Cell Stress and Chaperones (2013) 18:65–74
DOI 10.1007/s12192-012-0358-y

ORIGINAL PAPER

Changes in anti-heat shock protein 27 antibody and C-reactive protein levels following cardiac surgery and their association with cardiac function in patients with cardiovascular disease

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Received: 7 June 2012 / Revised: 12 July 2012 / Accepted: 13 July 2012 / Published online: 30 July 2012

Abstract The relationship between serum anti-heat shock protein (Hsp)27 antibody and high sensitive C-reactive protein (hs-CRP) levels and indices of cardiac function were investigated in patients undergoing coronary artery bypass grafting (CABG) or heart valve replacement. The changes in anti-Hsp27 antibody titers and hs-CRP levels were compared among patients undergoing off-pump and on-pump CABG or valvular heart replacement. Fifty-three patients underwent off-pump, on-pump CABG, and heart valvular replacement in each group. Serum anti-Hsp27 titers and hs-CRP values were measured 24 h before and after the operation and at discharge. Echocardiography was performed before surgery and before discharge. The results were compared with values from 83 healthy controls. hs-CRP levels increased and anti-Hsp27 antibody decreased following surgery ($P<0.001$ and $P<0.05$, respectively), although these changes were independent of operative procedure ($P=0.361$ and $P=0.120$, respectively). Anti-Hsp27 antibody levels were higher at the time of discharge ($P=0.016$). Only in coronary patients were anti-Hsp27 antibody levels negatively associated with E/E′ ($r=-0.268$, $P=0.022$), a marker of pulmonary capillary wedge pressure. In conclusions, anti-Hsp27 antibody levels are associated with indices of cardiac function in coronary patients. Cardiopulmonary bypass had no significant effect on the induction of changes in anti-Hsp27 levels. Moreover, anti-Hsp27 antibody levels fell in all groups postoperatively; this may be due to the formation of immune complexes of antigen–antibody, and antibody levels were higher at the time of discharge.

Keywords Heat shock protein 27 · Valvular heart disease · Coronary artery bypass grafting · Cardiac function

Electronic supplementary material The online version of this article (doi:10.1007/s12192-012-0358-y) contains supplementary material, which is available to authorized users.

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Introduction

Myocardial revascularization can be performed by using minimally invasive surgical techniques, or by open surgical treatment, known as coronary artery bypass grafting (CABG). Patients can be either operated with or without the use of cardiopulmonary bypass (CPB) during CABG, defined as "on-" and "off-pump" CABG, respectively.

Degenerative and rheumatic valvular heart disease (VHD) has several known risk factors that include genetic factors, inflammation, autoimmunity, infection, and oxidative stress (Davutoglu et al. 2005; O'Brien 2006; Stollerman 1997). Both heart valvular replacement and on-pump CABG require CPB. Coronary revascularization and valvular replacement with the use of CPB have a comparable intraoperative procedure and technique. One of the complications that may occur following CPB is ischemia–reperfusion syndrome (Bolli 1991; Vaage and Valen 1993). During this condition, functional, structural, and metabolic changes occur, one of which is an increase in the free radicals production (Bolli 1991; Vaage and Valen 1993), which may subsequently lead to tissue injury (Hearse 1990). In addition, it has been previously reported that epithelial/endothelial apoptosis occurs in patients undergoing CABG, irrespective of which CABG procedure (on or off-pump) is selected (Szerafin et al. 2006). Off-pump CABG has been proposed to reduce ischemia–reperfusion injury in patients with acute coronary syndrome (ACS) and thereby may improve the outcomes following surgery (Rastan et al. 2006). It has been suggested that the avoidance of CPB may prevent subsequent ischemia–reperfusion injury and hence reduce postoperative systemic complications (Cleveland, Jr. et al. 2001; Sabik et al. 2002).

Serum C-reactive protein (CRP) is a biomarker of systemic inflammation (Atar et al. 2005) and is believed to play an active role in cardiovascular disease (CVD), from initial recruitment of circulating leukocytes to the arterial wall to eventual rupture of the unstable plaque (Burke et al. 2002). Furthermore, we (Kazemi-Bajestani et al. 2007) have previously shown that high sensitive (hs)-CRP is associated with CVD in Iranian patients with angiographically defined coronary artery disease (CAD). Moreover, previous studies observed that CRP levels are a predictor of mortality and subsequent clinical events after angioplasty and stenting (Gaspardone et al. 1998; Gottsauner-Wolf et al. 2000) and reflect the instability of the atherosclerotic plaques.

Several potential auto-antigens have now been identified to be involved in the pathogenesis of atherosclerosis including heat shock proteins (Hsps), modified low-density lipoprotein (LDL) (oxidized LDL and malondialdehyde modified LDL), and beta-2-glycoprotein-I (Ghayour-Mobarhan et al. 2009; Mandal et al. 2005). The Hsps are a highly conserved family of proteins expressed by a number of cell types following exposure to stressful environmental conditions. These proteins enhance the ability of cells to overcome the effects of the stress (Lindquist 1986). A number of the Hsps have been shown to be molecular chaperones that are involved in protecting the cellular proteins from denaturation and refolding of other damaged protein molecules. Over the past two decades, there has been an increasing interest to study the relationship between Hsps and anti-Hsp antibodies with CVD and particularly to delineate whether an autoimmune response may be implicated or not. While most of the past studies have focused on Hsp65 and Hsp70 and antibody levels against them, there has been recent interest and investigations of the possible role of the smaller Hsps, such as Hsp27 in CVD (reviewed by Ghayour-Mobarhan) (Ghayour-Mobarhan et al. 2009).

