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Serum High-Sensitivity C-Reactive Protein and Heat Shock Protein 27 Antibody Titers in Patients With Stroke and 6-Month Prognosis

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Abstract

Serum heat shock protein 27 immunoglobulin G (IgG) antibody titers (anti-HSP27) and high-sensitivity C-reactive protein (hsCRP) concentrations were measured in samples from 168 patients in the first 24 hours after the onset of stroke and 80 age- and sex-matched control participants. In patients with stroke, median serum anti-HSP27 titer was significantly higher than that of the control group (0.18 [0.14-0.28] vs 0.08 [0.04-0.12], $P < .001$). Median serum hsCRP concentration was also significantly higher in patients compared with the control group (11.43 [8.07-13.53] vs 3.23 [1.66-6.24], $P < .001$). Serum anti-HSP27 and hsCRP concentrations did not differ significantly among patients with different stroke types. Neither serum anti-HSP27 nor hsCRP levels predicted 6-month prognosis in the patients with stroke. We conclude that serum anti-HSP27 titers and hsCRP concentrations are elevated in patients with stroke but do not distinguish between stroke types or predict 6-month prognosis.

Keywords

heat shock protein 27, high sensitive C-reactive protein, stroke, antibody

Introduction

Stroke is a common life-threatening event that leads to death in about 25% of cases.[1] Early detection of those at a high risk of stroke would be a prerequisite for disease prevention.

Serum C-reactive protein (CRP) is a marker of inflammation that is reported to be positively associated with the risk of cardiovascular disease (CVD) and cerebrovascular events[2,3] and is frequently elevated in the circulation of patients after acute ischemic stroke.[4-7] According to previous reports, high serum concentrations of CRP were positively associated with larger brain infarcts,[5,8] stroke severity, and subsequent neurological disability.[4,5,7-9]

Heat shock proteins (HSPs) are highly conserved proteins that are expressed in response to several environmental stresses and function as molecular chaperones. Among HSPs, there is evidence that HSP27 and 70 have putative neuroprotective properties against ischemic injury, which is more prominent for HSP27.[10-12] HSP27 and 70 have been used in vitro and in vivo to protect neural cells against ischemia or injury in animal models and have shown potential beneficial effects in reduction of ischemic injury and lesion size.[13] Anti-HSP titers, including anti-HSP60, 65, and 70, have been reported to be associated with cerebrovascular disease.[14,15]

We have previously reported that anti-HSP27 and hsCRP were risk predictors for coronary disease in an Iranian population.[16,17] In the current study, we extend our findings to stroke and evaluate the changes in serum level of anti-HSP27 and hsCRP as well as the prognostic value of these serum markers in patients with stroke.

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Methods

Participants

The study was carried out on 168 patients (86 males and 82 females) with documented acute cerebrovascular events from the Mashhad Stroke Incidence Study (MSIS), a large population-based study of stroke conducted in Mashhad, Iran.[18] All patients who were hospitalized after an established acute cerebrovascular event between October 2007 and September 2008 were enrolled. After hospital admission, the medical history was taken and a complete physical examination was undertaken. Computed tomography or magnetic resonance imaging of the brain was performed for all patients. In addition, some patients underwent duplex sonography of the extracranial carotid arteries if clinically indicated. Participants taking anti-inflammatory drugs except low doses of aspirin or a statin were excluded from the study. Furthermore, patients who had unstable angina, recent or old myocardial infarction (MI), acute infection, or any acute illness were also excluded. Demographic and clinical information were obtained by direct interviewing of patients or their family.

Each participant gave their informed written consent to participate in the study, which had been approved by the Mashhad University of Medical Science Ethics Committee.

Eighty age- and sex-matched healthy control participants (41 males and 39 females) who lived in the same city were recruited as the control group. Exclusion criteria for the controls were any known acute or chronic disease, including diarrhea, weakness, headache, dizziness, nausea, vomiting and confusion, cancer, hypertension, coronary heart disease (CHD), and psychiatric or central nervous system diseases.

Anthropometric and Other Measurements

Anthropometric parameters as well as systolic and diastolic blood pressures were measured as previously described.[17]

Blood Sampling

Fasted blood samples from patients were collected in the first 24 hours after the onset of stroke, and for the control group, 1 blood sample was taken on the day of laboratory sampling after a 12-hour fast. After being allowed to clot, the blood was centrifuged at 2500 rpm for 15 minutes at room temperature to obtain serum. Hemolyzed samples were excluded. Samples for lipid profile and measurement of serum HSP27 were taken into plain Vacutainer tubes, and those for measurement of glucose were taken into fluoride–oxalate Vacutainer. After separation, aliquots of serum were frozen at –80°C until the analysis.

