for several clinical factors (OR = 1.084, P<0.05). The lack of circulating biomarkers for early-stage of

atherosclerosis in diabetes requires further clinical investigation. Therefore, our findings might suggest the diagnostic value of elevated circulating HSP27.

Type 2 diabetes has been widely accepted as a metabolic disorder, characterized by insulin resistance [34], oxidative stress and inflammation [35]. Atherosclerosis, as the major macrovascular complication of diabetes, is considered as a chronic inflammatory disease within the wall of arteries [36]. Oxidative stress is responsible for the pathogenesis of the process [37–39]. Park et al reported that circulating HSP27 levels in acute coronary syndrome (ACS) patients were remarkably higher compared to age-and sex-matched healthy control group. In addition, the concentration of HSP27 positively correlated with C-reactive protein and CD40L [28]. Compared with controls, the significant increase in serum HSP27 in patients with coronary heart diseases (CHD) was also observed in a recent study [29]. Another research on coronary artery disease (CAD) reported that mRNA copy number of HSP27 in peripheral blood mononuclear cells (PBMCs) was elevated in patients with CAD and was significantly associated with the severity in patients having ≥ 50% stenosis [40], indicating that HSP27 may be used as an early predictive marker for the oxidative stress status in CAD patients. Consistent with these findings, our data showed that in type 2 diabetes, serum HSP27 concentrations had a significantly positive correlation with carotid IMT. Given carotid IMT as a critical marker in subclinical arthrosclerosis [2, 41], elevated serum HSP27 provides a clue for the diagnosis and treatment in subclinical atherosclerosis in type 2 diabetes.

Although both in vivo and in vitro studies have reported the protective effects of HSP27 on atherosclerosis, the elevation of serum HSP27 may be a consequence of a compensatory response to inflammation and oxidative stress in the context of type 2 diabetes. HSP27 resistance due to the compromised HSP27 signaling pathway may also explain this phenomenon, especially in the initiation of diabetic atherosclerosis. Given the protective role of HSP27 in protecting vessels from oxidative stress [42] and inhibiting inflammation [43], the elevation of serum HSP27 in IMT (+) patients in our study is proposed to counteract with these unfavorable factors. In a case-control study, evident higher plasma HSP27 levels were found in patients with diabetic nephropathy compared to controls [33]. The mechanisms by which HSP27 exerted an atheroprotective impact were probably attributed to its binding to and/or reducing scavenger receptor-A (SR-A), thus leading to the prevention of acetylated low-density lipoprotein (acLDL) uptake and attenuation of foam cell formation in vitro [21]. HSP27 could reduce the cholesterol content in plaques by more than 30 percent as well. In ApoE mouse model, extracellular HSP27 activated NF-κB signaling pathway to induce an increased expression of granulocyte-monocyte colony-stimulating factor (GM-CSF), ATP-binding cassette transporter A1 (ABCA1) and ATP-binding cassette transporter G1 (ABCG1) to facilitate cholesterol efflux [44]. Stimulation of the release of anti-inflammatory IL-10 via p38 signaling pathway was considered to be another mechanism [45].

Contrary to these results, circulating HSP27 levels were reported decreased in patients with carotid stenosis [23] and coronary artery diseases (CAD) compared with healthy subjects [46]. In hemodialysis patients, low HSP27 levels correlated with cardiovascular diseases and sudden cardiac death [47]. Immediate responses to stress, inflammation and cellular damage are to secret HSP27 into the blood to protect the body [48], and this may provide a possible explanation for the early-phase elevation of HSP27

in atherosclerosis. In the progression of arthrosclerosis, the degradation of extracellular HSP27 by proteases such as upregulated plasmin in plaques [24], matrix metalloproteases (MMPs) [49, 50] accounted for the subsequent decline of serum HSP27.

There were several limitations of our study. First, this cross-sectional study was confined to a specific time point and was unable to indicate cause-effect relationships. Second, there was only a small number of participants in our research. Subjects without type 2 diabetes were not included. Finally, although we have identified the relationship between circulating HSP27 and carotid IMT in type 2 diabetes, underlying mechanisms of the direct role of HSP27 remains to be revealed.

Conclusions

In summary, this is the first study assessing the correlation between circulating HSP27 and carotid IMT in the context of type 2 diabetes. Our research suggests that serum HSP27 is a compensatory protein increased in the early stage of arthrosclerosis in diabetes and it serves as a novel biomarker in the progression and the diagnosis of subclinical atherosclerosis in type 2 diabetes.