We have previously reviewed the potential relationship between soluble Hsps and atherosclerosis (Ghayour-Mobarhan et al. 2009). Martin et al. (1997, 1999) reported that both Hsp27 and Hsp70 were able to protect cardiomyocytes from the effect of ischemia and that decreasing the level of endogenous Hsp27 results in an enhancement of the damaging effects of a subsequent ischemic stimulus, suggesting the protective effects of Hsp27 on myocardial cells. We have also found that anti-Hsp27 antibody titers are higher in patients with ACS than healthy controls, and the levels of this antibody rise and fall rapidly after the onset of ACS and could be an early marker of myocardial ischemia (Ghayour-Mobarhan et al. 2008).

The changes in expression of anti-Hsps after CABG have not been described before and might be a structural marker for transient myocardial ischemia during CABG. Hence, in this study, we aimed to assess the changes in serum anti-Hsp27 antibody levels in relation to indices of cardiac function in patients undergoing CABG and heart valvular replacement and to compare the changes in anti-Hsp27 antibody among patients who were treated by different operative techniques.

Methods

Study population

The study subjects (n=159) were selected from individuals who were candidates for open heart surgery and had proven CAD or VHD. All the subjects had previously undergone coronary angiography. The indication for coronary revascularization and the type of surgery (off- or on-pump CABG) was determined on the basis of clinical judgment by the attending cardiac surgeon. Fifty-three patients were recruited to each group of the study. Because of the high prevalence of rheumatic fever in Iran some decades ago, there are many patients who have VHD and need valvular

replacement surgery. Typically, these patients do not have the same profile of traditional coronary risk factors as CAD patients.

Patients with VHD consisted of individuals with one or two diseased valves. Percutaneous coronary angiography was indicated for all patients with VHD in order to rule out the presence of CAD. The results of angiography showed that all the patients with VHD were free from significant CAD. The exclusion criteria for the groups were; taking oral contraceptives or hormone replacement therapy, pregnancy, a prior history of coronary angioplasty or CABG, chronic pulmonary obstructive disease, having overt clinical features of infection or chronic inflammatory disease. All subjects were negative for viral markers of hepatitis and anti-HIV antibody. Moreover, patients with ACS within the previous 3 months, renal, hepatic, or malignant diseases were also excluded, as were cases admitted as emergencies.

Eighty-three healthy volunteers were also recruited as a normal control group. The control subjects underwent a full clinical examination by a physician, and a careful medical history was recorded for all these subjects. None of the control subjects disclosed any symptom, nor had any signs of CVD. These subjects had no other apparent major disease. Information on smoking and drug treatment of patients was obtained via a questionnaire. Demographic and intraoperative data were recorded prospectively, including aortic cross clamp time, CPB time, duration of surgery, and number of grafts. Echocardiography was performed for all the patients. The study protocol was approved by the Ethics Committee of Mashhad University of Medical Sciences, and written informed consent was obtained from each participant.

The following conventional cardiovascular risk factors were defined: dyslipidemia (total cholesterol≥200 mg/dl and/or LDL cholesterol (LDL-C)≥130 mg/dl and/or use of cholesterol lowering drugs), diabetes (fasting blood glucose≥126 mg/dl and/or use of pharmacological treatment), and arterial hypertension (systolic blood pressure>140 mmHg and/or diastolic blood pressure >90 mmHg and/or use of antihypertensive drugs).

Blood sampling, biochemical analysis, and measurements

The first blood sample was taken from each patient for analysis after 12 h of fasting in the morning on the day of surgery. The second sample was taken on the first postoperative day at 24 h after surgery in ICU from a peripheral vein. The third blood sample was taken on the day of discharge. Following venepuncture, blood samples were collected into Vacutainer® tubes and centrifuged at 5,000×*g* for 15 min at 4 °C. After separation, aliquots of serum were frozen at −80 °C until analysis. A full-fasted lipid profile comprising total cholesterol, triglycerides, high-density lipoprotein-cholesterol, and LDL-C was determined for each subject. Serum lipid and fasting blood glucose (FBG) concentrations were measured enzymatically with the use of commercial kits using the BT-3000 auto-analyzer machine (Biotechnica, Rome, Italy). hs-CRP was measured by a PEG-enhanced immunoturbidimetry method using an Alycon analyzer (ABBOTT, Chicago, IL, USA). For all patients, anthropometric parameters including weight, height, and body mass index (BMI) were measured preoperatively. BMI was calculated as weight (in kilograms) divided by height squared (in square meters). Blood pressure was measured twice while the patients were seated and rested, using a standard mercury sphygmomanometer.