Routine Biochemical Analysis

A fasting lipid profile was obtained for each participant. Serum lipid and fasting blood glucose (FBS) concentrations were measured by enzymatic methods. hsCRP was measured by a PEG (polyethylene glycol)-enhanced immunoturbidimetry method with an Alcyon analyzer (Abbott, Chicago, Illinois).

Serum Anti-HSP27 Titers

Serum HSP27 antibody titers were measured using an in-house enzyme-linked immunosorbent serologic assay (ELISA).[17]

Statistical Analysis

All statistical analyses were performed with SPSS16. Values were expressed as mean $\pm$ SD or, in the case of nonnormally distributed data, as median and interquartile range. Data that were normally distributed were analyzed using Student t test (for 2 groups) or 1-way analysis of variance (ANOVA; for $\geq$3 groups). Data found to be nonnormally distributed were analyzed using the nonparametric Mann-Whitney U test (for 2 groups) or Kruskal-Wallis test (for $\geq$3 groups). A 2-sided $P < .05$ was considered significant. To analyze the relationship between hsCRP or anti-HSP27 antibody and individual stroke risk factors, Spearman correlation was used due to the nonnormal distribution of hsCRP and anti-HSP27. Logistic regression analysis was used to assess whether stroke was related to traditional atherosclerotic risk factors and hsCRP or anti-HSP27 titers.

Results

Demographic Data

The frequency of hyperlipidemia (25.6%), hypertension (71.3%), and diabetes mellitus (29.2%) in the patient group was significantly higher than that of the control group ($P < .001$), but smoking habit did not differ significantly between the groups. Serum low-density lipoprotein cholesterol (LDL-C), FBS, and systolic and diastolic blood pressure were also significantly higher in patients compared with controls ($P < .05$ for LDL-C and $P < .001$ for other parameters). No significant differences in age, body mass index (BMI), serum triglycerides, high-density lipoprotein cholesterol (HDL-C), and total cholesterol were observed between the groups (Table 1).

Serum hsCRP and Anti-HSP27 Titers in Patients and Controls

Median serum anti-HSP27 titers were significantly higher in patients compared with that of the control group ($P < .001$, Table 2). Likewise, median serum hsCRP concentration was significantly higher in patients than in the controls ($P < .001$).

Serum hsCRP and Anti-HSP27 Titers in Different Subtypes of Stroke

Patients were divided into 3 subgroups according to the etiology of stroke (Table 3): patients with transient ischemic attack (TIA; n = 14), patients with cerebral infarction (n = 120), and patients with cerebral hemorrhage (n = 29). Comparison

Table 1. Comparison of Clinical and Biochemical Characteristics of Patients and Controls[a]

	Control Group	Stroke Group
N	80	168
Female (%)	39 (47.5)	82 (48.8)
Smoker (%)	11 (13.3)	32 (19.0)
Diabetic patients[b] (%)	6 (8.3)	49 (29.2)[c]
Hyperlipidemic[d] (%)	11 (13.4)	43 (25.6)[c]
Hypertensive[e] (%)	7 (8.4)	117 (69.6)[c]
Age (year)	56.13 ± 7.48	57.08 ± 9.53
SBP (mm Hg)	123 ± 11	157 ± 31[c]
DBP (mm Hg)	77 ± 10	90 ± 14[c]
BMI (kg/m^2)	26.8 ± 4.1	26.6 ± 4.4
FBS (mg/dL)	101 ± 22	142 ± 65[c]
TC (mg/dL)	187 ± 32	192 ± 45
LDL-C (mg/dL)	139 ± 26	116 ± 33[f]
HDL-C (mg/dL)	46 ± 6	46 ± 11
TG (mg/dL)	145 (93–174)	126 (92-210)

NOTES: BMI = body mass index; DBP = Diastolic blood pressure; FBS = fasting blood sugar; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol; SBP = systolic blood pressure; TC = total cholesterol; TG = triglyceride.

[a] Values are expressed as mean ± SD for normally distributed data, median and interquartile range for non-normally distributed data, and number (%) for categorical data. Between-groups comparisons were assessed by *t*-test, chi-square test, or Mann-Whitney U test.