Declarations

Availability of data and materials

Datasets are available from the corresponding author upon reasonable request.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

XRW, ZY, XCW proposed the idea and designed the study; BL, RH, JS collected and organized the database; XRW, JS contributed to the statistical analysis and the interpretation of the data; ZY, XCW, WZ, JW and YY supervised the project administration; XRW wrote the first draft of the paper; ZY, XCW revised the article. All authors commented on the results and read the final manuscript.

Abbreviations

intima-media thickness (IMT), body mass index (BMI), waist circumference (WC), waist-to-hip ratios (WHR), systolic blood pressure (SBP), diastolic blood pressure (DBP), fasting blood glucose (FBG), postprandial blood glucose (PBG), homeostatic model assessment of insulin resistance (HOMA-IR), glycated hemoglobin A1c (HbA1c), blood urea nitrogen (BUN), serum creatinine (Scr), estimated glomerular filtration rate (eGFR), total cholesterol (TC), triglycerides (TG), low density lipoprotein-cholesterol (LDL-C), high density lipoprotein-cholesterol (HDL-C), C-reactive protein (CRP), urine albumin-to-creatinine ratio (UACR), odds ratio (OR), confidence interval (CI)

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Tables

Table 1. General characteristics of the study subjects

Characteristic	Total	IMT (-)	IMT (+)	P value
Subjects	186	110	76	-
Age (years)	67 (57-73)	64 (53.75-71)	70 (64.25-75)	0.000*
Sex (male/female)	74/112	39/71	35/41	0.147
Onset age of diabetes (years)	57.12±11.16	54.76±11.29	60.47±10.13	0.001*
Duration of diabetes (years)	6 (3-10)	6 (3-10)	7 (4-12)	0.204
BMI (kg/m^2)	24.63±3.21	24.46±3.19	24.88±3.23	0.392
WC (cm)	81.43±8.76	81.10±8.75	81.90±8.81	0.544
WHR	0.87±0.06	0.87±0.07	0.88±0.06	0.077
Smoking (%)	76.88	78.18	75	0.613
SBP (mmHg)	140 (130-150)	140 (129.5-150)	142 (135-160)	0.006*
DBP (mmHg)	84 (78-92)	82 (76-90)	86 (80-94.75)	0.034*
FBG (mmol/L)	7.80 (6.45-10.00)	7.55 (6.3-9.65)	8.60 (6.90-10.10)	0.067
PBG (mmol/L)	15.73±6.08	15.60±6.16	15.93±6.00	0.717
Fasting insulin (pmol/L)	12.20 (7.44-20.06)	12.22 (7.35-18.81)	12.20 (7.54-21.77)	0.562
Fasting C peptide (ng/mL)	2.94 (2.22-3.78)	2.96 (2.23-3.72)	2.91 (2.15-3.86)	0.984
2h insulin (pmol/L)	46.49 (28.06-69.77)	47.46 (29.43-70.23)	43.99 (25.77-69.63)	0.763
2h C peptide (ng/mL)	8.88 (6.61-11.74)	8.84 (7.07-10.92)	8.88 (6.45-12.67)	0.580
HOMA-IR	4.56 (2.39-7.99)	4.11 (2.23-7.42)	5.27 (2.72-9.22)	0.169
HbA1c (%)	6.70 (6.00-7.65)	6.70 (6.00-7.65)	6.80 (6.03-7.75)	0.473
BUN (mmol/L)	6.00 (5.00-7.15)	5.85 (5.00-6.70)	6.20 (5.20-7.70)	0.206
Scr (μmol/L)	65 (54-78)	62 (52-76)	68 (58-80)	0.054
Serum uric acid (μmol/L)	0.27 (0.24-0.33)	0.28 (0.23-0.33)	0.27 (0.24-0.33)	0.768
eGFR (mL·min^{-1} 1.73m^{-2})	121.15±30.98	124.15±31.80	116.74±29.41	0.111
TC (mmol/L)	5.26±1.18	5.07±0.99	5.54±1.38	0.008*
TG (mmol/L)	1.60 (1.07-2.16)	1.50 (1.09-2.11)	1.63 (1.01-2.28)	0.778
LDL-C (mmol/L)	3.00 (2.40-3.40)	2.90 (2.40-3.30)	3.00 (2.40-3.60)	0.222
HDL-C (mmol/L)	1.30 (1.05-1.50)	1.20 (1.00-1.50)	1.30 (1.10-1.60)	0.116
UACR (μg/mg)	14.42 (6.04-31.06)	11.29 (4.59-24.94)	20.17 (7.38-46.28)	0.006*
HSP27 (ng/mL)	7.85 (4.78-10.92)	6.93 (4.23-9.60)	8.80 (5.62-12.25)	0.006*

Data are presented as means ± standard deviation, percentage or median (interquartile range); $P \le 0.05$ (*)