Determination of anti-Hsp27 antibody levels

Serum Hsp27 antibody titers were measured using an in-house ELISA assay, as we have previously described (Pourghadamyari et al. 2011; Rahsepar et al. 2012). Briefly, serum Hsp27 antibody titers were measured using an in-house ELISA assay. Microtiter plates (Nunc Maxisorp, 3Nunc) were coated with 100 ng per well recombinant human Hsp27 dissolved in 50 μl carbonate buffer pH 9.6 incubated for 18 h at 4 °C under humidified conditions. The wells were washed three times in wash buffer (PBS containing 0.05 % Tween-20). Non-specific binding was reduced by blocking each well with 2 % goat serum in PBS and 250 μl added to each well and incubated for 30 min in 37 °C and 30 min at room temperature. Wells were washed three times with PBS. Serum was diluted 1:100 with 2 % goat serum in PBS and 100 μl added to the each wells in duplicated and incubated for 30 min at room temperature. After washing (four times in wash buffer and two times in PBS), 100 μl peroxidase conjugated goat anti-human IgG (Sigma-Aldrich, 4Inc., USA) diluted 1:500 with 2 % goat serum in PBS, was added to each wells, and incubated for 30 min at room temperature. After washing (four times in wash buffer and two times in PBS), 100 μl of TMB substrate (200 μl of 6 mg⁄mL TMB in DMSO was added to 10 mL of 50 mM acetate buffer, pH 4.5, containing 6 μl H2O2) was added per well and plate incubated for 15 min in the dark at room temperature. The reaction was terminated by adding 50 μl 2 M HCl per well. Optical density at 450 nm was measured using a Labsystems iEMS Reader MF Microtitre plate reader with a reference wavelength of 620 or 570 nm. The within-assay and between-assay precision was 3.5 and 5.2 %, respectively. After correction for the non-specific background absorbance (subtracting the absorbance of uncoated wells from the antigen-coated wells for each sample), the results were expressed in optical density units.

Cardiopulmonary bypass

CPB was initiated by cannulation of the distal ascending aorta and insertion of a single two-stage cannula into the right atrium. A membrane oxygenator (model Didico; Surin group, USA) was used. Intravenous heparin (300 IU/kg) was administered immediately before cannulation for CPB (Stockert SIII and SV, Medtronic, USA), and additional doses were given to maintain an activated clotting time of 480 s or greater. Non-pulsatile flow rates of 2.2 to 2.4 L/min/m^2 and temperatures between 28 and 30 °C were used. The mean arterial pressure was maintained between 50 and 60 mmHg, with administration of sodium nitroprusside or norpheny-lephrine as required, and the hematocrit was kept higher than 27 % by adding concentrated red blood cells if necessary. A 14-Fr retrograde coronary sinus perfusion catheter (Callmed, USA) was inserted by palpation of the coronary sinus for blood collection for retrieving coronary sinus blood samples. The basic cardioplegic solution used in both groups was prepared by mixing 500 mL of whole blood withdrawn directly from the pump oxygenator to a reservoir to which KCl (10 mEq), lidocaine hydrochloride (60 mg), and magnesium sulfate (8 mEq) were added. After cross-clamping of the ascending aorta, cardioplegia was induced in all patients by antegrade infusion of 1,000 mL of the cold cardioplegic solution obtained by use of a roller pump. Additional doses of 600 mL were infused after each distal anastomosis, or after 20 min of ischemia, and immediately before releasing the aortic cross-clamp.

During off-pump surgery, all operations were performed through a median sternotomy. Intravenous heparin (150 IU/kg) was administered. All anastomoses were sutured by hand. The proximal anastomosis was performed using a site clamp on the aorta. At the end of the surgical procedure, protamine sulfate was administered to reverse the heparin effect for both on-pump and off-pump patients.

Cardiac and vascular imaging

Resting echocardiographic examination was done for all the patients before surgery and on the day of discharge. The equipment used was VIVID 3, GE Vingmed Ultrasound, USA. Left ventricular (LV) ejection fraction, LV end systolic and diastolic diameters and volumes, early (E) and late (A) mitral forward Doppler flow, early (E′) and late (A′) diastolic mitral annulus pulsed wave tissue Doppler, and end systolic and diastolic volume were measured. E/E′ was indicated as pulmonary capillary wedge pressure. Diastolic functions as previously have been described (Garcia et al. 1998) were categorized to normal, stage I: impaired, stage II: pseudonormal, and stage III: restrictive.