[b] Defined as FBS ≥ 126 mg/dL.

[c] $P < .001$.

[d] Defined as TC ≥ 200 mg/dL or TG ≥ 150.

[e] Defined as SBP ≥ 140 mm Hg or DBP ≥ 90 mm Hg.

[f] $P < .01$.

Table 2. Comparison Between Serum Levels of hsCRP and Anti-HSP27 in Patients and Controls[a]

	Stroke Group	Control Group
N	168	80
hsCRP (mg/L)	11.43 (8.07-13.53)	3.23 (1.66-6.24)[b]
Anti-HSP27 (AU)	0.18 (0.14-0.28)	0.08 (0.04-0.12)[b]

NOTES: AU = absorbance unit; hsCRP = high-sensitivity C-reactive protein; HSP = heat shock protein.

[a] Values are expressed as median and interquartile range. Between-group comparisons were made using Mann-Whitney *U* test, as data were not normally distributed.

[b] $P < .001$.

among these 3 groups showed no significant difference for serum anti-HSP27 or hsCRP levels.

Serum hsCRP and Anti-HSP27 Titers and 6-Month Prognosis of Stroke

The patients with stroke were divided into 3 groups depending on their clinical outcome over the 6 months after their stroke: patients who survived without a recurrent event, those with a recurrent stroke, and those who died. Among these 3 groups, there were no significant differences for serum anti-HSP27 titers or hsCRP levels (Table 4).

Serum hsCRP and Anti-HSP27 Titers in Patients With and Without Risk Factors

There was no significant difference between the serum levels of anti-HSP27 and hsCRP in patients with and without diabetes mellitus or obesity (BMI >30) nor were they related to smoking habit. The levels of hsCRP and anti-HSP27, however, were significantly higher in hypertensive patients compared with nonhypertensive patients ($P < .001$; Table 5).

Correlation Between Serum hsCRP and Anti-HSP27 Titers and Risk Factors

Univariate analysis between serum anti-HSP27 titers and hsCRP with other cardiovascular risk factors showed that hsCRP correlated with serum triglycerides in the control group ($P < .05$). There was no other significant correlation between anti-HSP27 titers and hsCRP with vascular risk factors in patients or controls ($P > .05$; Table 6).

Multivariate Analysis of Anti-HSP27 and CRP

According to multivariate analysis, there was no variable with a significant influence on serum anti-HSP27 titers or hsCRP concentrations.

Discussion

We found significantly higher serum levels of hsCRP and anti-HSP27 titers in patients with stroke compared with healthy participants. However, there were no significant differences in serum anti-HSP27 and hsCRP among subgroups of patients with stroke. Moreover, no significant association between serum levels of anti-HSP27 and hsCRP and 6-month prognosis of the disease was observed.

In our study, the control group had significantly lower levels of systolic and diastolic blood pressure, FBS, and LDL-C, together with lower prevalence of diabetes mellitus, hyperlipidemia, and hypertension, as would be expected. The median serum hsCRP levels in the patients was similar to those previously reported for patients with stroke.[19] C-reactive protein is a predictor of stroke, and previous studies have shown elevated levels of serum CRP in patients after acute ischemic stroke.[4-7] These elevated levels of CRP in patients with stroke may reflect the extent of cerebral tissue injury, systemic infection, or inflammatory disease.[20]

HSP65 and 70 antibody titers were found to be elevated in the first 48 hours after the onset of ischemic stroke, and elevated levels of these antibodies were independent predictors of stroke.[14] It has also been reported that detectable levels of immunoglobulin G (IgG) antibody against HSP60/65 were independently associated with an increased risk of stroke.[15] Formation of these antibodies could be attributed to the chronic humoral immunity against HSPs that are expressed on endothelial cells and which may lead to the development of vascular disease.[14] However, these findings have not been consistently

Table 3. Comparison of Serum hsCRP and Anti-HSP27 Levels Among Different Subtypes of Stroke[a]

	TIA	Infarction	Hemorrhage
N (%)	14 (8.33)	120 (71.42)	29 (17.26)
hsCRP (mg/L)	8.44 (6.1-13.85)	11.48 (8.21-13.51)	10.65 (7.42-14.28)
Anti-HSP27 (AU)	0.18 (0.12-0.39)	0.18 (0.14-0.28)	0.17 (0.13-0.26)

NOTES: AU = absorbance unit; hsCRP = high-sensitivity C-reactive protein; HSP = heat shock protein; TIA = transient ischemic attack. Values are expressed as median and interquartile range.
[a] Comparisons were made using Kruskal-Wallis test as data were not normally distributed. No significant difference was observed ($P > .05$).

