

High Serum Levels of Rheumatoid Factor and Anti-Phosphatidylserine Antibody in Patients with Ischemic Heart Disease

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ABSTRACT

Background: Immunopathological and inflammatory processes play important roles in the initiation and development of Ischemic Heart Disease (IHD). **Objective:** The aim of this study was to evaluate the serum levels of several autoantibodies including rheumatoid factor (RF), anti-nuclear antibodies (ANA), anti-small nuclear ribonucleoprotein (anti-Sm), anti-phosphatidylserine (anti-PS) and anti-cardiolipin (anti-CL) antibodies in patients with IHD. **Methods:** A total of 120 patients with IHD with acute myocardial infarction (AMI; n=60) or unstable angina (UA; n=60) and 60 sex- and age-matched healthy subjects were enrolled in this study. Serum samples of participants were tested for the ANA, anti-Sm, anti-PS and anti-CL antibodies by ELISA. Serum level of RF was measured by a turbidometric method. **Results:** The mean serum levels of RF and anti-PS antibodies in AMI group and UA group were significantly higher than those observed in the control group ($p<0.0001$). The mean serum levels of RF and anti-PS antibodies in AMI patients were significantly higher than the UA group ($p<0.01$ and $p<0.001$, respectively). The mean serum levels of RF in men with AMI or UA diseases were significantly higher as compared to healthy control men ($p<0.0001$ and $p<0.003$, respectively). The differences of the serum levels of ANA, anti-Sm and anti-CL antibodies were not significant between AMI, UA and the control groups. There was no difference in the serum levels of RF, ANA, anti-Sm, anti-PS or anti-CL antibodies in patients with traditional risk factors, including hypertension, dyslipidemia, diabetes and smoking, and those without a certain risk factor. **Conclusion:** Higher serum levels of RF and anti-PS antibody in patients with IHD may be considered as independent risk factors for IHD.

Keywords: Anti-Nuclear Antibody, Myocardial Infarction, Rheumatoid Factor, Unstable Angina

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INTRODUCTION

Traditional risk factors, such as hypercholesterolemia, hypertension, diabetes, genetic abnormalities, smoking and obesity can explain only about half of the cases of coronary heart disease (1). It has been demonstrated that the immunopathological and inflammatory processes play important roles in the initiation and development of Ischemic Heart Disease (IHD). Accumulation of leukocytes including monocytes/macrophages, T cells, B cells and PMN cells has been shown in atherosclerotic lesions (1,2). Moreover, C-reactive protein (CRP), a sensitive marker of inflammation, has been reported to increase in patients with IHD and is considered as a valuable tool for the estimation of at-risk populations (3,4). It has also been reported that serum levels of CRP using a high sensitivity assay (hs-CRP) can identify sub-clinical inflammatory states, reflecting vascular inflammation (4,5).

Some of the noticeable data that link autoimmunity and atherosclerosis originate from epidemiological studies of patients with rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) (6,7). RA patients are at higher risk of developing acute myocardial infarction and the risk increases with disease duration (8). The prevalence of silent myocardial infarction and abrupt death is also higher in patients with RA than in the general population. This excess cardiovascular risk is only partially due to traditional risk factors, therefore suggesting a principal role of RA-associated risk factors (8). Similar pathological mechanisms have been reported for RA and atherosclerosis including the participation of proinflammatory cytokines such as tumor necrosis factor (TNF)- α and autoreactive T cells (9,10). These similarities indicate that the inflammatory mechanisms responsible for synovial injuries also lead to cardiovascular disease in patients with RA (10). The RF autoantibody that is strongly associated with RA, may exist in subjects many years before they exhibit RA and its appearance awards a risk of developing RA that increases with rising titer (11). Recently, RF has been shown to associate with an increased probability of IHD in patients with inflammatory polyarthritis (12). Based on these observations it seems that RF may have a special role in the pathogenesis of IHD. Patients with SLE are also 5-6 times more likely to have a cardiovascular event than people in the general population (13). The production of autoantibodies against nuclear antigens such as ANA and anti-Sm is the hallmark of SLE (14). It has been suggested that ANA may contribute to the pathogenesis of atherosclerosis and that ANA positivity is associated with the presence of coronary atherosclerosis (15).