Table 1 Comparison of clinical and biochemical characteristics of patients and controls

Groups	On-pump CABG	Off-pump CABG	VHD patients	Controls
N	53	53	53	83
Female, *n* (%)	26 (49.06)	20 (37.73)	29 (55)	34 (40.96)
Smokers, *n* (%)	14 (26.41)	20 (37.73)*	13 (24.5)	13 (15.66)
Diabetics, *n* (%)	19 (35.85)*	20 (37.73)*	3 (6)	5 (6.02)
Dyslipidemic, *n* (%)	23 (43.40)*	32 (60.38)*	6 (11)	7 (10.45)
Hypertensive, *n* (%)	33 (62.26)*	33 (62.26)*	12 (23)	12 (26.67)
Age (years)	61.70±9.11*	58.77±9.33	45.09±14.29*	56.80±8.70
BMI (kg/m^2)	26.5 (22.36–29.71)	27.18 (24.77–29.01)	22.23 (19.51–26.45)*	27.08 (24.95–30.94)
WC (cm)	97.37±11.7	94.39±9.86	85.14±11.96*	98.76±14.17
HC (cm)	106.5 (98.25–111.37)	101 (97–107)	95.5 (86.25–103)*	102 (97–108)
FBG (mg/dl)	111.5 (99.25–147.5)*	115.5 (94.25–146.75)*	100.5 (87.75–117.75)*	92 (80.5–100)
TG (mg/dl)	136 (88–198)	128 (78.25–181.75)	100 (84.5–123.5)*	132 (94.5–167.5)
HDL-C (mg/dl)	42.98±8.03	39.68±9.4*	42.97±10.17	44.20±6.45
LDL-C (mg/dl)	105.5 (86.05–123.90)	90.4 (66.5–109)	97.4 (78.25–120.57)	101.2 (85.75–112.75)
DBP (mmHg)	80 (70–80)	80 (70–80)	70 (67.5–80)*	80 (70–90)
SBP (mmHg)	130 (115–135)	120 (110–130)	115 (110–130)*	120 (115–130)

Values are expressed as mean±SD, or median and interquartile range. Chi-square test was used to compare qualitative data. Student's *t* test and Mann–Whitney test were used for normally and non-normally distributed data, respectively

CABG coronary artery bypass grafting, *VHD* valvular heart disease, *BMI* body mass index, *FBG* fasting blood glucose, *TG* triglyceride, *HDL-C* high-density lipoprotein cholesterol, *LDL-C* low-density lipoprotein cholesterol, *DBP* diastolic blood pressure, *SBP* systolic blood pressure, *WC* waist circumferences, *HC* hip circumference

*$P<0.05$ means significant difference between coronary and valvular patients with controls

Statistical analysis

All statistical analyses were performed using the SAS for Windows™, version 9.2 software package. Data were expressed as means±SD (for parameters with a symmetric distribution) or median and interquartile range (in the case of non-symmetrically distributed data). For parameters with a normal distribution, group comparisons were performed using Student's *t* test (between two groups) and ANOVA (for ≥3 groups). For parameters with a non-normal distribution, unequal variances, and for ordinal variables, Mann–Whitney (between two groups) and Kruskal–Wallis (for ≥3 groups) test were used. A two-sided *P* value <0.05 was considered statistically significant. Bivariate correlations between different parameters and anti-Hsp and CRP values were performed using Spearman's rank correlation (for non-normally distributed data). In order to compare anti-Hsp and CRP values between the three samples of the patients between two groups (on and off-pump CABG) and also VHD patients, repeated measure test was used. Pre-treatment (baseline) measure of anti-Hsp and hs-CRP levels was used as covariates variables. This permits using each subjects as its "own control" to assess the effect of treatment over time. As missing data can have an important effect on multivariate

Table 2 Echocardiographic findings and pump and aortic clamp time of patients

Groups		On-pump	Off-pump	VHD
Number		53	53	53
Pre-ejection fraction, (%)		51.75 (43.12–59.75)	55 (45–60)	55 (51.25–60)
Post-ejection fraction, (%)		46.25 (35–57.62)	50 (41–55)	55 (45–59)
Pre-E (cm/s)		62 (50.25–81.5)	68.5 (51.5–83.25)	110 (79–200)*
Post-E (cm/s)		73 (60–83.75)	70.5 (59.5–89.25)	119 (88–161)
Pre-E′ (cm/s)		6 (5–8)	7 (6–9.5)	7 (5–10)
Post-E′ (cm/s)		6 (5–7)	6.5 (5–7)	7.8 (6–10)
Pre-A (cm/s)		90 (70–100.75)	76 (66–90)	97 (55.25–145.25)
Post-A (cm/s)		75 (63.5–85.5)	70 (59–80)	75 (58–93)
Pre-A′ (cm/s)		9 (6.25–10)	10 (8–11)	7 (6–9.75)*
Post-A′ (cm/s)		6.5 (5–8.3)	8 (7–10)	5 (5–7.5)
Pre-E/E′		10.29 (7.93–12.29)	9.17 (7.20–11.75)	15.07 (9.33–30.97)*
Post-E/E′		11.83 (10.48–14.03)	11.07 (8.96–14.27)	15.29 (10.79–22.26)
Pre-LVDD (mm)		51 (45.2–57.15)	49.5 (44.95–54)	49.4 (40–53)
Post-LVDD (mm)		49 (45.5–55)	47.15 (40–55.75)	44.85 (41.23–51.20)
Pre-LVSD (mm)		36 (29.17–43)	35 (31–38)	33.7 (28.12–40.82)
Post-LVSD (mm)		36.5 (30.25–44)	34.9 (28.75–41.5)	31.85 (28.75–39.07)
Pre-EDV (cc)		82 (61–110)	84 (64–104)	81 (58–120.5)
Post-EDV (cc)		84 (60.25–120.75)	76 (57.75–91.25)	78 (64–104)
Pre-ESV (cc)		32 (25.5–50.15)	36.5 (26.5–46.5)	33.90 (24–56.75)
Post-ESV (cc)		40 (25–68)	35.5 (23–46.75)	37 (27–47.25)
Pre-diastolic function	Normal, %	10.8	29.7	30
	Grade I, %	78.4	59.5	33
	Grade II, %	8.1	8.1	30
	Grade III, %	2.7	2.7	7
Post-diastolic function	Normal, %	8	15.8	45
	Grade I, %	56	47.4	35
	Grade II, %	32	36.8	20
	Grade III, %	4	0	0
Aorta clamp time (min)		50 (44.5–64.5)	–	58 (39–95.5)
Pump time (min)		82.5 (66.5–107.25)	–	74 (56.75–115)

