Correlation Between Heat-Shock Protein 27 Serum Concentration and Common Carotid Intima-Media Thickness in Hemodialysis Patients

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Introduction. Cardiovascular disease is a major factor in the deterioration of the health and the death of hemodialysis patients. Previous studies have mainly shown a decreased level of heat shock protein 27 (HSP27) in patients with cardiovascular disease. We conducted this study to investigate whether HSP27 correlates with common carotid intima-media thickness (CCIMT) and if it has a potential to be a biomarker for cardiovascular disease.

Materials and Methods. In this cross-sectional study, the correlation between HSP27 serum concentration and CCIMT was investigated in 42 hemodialysis patients. An enzyme-linked immunosorbent assay method was used to measure HSP27 level in the plasma of the patients, and a high-resolution B-mode ultrasonography was applied to measure CCIMT.

Results. There was an inverse significant correlation between serum concentration of HSP27 and CCIMT only in patients that had hypertension as their only cardiovascular risk factor (r = -0.61, P = .02).

Conclusions. According to our results and the fact that HSP27 has been shown to be expressed in atherosclerotic plaques of both experimental animals and humans, we suggest that circulatory HSP27 concentration has a potential of being used as a marker for cardiovascular events.

INTRODUCTION

Hemodialysis patients suffer from a high prevalence of cardiovascular disease. More than half of the deaths in hemodialysis patients are attributed to cardiovascular disease. Atherosclerosis is the most frequent cause of cardiovascular mortality in patients with end-stage renal diseases.1-3 Atherosclerosis is a multifactorial disease in which hypertension, diabetes mellitus, dyslipidemia, and other risk factors are thought to play a role.4,5 Atherosclerotic plaques develop as a consequence of the accumulation of circulating lipid and the subsequent migration of inflammatory cells (macrophages and T lymphocytes) and vascular smooth muscles cells (VSMCs). Advanced plaques consist of a lipid-rich core, separated from the lumen by a fibrous cap composed of VSMCs, collagen, and extracellular matrix.6-9

Heat-shock protein 27 (HSP27) is a molecular chaperone involved in different processes, such as the response to stress, modulation of the actin cytoskeleton, and apoptosis.7,10 Heat-shock
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...protein 27 can block the apoptotic pathway at different levels. It can interact with death domain associated protein (it is a pro-apoptotic protein) and prevent Fas-mediated apoptosis. Moreover, HSP27 can block the intrinsic pathway by promoting the retention of cytochrome C and inhibiting the release of pro-apoptotic molecule known as second mitochondrial-derived activator of caspase/direct inhibitor of apoptosis protein-binding protein with low pi. On the other hand, HSP27 can downregulate the apoptotic signaling pathway and could thus contribute to stabilizing atherosclerotic lesions. It was found that HSP27 secretion was decreased in atherosclerotic lesions. Therefore, it was identified as a potential marker of atherosclerosis. Recently, several studies have shown a significant decrease of HSP27 in atheroma plaque secretion versus the nondamaged arterial segment demonstrated by proteomic approach and 2-dimensional electrophoresis.

Among noninvasive diagnostic methods for atherosclerosis, ultrasonography of the carotid artery is useful for measuring common carotid intima-media thickness (CCIMT). Increased CCIMT is associated with vascular risk factors and the presence of more advanced atherosclerosis, including coronary artery disease. Common carotid intima-media thickness is a strong predictor for cardiovascular events in the general population and is an independent predictor of cardiovascular mortality in hemodialysis patients.

**MATERIALS AND METHODS**

**Participants**

In a cross-sectional study, 41 patients (26 men and 16 women) that were receiving maintenance hemodialysis therapy for at least 6 months were enrolled in the hemodialysis unit of Imam Reza Hospital in Mashhad, Iran, between July 2007 and November 2008. This study was accepted by the Ethics Committee of Mashhad University of Medical Science. Written informed consent for the study was obtained from all patients.

**Biochemical Assays**

Venous blood was taken in the morning after an overnight fasting at least 12 hours before a hemodialysis session. Serum samples were immediately removed by centrifugation (10 minutes at 10000 g) and stored at -70°C until to 1.6 m² surface area; Fresenius Medical Care, Homburg, Germany) using bicarbonate dialysis fluid. All patients were undergoing hemodialysis with a mean value of KT/V of 2.28 ± 0.91 (Table 1). The patients’ age was in the range of 18 to 60 years. Patients with heart failure (ejection fraction < 40% based on echocardiography), thyroid disease, liver dysfunction, diabetes mellitus, acute infection, malignancy, and autoimmune diseases (except the ones of renal origin such as glomerulonephritis) were excluded from the study.