Antiphospholipid (anti-PL) antibodies constitute a heterogeneous group of autoantibodies that are able to bind phospholipids (PL) alone, protein-PL complexes or PL-binding proteins (16). Recent observations suggest that anti-PL antibodies may involve in the pathogenesis of atherosclerosis (16,17). Laboratory findings and murine models also support the pro-atherogenic role of anti-PL antibodies, as they are involved in the uptake of oxidized LDL into macrophages, and immunization of mice with them results in enhanced atherosclerosis (18). One of the most commonly reported anti-PL antibodies is the anti-PS antibody type (19). The importance of these antibodies is due to their specific binding to phosphatidylserine molecule which shifts from the inner to the outer leaflet of cell membranes during early apoptosis. This event activates the coagulation cascade and thus may cause blood clotting (20). The results of some studies also demonstrated an association between anti-CL antibody and IHD (21). It should be noted that genetic factors as well as other traditional risk factors such as smoking, hypercholesterolemia,

diabetes mellitus and hypertension may contribute to IHD development and these parameters differ among various population. Although there are a few studies on the association of some autoantibodies with IHD, more epidemiological data are required to confirm their significance as independent risk factors in cardiovascular diseases. Moreover, the data on the relationship of autoantibodies with traditional risk factors of IHD is scarce. Therefore, this study was conducted to evaluate the serum levels of RF, ANA, anti-Sm, anti-PS and anti-CL antibodies in Iranian patients with IHD and also to clarify their association with traditional risk factors of the disease.

MATERIALS AND METHODS

Subjects. A total of 120 patients (aged 40-60 years) with IHD who were admitted to Ali-ebne-Abitaleb hospital of Rafsanjan, in Southeast of Iran, were enrolled to this cross-sectional, case-control study. Patients were classified into 2 groups according to well established criteria, as having AMI (n=60) or unstable angina (n=60). AMI was diagnosed by the presence of two of the following criteria: i) prolonged chest pain compatible with AMI, ii) typical ECG changes, iii) rising of cardiac enzymes such as creatine kinase and lactate dehydrogenase. UA was defined according to the Braunwald's classification and all patients had chest pain at rest with definite ischemic electrocardiographic changes such as ST-segment changes and/or T-wave inversion. UA patients were in class IIIB according to Braunwald's classification (22). Exclusion criteria were valvular heart disease, surgery, trauma during the prior month, cardiomyopathy, liver disease, renal failure, arthritis, malignant diseases, other inflammatory diseases (such as SLE and RA) and oral anticoagulant therapy. In patients with AMI, the serum concentration of autoantibodies was measured during 3-5 days after admission. In patients with UA the measurements were done at admission time. A third sex and age-matched group (n=60) with similar geographic and socioeconomic backgrounds without any ischemic heart disease were used as a control group. All control subjects were basically healthy, with no acute or chronic illnesses and did not use any drugs. The healthy control group was recruited from blood donors attending Rafsanjani Blood Transfusion Center. Peripheral blood (2-4 milliliter) was collected from all 3 groups and the serum was separated and stored at -20°C. This study was evaluated and approved by the Ethical Committee of Rafsanjan University of Medical Sciences. Moreover, patients were only recruited if they agreed to blood sampling.

Detection of the Serum Levels of Autoantibodies. Serum RF levels were measured by turbidimetric method using commercial kits (Biosystem, Barcellona, Spain). RF causes agglutination of the latex particles coated with human gammaglobulin. The agglutination of the latex particles is proportional to RF concentration which is measured by turbidimetry. The serum levels of RF were quantitated using standard samples with known concentrations of the factor expressed as IU/mL, provided by the manufacturer. Although, RF is mainly in the IgM class, it can be of other isotypes such as IgG and IgA (23). It should be noted that turbidimetry does not differentiate between RF isotypes. Accordingly, all isotypes of RF were measured by this method (24). The normal range of RF has been reported to be <20 IU/mL (25).

The serum levels of ANA and anti-Sm, anti-CL and anti-PS antibodies were also measured using the commercial ELISA kits (ANA, Genesis, Cambridgeshire, UK; anti-Sm,