Kruskal–Wallis test was used to compare the pre-echocardiographic findings among three groups. Pre-E, pre-E/E′, and pre-A′ were significantly different ($P<0.05$) between three groups of patients

VHD valvular heart disease, *EF* ejection fraction, *ESV* end systolic volume, *EDV* end diastolic volume, *LVDD* left ventricular diastolic diameter, *LVSV* left ventricular systolic diameter, *E/E′* pulmonary capillary wedge pressure, *E* peak early trans-mitral flow velocity, *E′* peak early diastolic myocardial velocity

methods of repeated measures analysis and due to presence of some missing data, we used the Proc Mixed routine in SAS.

Results

Demographic data

Overall, 53, 53, 53, and 83 subjects were evaluated in on-, off-pump CABG, valvular replacement, and control groups, respectively. Demographic data and biochemical characteristics of patients and controls have been summarized in Table 1. Angiographic findings showed that the VHD patients did not have significant stenosis of their coronary arteries; our results also showed that these patients had significantly lower rates of traditional cardiovascular risk factors than coronary patients. The first and second echocardiographic measurements of patients have been summarized in Table 2.

Determination of serum markers

Baseline values of hs-CRP and anti-Hsp27 antibody titers were not significantly different among patients. CAD and VHD patients had higher values of anti-Hsp27 antibody and hs-CRP levels in comparison with healthy control subjects (P=0.005 and P<0.001 in CAD patients and P<0.001 for anti-Hsp27 antibody and hs-CRP levels in VHD patients, respectively). We observed that changes in hs-CRP levels (P=0.361) and anti-Hsp27 antibody titers (P=0.120) were not significantly different between on-pump and off-pump patients. hs-CRP levels increased significantly 24 h following surgery (second sample) in both groups of on- and off-pump CABG (P<0.001) and remained in high levels at the time of discharge (third sample). Anti-Hsp27 antibody levels decreased significantly 24 h post-operation in both groups of on- and off-pump CABG (P=0.012), while in the third sample, at the time of discharge, anti-Hsp 27 antibody titers increased statistically to almost reach their baseline levels (P=0.016). A similar pattern was found for VHD patients as hs-CRP values increased significantly in the second and third samples in comparison with baseline values (P<0.001); in the VHD patients, hs-CRP decreased in the third sample when compared with second samples (P=0.017). Anti-Hsp27 antibody titers were also decreased insignificantly in the second and significantly in the third samples (P=0.194, and P=0.033, respectively) in comparison with baseline values; however, this reduction was only significant between first and third samples (Fig. 1).

Bivariate correlations

We did not observed any significant association between age, anthropometric factors, biochemical measurements, and antibody levels against Hsp27 in CAD and VHD patients, while hs-CRP values were positively correlated with total cholesterol, triglyceride, and FBG in CAD group and negatively with systolic blood pressure in VHD patients. Despite the above findings, in CAD patients, anti-Hsp27 antibody levels were negatively associated with E/E′ (r=−0.268, P=0.022), a marker of pulmonary capillary wedge pressure, and positively with changes in end diastolic volume before and after surgery (r=0.311, P=0.038). No