Data on demographic characteristics, laboratory studies, medication history, past medical history, familial history, and cardiovascular risk factors were collected for each patient.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>36.2 ± 13.2</td>
</tr>
<tr>
<td>Female-male ratio</td>
<td>0.62</td>
</tr>
<tr>
<td>Dialysis duration, mo</td>
<td>20.5 ± 20.2</td>
</tr>
<tr>
<td>Plaque occurrence, %</td>
<td>24.5</td>
</tr>
<tr>
<td>Laboratory tests</td>
<td></td>
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<tr>
<td>KT/V</td>
<td>2.28 ± 0.91</td>
</tr>
<tr>
<td>Serum calcium, mg/dL</td>
<td>9.50 ± 0.62</td>
</tr>
<tr>
<td>Serum phosphorus, mg/dL</td>
<td>5.84 ± 1.96</td>
</tr>
<tr>
<td>Plasma parathyroid hormone, pg/mL</td>
<td>256.97 ± 253.50</td>
</tr>
<tr>
<td>Serum creatinine, mg/dL</td>
<td>9.98 ± 2.81</td>
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<tr>
<td>HDLC, mg/dL</td>
<td>41.14 ± 7.99</td>
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<td>LDLC, mg/dL</td>
<td>82.5 ± 28.83</td>
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<tr>
<td>Serum cholesterol, mg/dL</td>
<td>159.62 ± 40.09</td>
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<tr>
<td>Serum triglycerides, mg/dL</td>
<td>163.65 ± 101.63</td>
</tr>
<tr>
<td>Serum HSP27, ng/mL</td>
<td>10.58 ± 3.50</td>
</tr>
<tr>
<td>CCIMT, mm</td>
<td>0.88 ± 0.12</td>
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**Cardiovascular risk factors**

<table>
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<th></th>
<th>Value</th>
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<tr>
<td>Hypertension, %</td>
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<tr>
<td>As only risk factor</td>
<td>34.0</td>
</tr>
<tr>
<td>Dyslipidemia, %</td>
<td>7.3</td>
</tr>
<tr>
<td>Positive family history, %</td>
<td>22.0</td>
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<tr>
<td>Diabetes mellitus, %</td>
<td>0</td>
</tr>
<tr>
<td>Active smoking, %</td>
<td>9.8</td>
</tr>
<tr>
<td>BMI &gt; 27 kg/m², %</td>
<td>17.0</td>
</tr>
</tbody>
</table>

*HDLC indicates high-density lipoprotein cholesterol; LDLC, low-density lipoprotein; HSP27, heat-shock protein 27; CCIMT, common carotid intima-media thickness; and BMI, body mass index.
analyzed. Biochemical measurements including plasma glucose, total cholesterol, triglyceride, low-density lipoprotein cholesterol (LDLC), high-density lipoprotein cholesterol (HDLC), creatinine, calcium, phosphorus, urea, parathyroid hormone, and albumin were carried out by routine laboratory methods.

Heat-Shock Protein 27 Serum Concentration

Serum levels of soluble HSP27 were measured with an enzyme-linked immunosorbant assay kit (Calbiochem, London, UK). Each assay was calibrated using an HSP27 standard curve according to the manufacturer’s instruction. Each sample was measured in duplicate.

Common Carotid Intima-Media Thickness Measurement

High resolution B-mode ultrasonography was carried out to take the image of the right and left common carotid arteries. Common carotid intima-media thickness was measured between the lumen intima and media-adventia interfaces of the far wall of the common carotid arteries by a single reader, using an automated edge detection system. The mean CCIMT of this 1-cm segment was measured on 2 separate images of the left and right common carotid arteries, and the peak of the R wave of these four measurements was used as the CCIMT.

Statistical Analyses

Statistical analyses were performed using the SPSS software (Statistical Package for the Social Sciences, version 11.5, SPSS Inc, Chicago, Ill, USA). All measured values were presented as the mean ± standard deviation. Correlation between serum concentration of HSP27 and CCIMT was analyzed using the Pearson correlation coefficient. For comparison of serum concentration of HSP27 between different groups, the 1-way analysis of variance test was used. P values less than .05 were taken as significant.