Table 4. Comparison of Serum hsCRP and Anti-HSP27 Levels Between Patients With Stroke Who Survived, Had a Recurrent Event and Those Who Died After 6 Months[a]

	Survived Without Event	Survived With Recurrent Disease	Died
N (%)	113 (67.3)	10 (6)	45 (26.8)
hsCRP (mg/L)	11.41 (8.08-15.13)	11.69 (10.76-12.58)	11.43 (6.93-13.24)
Anti-HSP27 (AU)	0.18 (0.14-0.35)	0.21 (0.17-0.27)	0.17 (0.14-0.27)

NOTES: AU = absorbance unit; hsCRP = high-sensitivity C-reactive protein; HSP = heat shock protein.
[a] Values are expressed as median and interquartile range. Comparisons were made using Kruskal-Wallis test as data were not normally distributed. No significant difference was observed ($P > .05$).

reported. Others found no significant difference in the average serum levels of either anti-HSP60 or anti-HSP65 antibodies between patients with cerebrovascular diseases and age-matched healthy participants.[21] Mantle and colleagues also reported that despite being higher in the patients with stroke, anti-HSP65 IgG titers increase with age in both patients with stroke and controls and concluded that this antibody is a marker of aging rather than stroke.[22] In contrast to the latter finding, the association between anti-HSP65 and stroke was maintained after adjustment for age and other risk factors in another study.[15] The high titers of anti-HSP27 and hsCRP in the current study support their value as biomarkers of vascular risk and our previous studies.[16,17]

To date, few studies have evaluated the relationship between CRP and stroke subtype.[5,19,23-25] Terruzzi et al found that in the acute phase of cerebral infarction CRP might be a predictive factor for short-term mortality or a marker of cardioembolism.[19] Another study failed to observe significant differences in CRP levels among stroke subtypes[5] and others found elevated CRP levels across all subtypes, with cardioembolic strokes having the highest values.[24] Furthermore, Eikelboom et al reported elevation of CRP both in cardioembolic and large-vessel strokes but not in small-vessel strokes.[23] Masotti et al found the highest levels of CRP in cardioembolic strokes compared with large-artery and small-artery strokes.[25] In our study, median serum levels of hsCRP and anti-HSP27 titers did not differ significantly among subtypes of stroke. Although median serum hsCRP was lower in patients with TIA compared with subgroups of infarction and hemorrhage, this difference did not reach significance. However, this may be due to the small number of patients in this group.

Moreover, we found no significant relationship between serum hsCRP and anti-HSP27 titers with 6-month prognosis of stroke. These results regarding hsCRP and prognosis are consistent with the finding of others who reported that, unlike for acute coronary events, routine CRP measurement is not a useful marker for the prediction of outcome in acute cerebrovascular events on hospital admission.[4,26,27] In addition, it has been suggested that it is not possible to transfer concepts that are valid for CHD directly to stroke.[28] Canova et al did not use a highly sensitive laboratory assay for further quantification of CRP within normal limits and below the detection limit of 2.4 mg/L.[4] Thus, they suggested that very small changes could be missed within the normal range. These small changes seem to be important as Ridker et al found that small changes may predict the risk of myocardial infarction and stroke in otherwise healthy men.[29] However, despite the aforementioned recommendation to assess risk with hsCRP, we did not find a significant relationship between serum hsCRP values and 6-month prognosis of stroke.

Table 5. Median Serum hsCRP and Anti-HSP27 Levels in Patients With and Without Risk Factors[a]

Subgroup	N	hsCRP (mg/L)	Anti-HSP27 (AU)
Diabetic	56	10.85 (7.69-13.03)	0.17 (0.12-0.26)
Nondiabetic	112	11.33 (6.69-13.59)	0.17 (0.14-0.27)
Hypertensive	99	11.06 (6.68-13.31)	0.18 (0.14-0.28)[b]
Nonhypertensive	69	11.63 (10.10-13.92)	0.17 (0.13-0.27)
Smoker	32	11.56 (7.38-13.21)	0.16 (0.13-0.31)
Nonsmoker	136	11.38 (8.16-13.54)	0.18 (0.14-0.27)
Obese (BMI > 30)	51	11.26 (8.01-13.57)	0.18 (0.14-0.26)
Nonobese	117	11.15 (6.93-13.46)	0.17 (0.14-0.27)

NOTES: AU = absorbance unit; BMI = body mass index; hsCRP = high sensitive C-reactivity protein; HSP = heat shock protein.
[a] Values are expressed as median and interquartile range. Between-group comparisons were made using Mann-Whitney *U* test as data were not normally distributed.
[b] $P < .001$).