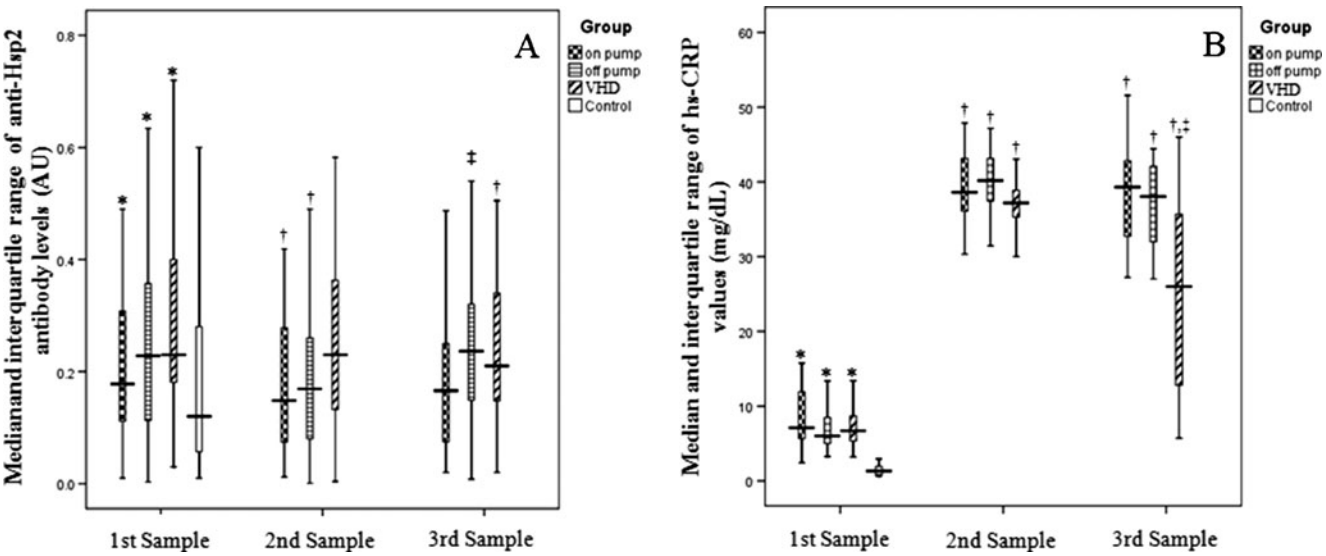

Fig. 1 **a**, **b** Compare anti-Hsp27 antibody levels and hs-CRP values, respectively, for the patients with coronary and valvular heart disease and controls. *, †, and ‡ Significant difference between patients and controls (P<0.05). *AU* absorbency unit, *VHD* vascular heart disease

Table 3 Correlation between baseline anti-Hsp27 and hs-CRP values and different parameters

	CAD patients				VHD patients			
	Anti-Hsp27		hs-CRP		Anti-Hsp27		hs-CRP	
Factor	*r*	*P* value	*r*	*P* value	*r*	*P* value	*r*	*P* value
Age	0.141	0.154	0.044	0.664	−0.075	0.598	0.090	0.556
BMI	0.065	0.514	0.053	0.604	0.041	0.781	−0.167	0.291
WC	−0.031	0.806	0.170	0.176	−0.130	0.565	−0.214	0.364
HC	0.061	0.628	0.202	0.106	−0.094	0.676	−0.180	0.448
DBP	−0.191	0.056	0.087	0.396	−0.162	0.272	−0.054	0.736
SBP	−0.097	0.337	−0.019	0.851	−0.072	0.619	−0.329	0.041
TC	−0.135	0.193	0.222	0.033	−0.067	0.676	0.296	0.079
LDL-C	−0.179	0.101	0.103	0.355	−0.227	0.190	0.191	0.295
HDL-C	−0.129	0.229	0.066	0.548	−0.240	0.147	−0.128	0.463
TG	−0.080	0.442	0.223	0.032	0.148	0.370	0.102	0.571
FBG	−0.020	0.851	0.232	0.027	0.157	0.327	0.00047	0.998
EF	0.038	0.704	−0.082	0.417	−0.200	0.155	0.095	0.538
ESV	−0.062	0.611	0.033	0.786	−0.041	0.798	−0.246	0.155
LVDD	−0.067	0.522	−0.114	0.284	−0.129	0.416	−0.211	0.203
E/E′	−0.268	0.022	0.209	0.081	−0.147	0.371	0.168	0.334
EDV	−0.180	0.128	0.018	0.878	−0.009	0.955	−0.226	0.192
LVSD	−0.035	0.736	−0.072	0.495	−0.001	0.994	−0.132	0.451
$EF_{1–2}$	−0.030	0.784	−0.030	0.789	0.239	0.128	−0.083	0.630
$ESV_{1–2}$	0.091	0.550	0.042	0.785	0.222	0.265	0.288	0.193
$LVDD_{1–2}$	0.042	0.752	0.001	0.992	0.247	0.205	0.327	0.111
$E/E'_{1–2}$	0.033	0.821	0.098	0.506	0.026	0.894	−0.155	0.461
$EDV_{1–2}$	0.311	0.038	−0.040	0.797	0.131	0.515	0.115	0.610
$LVSD_{1–2}$	0.064	0.625	0.038	0.778	0.124	0.538	0.056	0.796

Correlations were assessed by using Spearman's correlation test

CAD coronary artery disease, *VHD* valvular heart disease, *BMI* body mass index, *WC* waist circumferences, *HC* hip circumferences, *FBG* fasting blood glucose, *HDL-C* high-density lipoprotein cholesterol, *LDL-C* low-density lipoprotein cholesterol, *WBC* white blood cell, *DBP* diastolic blood pressure, *SBP* systolic blood pressure, *EF* ejection fraction, *ESV* end systolic volume, *EDV* end diastolic volume, *LVDD* left ventricular diastolic diameter, *LVSD* left ventricular systolic diameter, *E* peak early transmitral flow velocity, *E′* peak early diastolic myocardial velocity, *E/E′* pulmonary capillary wedge pressure. (1–2) changes between first and second echocardiographic findings

other significant association was found between echocardiographic measurements and anti-Hsp27 antibody levels or hs-CRP values in both VHD and CAD groups (Table 3). The correlation between changes in anti-Hsp27 and hs-CRP levels was also assessed, and we found that only changes in hs-CRP values were associated with echocardiographic measurements (Table 4).