Euroimmun, Lübeck, Germany, anti-CL, Genesis, Cambridgeshire, UK; anti-PS, Euroimmun, Lübeck, Germany). The serum samples were incubated in antigen-coated wells. After washing, the anti-human IgG conjugated with horseradish peroxidase (HRP) was added to the wells. After incubation the unbound conjugate was removed by washing and then the enzyme substrate was added. After color development, the optical density of samples was measured using a microplate reader. The optical density is directly proportional to the antibody concentration in the sample. The serum levels of ANA, anti-CL, anti-PS and anti-Sm antibodies were quantitated using standard samples with known concentrations of antibodies provided by the manufacturers. The serum levels of anti-CL IgG was expressed as GPLU/mL. According to the manufacturer's guideline, the normal range of anti-CL IgG has been reported to be <8 GPLU/mL. The serum levels of ANA IgG was expressed as U/mL with a normal range of <10 U/mL based on kits indicator. Moreover, according to the manufacturer's guidelines, the serum concentrations of anti-Sm IgG and anti-PS IgG were expressed as relative units per milliliter (RU/mL), as no International Standard is available. Their normal ranges were <20 RU/mL and <12 RU/mL, respectively.

Statistical Analysis. Differences in variables were analyzed using Student's t-test, ANOVA, Mann-Whitney U and Kruskal-Wallis as appropriate and p values of less than 0.05 were considered significant. All the available data were analyzed by a computer program (SPSS, Chicago, IL, USA).

RESULTS

Baseline Characteristics of Patients. Baseline characteristics of AMI, UA and healthy control groups are shown in Table 1. There were no significant differences among 3 groups for the age and the gender ratio. Moreover, no statistically significant differences were observed between the two groups of patients with respect to the age or the presence of traditional risk factors for atherosclerosis.

Table 1. Baseline characteristics of study patients.

	AMI (%) (n=60)	UA (%) (n=60)	Control (n=60)
Age (year), mean \pm SD	48.8 \pm 7.9	49.7 \pm 7.3	49.8 \pm 7
Sex (Men/Women)	39/21	36/24	37/23
Hypertension	30 (50.0)	25 (41.6)	0
Dyslipidemia	31 (51.6)	32 (53.3)	0
Diabetes mellitus	20 (33.3)	14 (23.3)	0
Current smoking	23 (38.3)	20 (33.3)	0
Medications:			
Aspirin	26 (43.3)	25 (41.6)	0
Statins	19 (31.6)	24 (40.0)	0

AMI: Acute myocardial infarction, SD: Standard deviation, UA: Unstable angina

Serum levels of Autoantibodies. The mean serum levels of RF, ANA, anti-Sm, anti-PS and anti-CL antibodies in healthy control and patient groups are demonstrated in Table 2. The mean serum levels of RF in AMI group (88.61 ± 34.88 IU/mL), UA group (72.78

± 12.84 IU/mL) and all the patients with IHD (80.70 ± 27.35 IU/mL) were significantly higher than that observed for the control group (60.71 ± 15.94 IU/mL, $p < 0.0001$). Moreover, the mean serum levels of RF in AMI patients was significantly higher than UA group ($p < 0.01$). The mean serum levels of RF in male subjects with AMI (95.15 ± 31.64 IU/mL) or UA (72.04 ± 14.56 IU/mL) were significantly higher as compared to healthy men (61.05 ± 15.26 IU/mL; $p < 0.0001$ and $p < 0.003$, respectively). Although in female patients with AMI or UA disease, the mean serum levels of RF was higher than that in the healthy group, but the differences were not statistically significant.

In AMI group, the mean serum levels of RF in men was significantly higher than that of women ($p < 0.04$). However, the mean serum levels of RF in male and female groups with UA or the control group did not differ significantly (Table 2).

Table 2. Serum levels of RF, ANA, anti-Sm, anti-PS and anti-CL antibodies in IHD and healthy groups according to gender.