RESULTS

Characteristics of Participants

The study sample contained 42 hemodialysis patients. Data on demographic characteristics, plaque occurrence (based on carotid ultrasonography), biochemical data (laboratory tests) including HSP27 levels, CCIMT, traditional cardiovascular risk factors, and dialysis duration are summarized in Table 1. Hypertension and dyslipidemia were determined based on the standard guidelines. All of the patients were taking an angiotensin-converting enzyme inhibitor and only dyslipidemic patients were taking statins.

Heat-Shock Protein 27 and Carotid Intima-Media Thickness

There was no significant correlation between serum concentrations of HSP27 and CCIMT (r = -0.008, P = .96). However, there was an inverse significant correlation between serum concentrations of HSP27 and CCIMT in 14 patients with hypertension as their only cardiovascular risk factor (r = -0.61, P = .02; Figure 1).

Heat-Shock Protein 27 and Cardiovascular Risk Factors

Cardiovascular risk factors measured in this study were hypertension, dyslipidemia, family history of heart disease, current active smoking, age above 55 years for women and 45 years for men, and a body mass index (BMI) greater than 27 kg/m². The patients were classified based on the number of their cardiovascular risk factors. Serum HSP27 concentrations were compared between these groups (Table 2). No significant difference was found in HSP27 levels between subgroups of the number of cardiovascular risk factors (P = .89).

No statistically significant difference was found in serum concentration of HSP27 between patients

Figure 1. Correlation between serum concentration of heat-shock protein 27 (HSP27) and common carotid intima-media thickness (CCIMT) in patients with hypertension as their only cardiovascular risk factor (r = -0.61, P = .02).
with hypertension \((P = .40)\), dyslipidemia \((P = .26)\), family history of heart disease \((P = .80)\), active smoking \((P = .37)\), and age groups \((P = .94)\) and those without each of these factors. Also, there was no correlation between the serum concentration of HSP27 and patients age \((r = 0.162, P = .32)\), BMI \((r = 0.107, P = .52)\), plaque occurrence \((P = .20)\), or serum concentrations of total cholesterol \((r = 0.30, P = .14)\), LDLC \((r = 0.406, P = .15)\), HDLC \((r = 0.114, P = .70)\), and triglyceride \((r = 0.059, P = .78)\).

**Carotid Intima-Media Thickness and Cardiovascular Risk Factors**

A significant difference was found in CCIMT values between patients based on their age group \((P < .001)\), dyslipidemia \((P = .005)\), and presence of atherosclerotic plaque \((P < .001; \text{Figure } 2)\). However, no significant difference was found in CCIMT values between patients with and without hypertension \((P = .11)\), family history of heart disease \((P = .93)\), current active smoking \((P = .11)\), and a BMI greater than 27 \((P = .10)\).

There was no correlation between CCIMT and dialysis duration \((r = 0.195, P = .23)\), gender \((P = .06)\), or serum concentrations of total cholesterol \((r = 0.278, P = .17)\), LDLC \((r = 0.205, P = .481)\), HDLC \((r = 0.327, P = .26)\), and triglyceride \((r = 0.310, P = .12)\), but a relationship between CCIMT with patient age \((r = 0.570, P < .001)\) was found (Figure 3).

**Carotid Intima-Media Thickness and Other Factors**

There was no correlation between the CCIMT and serum calcium \((r = 0.265, P = .10)\), phosphorus \((r = 0.293, P = .07)\), or parathyroid hormone \((r = -0.043, P = .81)\) concentrations.

**DISCUSSION**

Our results indicated that there was an inverse significant correlation between serum concentration of HSP27 and CCIMT only in hemodialysis patients with hypertension as a cardiovascular risk factor.
However, no overall significant correlation between serum concentration of HSP27 and CCIMT was found. We also investigated the correlation between CCIMT and some traditional cardiovascular risk factors. Among them, dyslipidemia, age, and atherosclerotic plaque correlated with CCIMT. A small subgroup of the patients (7.3%, 3 patients) were taking a statin to control their dyslipidemia. It has been found that atorvastatin reduces HSP27 level secreted by cultured atherosclerotic plaques. As no correlation was found between HSP27 concentrations and CCIMT in these patients, this drug treatment has no implication on our results.