Discussion

One of the novel aspects of the present study was the assessment of the relationship between immunologic and inflammatory markers with indices of cardiac function. We found that in CAD patients, anti-Hsp27 antibody levels were negatively associated with E/E′, a marker of pulmonary capillary wedge pressure, and positively with changes in end diastolic volume before and after surgery. No other significant association was found between echocardiographic measurements and anti-Hsp27 antibody levels or hs-CRP values in both VHD and CAD groups. Recently, it was found in patients undergoing cardiac surgery with cardioplegic arrest for CABG or valve repair/replacement that phosphorylation of Hsp27 and αB-crystallin was negatively correlated with cardiac function after surgery, indicating that increased phosphorylation of Hsp27 is correlated with depressed cardiac function after cardiac surgery (Clements et al. 2007). It has been previously reported that patients with more severe LV dysfunction and lower left ventricular ejection fraction had elevated circulating Hsp60 and anti-Hsp60 antibodies, Hsp72, and CRP levels (Giannessi et al. 2007). Moreover, in the latter study, the significant association between Hsp72 and anti-Hsp60 autoantibodies with left ventricular end diastolic diameter values was found (Giannessi et al. 2007).

Another interesting finding of this study was that changes in anti-Hsp27 antibody titers were not significantly different between on-pump and off-pump patients. Anti-Hsp27 antibody levels decreased significantly 24 h post-operation in both groups of on- and off-pump CABG, while at the time of discharge increased to almost its baseline levels. In VHD group, anti-HSP27 antibody titers were also decreased; however, this reduction was only significant between baseline and discharge samples. We have previously found that anti-Hsp27 antibody titers rise and fall rapidly after the

Table 4 Correlation between changes in anti-Hsp27 and hs-CRP values and different parameters

	CAD patients				VHD patients			
	Anti-Hsp27$_{(3–1)}$		hs-CRP$_{(3–1)}$		Anti-Hsp27$_{(3–1)}$		hs-CRP$_{(3–1)}$	
Factor	*r*	*P* value	*r*	*P* value	*r*	*P* value	*r*	*P* value
EF	−0.036	0.825	−0.013	0.939	0.141	0.426	−0.163	0.365
ESV	0.025	0.904	−0.167	0.426	0.052	0.793	0.223	0.262
LVDD	−0.065	0.697	−0.007	0.965	−0.039	0.844	0.280	0.158
E/E′	−0.158	0.388	0.260	0.173	0.165	0.393	−0.114	0.580
EDV	0.202	0.292	−0.291	0.140	0.042	0.831	0.199	0.320
LVSD	0.100	0.551	−0.067	0.698	−0.107	0.594	0.118	0.567
EF$_{1–2}$	−0.012	0.924	0.170	0.315	−0.016	0.932	0.262	0.162
ESV$_{1–2}$	0.142	0.586	−0.274	0.324	−0.071	0.758	−0.263	0.262
LVDD$_{1–2}$	0.026	0.893	−0.431	0.031	−0.156	0.477	−0.531	0.013
E/E′$_{1–2}$	−0.086	0.657	−0.187	0.371	−0.188	0.391	0.030	0.898
EDV$_{1–2}$	−0.007	0.978	−0.390	0.151	−0.076	0.743	−0.195	0.409
LVSD$_{1–2}$	0.058	0.765	−0.401	0.047	−0.115	0.610	−0.304	0.193

Correlations were assessed by using Spearman's correlation test

CAD coronary artery disease, *VHD* valvular heart disease, *EF* ejection fraction, *ESV* end systolic volume, *EDV* end diastolic volume, *LVDD* left ventricular diastolic diameter, *LVSD* left ventricular systolic diameter. *E* peak early trans-mitral flow velocity, *E′* peak early diastolic myocardial velocity, *E/E′* pulmonary capillary wedge pressure, *(3–1)* changes between serum measurement between the first and third sample of the patients. (1–2) changes between first and second echocardiographic findings

onset of ACS, suggesting that anti-Hsp27 antibody may be an early marker of myocardial ischemia (Ghayour-Mobarhan et al. 2008). Moreover, in ACS patients, elevated serum Hsp27 antigen concentrations in the early hours following ACS has been found, but its concentrations fell to levels near to those in healthy individuals after about 12 h from the onset of chest pain (Heidari-Bakavoli et al. 2012). In another study (Moohebati et al. 2011), we found that anti-Hsp27 antibody levels decreased significantly 24 h after percutaneous coronary intervention (PCI) in patients with stable CAD. In previous studies, it has been reported that cardiomyocyte injury is associated with a release of Hsp60 antigen into the systemic circulation and subsequent down-regulation of both humoral and cellular immune responses to Hsps. This finding supports our proposal that soluble and circulating Hsps are able to form immune complexes with their cognate antibody (Schett et al. 1999).