Table 2. Logistic regression analysis: independent predictors for carotid artery IMT among patients with type 2 diabetes

	B	OR	95%CI		F value
			Lower	Upper	
Age	0.067	1.069	1.033	1.108	0.000
DBP	0.04	1.041	1.009	1.073	0.012
TC	0.298	1.347	1.014	1.790	0.041
HSP27	0.08	1.084	1.012	1.161	0.022

Table 3. Correlation between serum HSP27 and other parameters in patients with type 2 diabetes

variable	HSP27	
	r	*P* value
Age	0.074	0.313
Onset age	0.091	0.217
Duration of diabetes	0.000	0.999
BMI	0.084	0.253
WC	-0.005	0.949
WHR	0.023	0.753
SBP	0.041	0.578
DBP	0.018	0.811
FBG	0.08	0.281
PBG	0.005	0.945
HbA1c	0.097	0.186
Fasting insulin	-0.142	0.054
Fasting C peptide	0.033	0.659
HOMA-IR	-0.083	0.262
BUN	0.170	0.020*
Scr	0.06	0.414
Serum uric acid	-0.075	0.312
eGFR	-0.018	0.812
TC	0.053	0.474
TG	0.035	0.637
LDL-C	0.092	0.215
HDL-C	0.070	0.341
Adiponectin	-0.079	0.583
CRP	0.104	0.333
UACR	0.098	0.182
carotid IMT	0.198	0.007*

r: Spearman's correlation coefficient. $P<0.05$ (*)