Table 6. Correlation Between Serum Levels of hsCRP and Anti-HSP27 With Individual Atherosclerosis Risk Factors in Patients and Controls

	hsCRP (mg/L)		Anti-HSP27 (AU)	
Parameter	Controls	Patients	Controls	Patients
Age (years)	0.222	–0.065	0.059	0.015
BMI (kg/m^2)	–0.190	0.078	–0.135	–0.061
SBP (mm Hg)	0.185	–0.102	–0.099	–0.006
DBP (mm Hg)	–0.108	–0.022	0.011	–0.090
FBS (mg/dL)	–0.021	–0.047	0.052	–0.077
TC (mg/dL)	–0.302	0.166	0.027	–0.231
TG (mg/dL)	–0.163[a]	0.230	0.002	–0.110
HDL-C (mg/dL)	–0.102	–0.082	0.113	0.091
LDL-C (mg/dL)	–0.271	0.226	0.251	–0.205
hsCRP (mg/L)	–	–	–0.095	–0.025
Anti-HSP27 (AU)	–0.095	–0.025	–	–

AU = absorbance unit; BMI = body mass index; DBP = Diastolic blood pressure; FBS = fasting blood sugar; HDL-C = high-density lipoprotein cholesterol; hsCRP = high-sensitivity C-reactive protein; HSP = heat shock protein; LDL-C = low-density lipoprotein cholesterol; SBP = systolic blood pressure; TC = total cholesterol; TG = triglyceride.

[a] Correlations were assessed using Spearman correlation coefficients ($P < .05$).

Despite the above findings, and although no large study has prospectively assessed the value of CRP for prognostic short-term and long-term stratification of patients with ischemic stroke, many data suggest that CRP might be of value in this group of patients.[5,7,9] However, even if prognostic value of elevated levels of CRP is confirmed, it remains to be established whether specific therapeutic options can be derived from this.[28]

In addition to hsCRP, we did not find any relationship between anti-HSP27 and 6-month prognosis of stroke. According to a previous study, high levels ($\geq$90th percentile) of antibodies against mycobacterial HSP65, but not those of anti-HSP60, predicted the development of new MI, stroke, or cardiovascular death.[30] Furthermore, Xu et al found that high anti-HSP65 levels predicted carotid atherosclerotic progression during 5-year follow-up.[31] In our study, multivariate analysis showed that none of the variables including age, systolic and diastolic blood pressure, fasting blood sugar, total cholesterol, LDL-C, HDL-C, and triglycerides had significant influence on hsCRP or anti-HSP27 titers. Similarly, others found that risk factors for vascular disease in general and stroke in particular had no visible influence on CRP levels.[5,26,27] They found no relationship between the time interval since the onset of symptoms and CRP concentration, which indicated that an acute cerebrovascular event has little impact on CRP values.[4] These results were confirmed by another study where after logistic regression analysis, CRP remained as an independent predictor of 14-day mortality.[19] Furthermore, the results of logistic regression analysis in 2 other studies indicated that IgG antibodies to HSP60, 65, and 70 are associated with increased risk of stroke independently of other risk factors.[14,15]

A limitation of our study is that we did not perform blood sampling sequentially following the onset of stroke. This would have identified the time course and pattern of anti-HSP27 and hsCRP rise and fall after stroke.

In summary, our findings showed that serum anti-HSP27 titers and hsCRP concentrations are elevated in patients with stroke compared with healthy participants. These elevated values may be "emerging" risk factors for stroke but are not related to the 6-month prognosis of the disease. Serum anti-HSP27 titers and hsCRP may be useful biomarkers for the diagnosis of patients with vascular events or participants who are prone to the development of these disorders. However, further studies are necessary to confirm our results in larger populations.

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Declaration of Conflicting Interests

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