As briefly reviewed in the "Introduction" section, irrespective of which CABG procedure is selected, cell injury occurs, particularly following CPB, which maybe associated with ischemia–reperfusion syndrome, cell necrosis, or apoptosis occurrence leading to tissue injury (Bolli 1991; Hearse 1990; Szerafin et al. 2006; Vaage and Valen 1993). In the latter studies investigating the changes in antibody levels following ACS and PCI, it is possible that the reduction in antibody titers could be ascribed to formation of immune complexes between antigen–antibody. If the cardiomyocytes do not survive from any possible mechanisms such as necrosis or apoptosis, then these high levels of intracellular Hsps will be released into the circulation (Beatty et al. 1994; Boulanger et al. 2001). Moreover, Eberhardt et al. (2000) have reported that after CPB, Hsp70 levels increased significantly compared to baseline levels, which supports our hypothesis that Hsps are released following cardiac surgery. Certainly following ACS, PCI, and open cardiac surgery, myocardial necrosis or apoptosis may occur leading to the release of Hsps from cardiomyocytes. This may then lead to the formation of immune complexes and a reduction in antibody levels.

hs-CRP levels increased significantly in both CAD and VHD following surgery and remained in high levels even at the time of discharge, and changes in hs-CRP levels were not significantly different between on-pump and off-pump patients. However, there are some reports indicating that use of CPB is associated with higher inflammatory response (Ascione et al. 2000; Biglioli et al. 2003), but most studies reported difference in the acute phase response in patients undergoing cardiac surgery irrespective of method of surgery. For example, Fransen et al. (1998) report a similar pattern of changes in the acute phase reactants, for both

on- and off-pump CABG groups. Karu et al. (2010) also report that hs-CRP and serum interleukin-6 concentrations increased statistically in both on- and off-pump CABG and valvular replacement groups and remained elevated during the first post-operative week, suggesting that cardiac surgery leads to extensive and complex inflammatory response regardless of the method of surgical procedure. The sustained inflammatory response is evident irrespective of whether operations are performed due to valvular or coronary disease, with or without CPB. The authors of the latter study proposed that even during uncomplicated cardiac surgery, the inflammatory response is extensive, as the initial high concentrations were decreasing on the second/third postoperative days but still remained higher than baseline by the end of the first week (Karu et al. 2010). Hence, our results and those previously reported (Biglioli et al. 2003; Fransen et al. 1998) establish that on- and off-pump surgeries have few differences in their effects on acute phase responses during coronary surgery and this inflammatory response is predominantly caused by the surgical procedure rather than CPB.

CAD and VHD patients had higher values of anti-Hsp27 antibody and hs-CRP levels in comparison with healthy controls, supporting our previous findings (Ghayour-Mobarhan et al. 2008; Kazemi-Bajestani et al. 2007; Rahsepar et al. 2012). The higher levels of anti-Hsp27 antibody levels in patients with VHD without any significant CAD indicate that the Hsp27 antibody levels are a marker of the cell injury in cardiac tissue following the exposure to different stressors. It has recently been reported that increases in serum markers such as those associated with oxidative stress may occur well before the development of aortic valve stenosis and are not merely epiphenomena related to hemodynamically significant valve stenosis (Miller et al. 2009). Hence, high levels of anti-Hsp27 antibody in VHD who were free from CAD probably reflect the high level of cell stress that cardiomyocytes are facing in these patients. The elevation in Hsp27 antibody levels may be explained by this state of stress in the VHD patients even in the absence of severe hemodynamic changes. Hence, we suggested that increased anti-Hsp27-antibody titers are a marker of changes in myocardial stress and are not specific for the presence of atherosclerotic lesions (Rahsepar et al. 2012).

In conclusion, we have found that serum anti-Hsp27 antibody titers were associated with indices of cardiac function in coronary patients. The changes in antibody titers in coronary patients were not significantly different between the groups of patients who were operated on- or off-pump, indicating that CPB has no significant effect on the changes in serum anti-Hsp27 levels. Moreover, anti-Hsp27 antibody levels fell in all groups of study; these may be caused by the formation of immune complexes of antigen–antibody but increased at the time of discharge.

Limitations

The major limitation of this study was that patients were pre-selected for the type of surgical procedure they subsequently underwent. It was not possible to randomize patients to group, and this may have resulted in a selection bias dependent on disease severity or other confounding criteria. A further limitation of this study was that we were unable to measure the level of Hsp27 antigen level, determining the levels of both antigen and antibody levels against Hsps would help to interpret the data more accurately with respect to the temporal changes that were observed.

Acknowledgments We are particularly grateful to the patients and their family members who volunteered to participate in this study. This work was supported by Mashhad University of Medical Science (MUMS), Iran. The results presented in this work have been taken from Amir Ali Rahsepar's thesis in MUMS, with the following ID number: 87065.

Conflict of interest The authors declare no conflict of interest.

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