Atherosclerosis, the major cause of cardiovascular disease, is a multifactorial condition with what appears to be a strong chronic inflammatory component. Renal insufficiency has been shown to be a significant and independent risk factor for carotid atherosclerosis, suggesting that renal insufficiency per se accelerates the atherosclerotic process. Recently, chronic kidney disease (CKD) has been recognized as an independent risk factor, as well. Epidemiologic studies have documented an extraordinarily high incidence of death from chronic vascular disease in patients with CKD.

In the general population, different markers of target-organ damage (such as echocardiographically determined left ventricular hypertrophy) have prognostic values. Common carotid intima-media thickness is also a marker of target-organ damage and preclinical asymptomatic atherosclerosis in the general population. Data from previous studies suggest that increased CCIMT can be used as an early marker for atherosclerosis. It is not exactly known how and why the level of kidney function is in direct association with an advanced atherosclerosis, expressed as an increasing CCIMT. A decrease of kidney function is associated with traditional risk factors for atherosclerosis, such as hypertension, diabetes mellitus, dyslipidemia, and obesity, and is probably also associated with an increase in nontraditional risk factors, including micro-inflammation and decreased anti-oxidative defenses. These novel risk factors may play a very important role, because there are many patients with classic risk factors for atherosclerosis with normal kidney function and also a normal CCIMT.

Experimental animal and human studies have implicated several HSPs, including Hsps-60/65 and Hsps-70/72 in atherosclerosis pathogenesis. The HSPs comprise several families of molecular chaperones, the expression of which are increased in cells of the artery wall following exposure to a variety of stressful stimuli that include free radicals, heat, toxins, ischemia (hypoxic stress, reperfusion, and oxidative stress), oxidized LDLc, oxidants, and cytokine stimulation, via the activation of heat-shock transcription factor 1. The chaperone functions of the HSPs protect cells from damage and allow denatured proteins to adopt their native configuration. Heat-shock proteins have been classified into 6 protein families on the basis of their molecular weights (HSP-20-100 kDa), of which HSP27 is a member of a small protein family. Heat-shock proteins have been shown to be expressed in atherosclerotic plaques of both experimental animals and humans. Heat-shock protein 27 has a molecular weight of approximately 27 kDa and is expressed at high levels in a variety of tumors and normal tissues including the heart. In addition to its role as a molecular chaperone, HSP27 has a number of other potentially important biological properties, including being anti-apoptotic and involvement in VSMC migration and proliferation, embryogenesis, cardioprotection, resistance to oxidative stress, and the modulation of inflammation. These properties may have an important bearing on atherogenesis. Intracellular HSP27 downregulation decreases VSMCs resistance to proteolytically induced apoptosis. The VSMCs apoptosis results in weakening the fibrous cap of the atheroma and subsequent plaque rupture. Furthermore, a proteomic study of stable and unstable atherosclerotic plaque demonstrated HSP27 downregulation in unstable plaques.

Hypertension has been considered as a predisposing factor for apoptosis of endothelial cells and cardiomyocytes. In our patients, hypertension was an important risk factor associated with asymptomatic atherosclerosis. Hypertension is a frequent finding in all stages of CKD. Because the pathogenesis of atherosclerosis in patients with CKD is multifactorial, it has been difficult to ascertain the precise role of hypertension in its development. Systolic blood pressure and pulse pressure are predictors of the carotid plaque burden and distribution of stenosis in hemodialysis patients. The findings of previous studies imply that CCIMT and hypertension may be important underlying signs and causes of an excess risk for
CVD among patients with CKD. Major limitation of our study is the small number of study patients who had hypertension as the only risk factor. We suggest study on a larger group of patients with hypertension to further evaluate the results of our study. Furthermore, based on the abovementioned studies and our results, we suggest an explanation for our observed results that high levels of HSP27 might inhibit the induction of apoptosis in patients with hypertension and lead to stabilization of atherosclerotic plaque and delayed cardiovascular events indicated by lower CCIMT values. This hypothesis also needs to be evaluated by future studies.

CONCLUSIONS

Our study showed that HSP27 level in hemodialysis patients with hypertension has a reverse correlation with CCIMT. Also, it is expected that dyslipidemia and age have a positive correlation with CCIMT. Confirmation of these findings requires studying on a larger patient population.

CONFLICT OF INTEREST

None declared.

REFERENCES


29. Shulman NB, Ford CE, Hall WD, et al. Prognostic value of serum creatinine and effect of treatment of hypertension on renal function. Results from the hypertension detection and follow-up program. The Hypertension Detection and