Figures
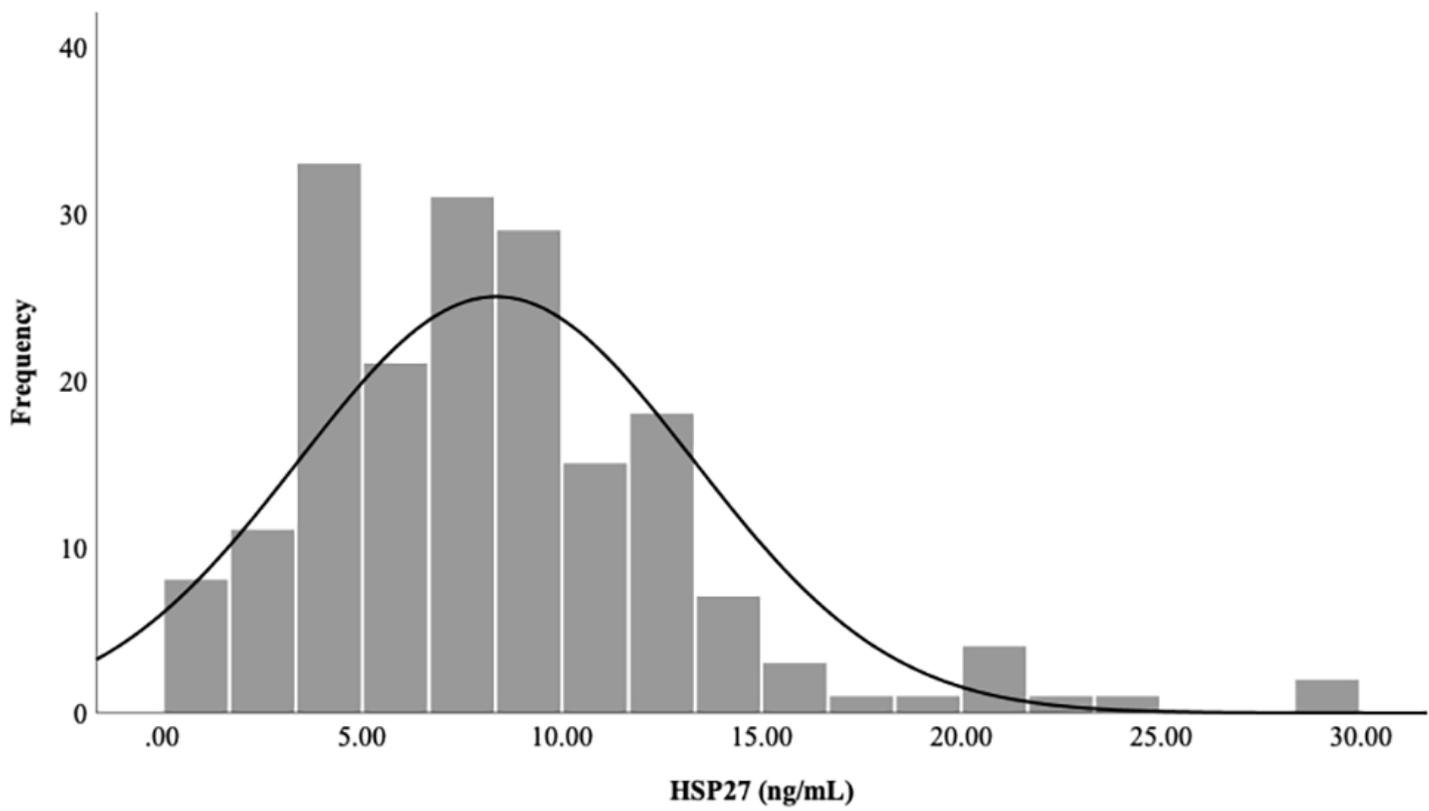
Figure 1

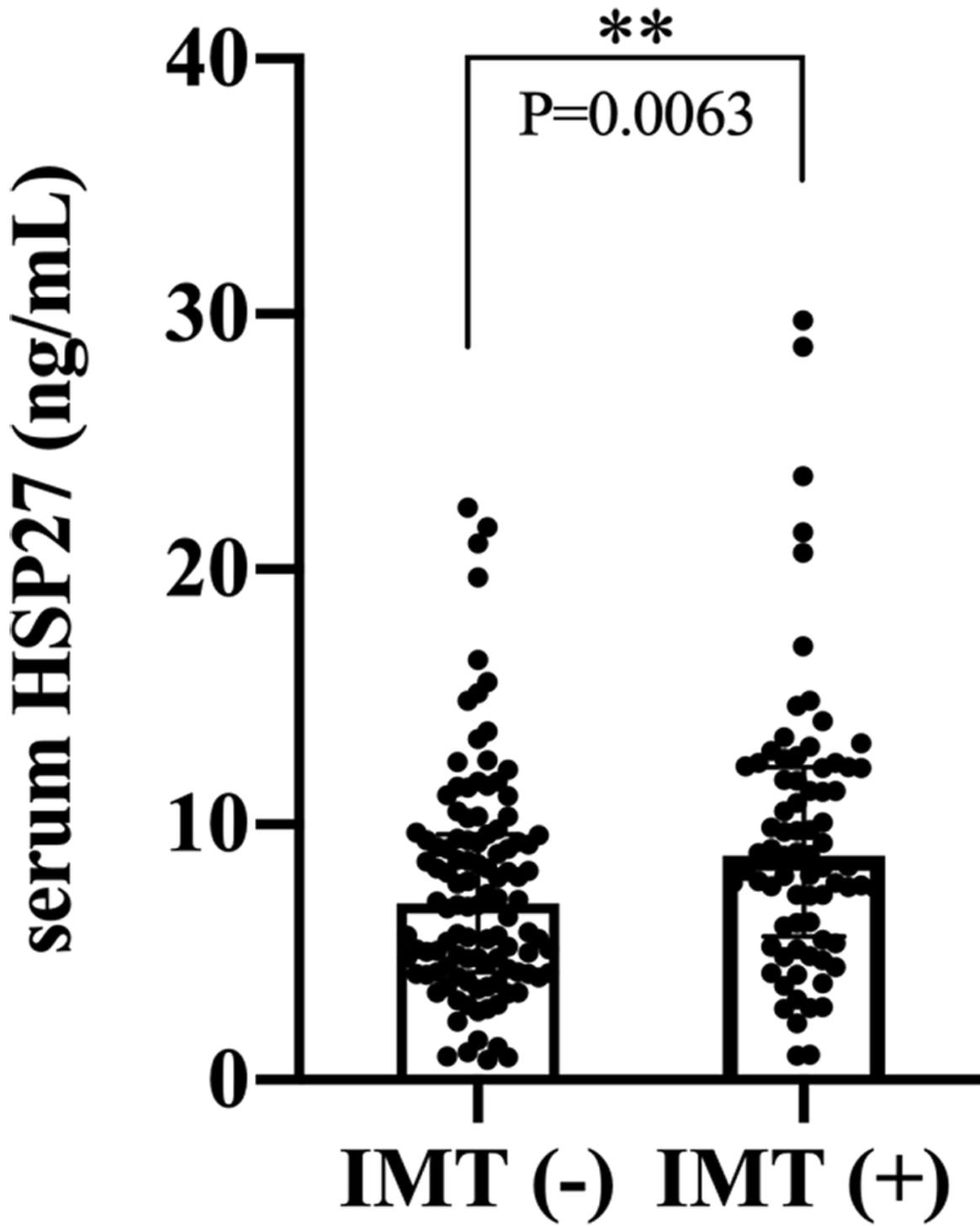Distribution of HSP27 levels in the study's population

Figure 2

Serum HSP27 levels according to the values of IMT 1.0 mm. Data are expressed as median (IQR)