Group	Sex	No.	RF (IU/mL) (mean \pm SD)	ANA (U/mL) (mean \pm SD)	Anti-Sm (RU/mL) (mean \pm SD)	Anti-PS (RU/mL) (mean \pm SD)	Anti-CL (GPLU/mL) (mean \pm SD)
AMI	Male	39	95.15 ± 31.64	3.48 ± 1.45	6.01 ± 3.72	7.39 ± 2.00	6.20 ± 1.33
	Female	21	76.47 ± 38.07	7.72 ± 14.91	7.10 ± 4.30	7.81 ± 2.60	6.84 ± 1.66
	Total	60	88.61 ± 34.88	4.96 ± 8.99	6.39 ± 3.93	7.53 ± 2.22	6.42 ± 1.47
UA	Male	36	73.27 ± 11.75	3.89 ± 1.76	5.52 ± 1.58	5.79 ± 1.52	6.16 ± 1.90
	Female	24	72.04 ± 14.56	5.22 ± 2.45	5.98 ± 2.36	5.16 ± 1.08	5.98 ± 2.33
	Total	60	72.78 ± 12.84	4.42 ± 2.15	5.70 ± 1.92	5.54 ± 1.38	6.09 ± 2.06
IHD	Male	60	84.65 ± 26.46	3.68 ± 1.61	5.77 ± 2.89	6.62 ± 1.95	6.18 ± 1.62
	Female	60	74.11 ± 27.83	6.39 ± 10.29	6.50 ± 3.41	6.40 ± 2.34	6.38 ± 2.06
	Total	120	80.70 ± 27.35	4.69 ± 6.52	6.05 ± 3.10	6.54 ± 2.10	6.26 ± 1.79
Healthy	Male	37	61.05 ± 15.26	3.59 ± 1.52	5.51 ± 1.94	3.52 ± 2.06	5.67 ± 2.88
	Female	23	60.17 ± 17.33	5.60 ± 3.77	5.71 ± 2.07	4.07 ± 2.88	6.94 ± 7.51
	Total	60	60.71 ± 15.94	4.36 ± 2.77	5.59 ± 1.98	3.73 ± 2.40	6.16 ± 5.15

AMI: Acute myocardial infarction, ANA: anti-nuclear antibodies, Anti-CL: anti-cardiolipin, Anti-PS: anti-phosphatidylserine, Anti-Sm: anti-small nuclear ribonucleoprotein, IHD: Ischemic heart disease, No: Number of subjects, SD: Standard deviation, UA: Unstable angina.

The mean serum levels of anti-PS in AMI group (7.53 ± 2.22 RU/mL), UA group (5.54 ± 1.38 RU/mL) and in all the patients with IHD (6.54 ± 2.10 RU/mL) were significantly higher than that observed for the control group (3.73 ± 2.40 RU/mL, $p < 0.0001$). Moreover, the mean serum levels of anti-PS antibody in AMI patients was significantly higher in comparison to UA group ($p < 0.001$). In patients and control groups, the differences of the mean serum levels of anti-PS antibody between men and women were not statistically significant (Table 2).

The differences of the serum levels of ANA, anti-Sm and anti-CL antibodies between AMI, UA and control groups were not statistically significant. No significant differences were observed between men and women of AMI, UA and control groups regarding the serum levels of ANA, anti-Sm and anti-CL antibodies (Table 2).

The serum levels of autoantibodies according to traditional risk factors of IHD have been summarized in Table 3. The differences of the mean serum levels of autoantibodies in patients with a traditional risk factor including hypertension, dyslipidemia, diabetes and smoking were not statistically significant as compared to corresponding groups without a certain risk factor.

Table 3. Serum levels of RF, ANA, anti-Sm, anti-PS and anti-CL antibodies in patients with IHD according to traditional risk factors.

Risk factor	Risk factor status	No.	RF levels (IU/MI) (mean ± SD)	ANA (U/mL) (mean ± SD)	Anti-Sm (RU/mL) (mean ± SD)	Anti-PS (RU/mL) (mean ± SD)	Anti-CL (GPLU/mL) (mean ± SD)
Without RF [#]	-----	60	60.71 ± 15.94	4.36 ± 2.77	5.59 ± 1.98	3.73 ± 2.40	6.16 ± 5.15
Hypertension	Positive	55	82.27 ± 26.10	5.56 ± 9.44	6.50 ± 3.70	6.57 ± 2.25	6.41 ± 1.67
	Negative	65	79.36 ± 28.50	3.96 ± 1.59	5.66 ± 2.45	6.51 ± 1.97	6.12 ± 1.89
Dyslipidemia	Positive	63	80.52 ± 26.81	4.25 ± 2.13	6.20 ± 3.16	6.51 ± 2.12	6.40 ± 1.85
	Negative	57	80.89 ± 28.18	5.18 ± 9.21	5.87 ± 3.06	6.56 ± 2.08	6.10 ± 1.73
Diabetes	Positive	34	79.58 ± 27.55	4.03 ± 2.18	6.77 ± 4.14	6.99 ± 2.27	6.26 ± 1.77
	Negative	86	81.13 ± 27.42	4.96 ± 7.57	5.76 ± 2.55	6.36 ± 2.01	6.26 ± 1.81
Smoking	Positive	43	82.11 ± 31.91	3.41 ± 1.39	6.00 ± 3.28	6.57 ± 2.00	6.11 ± 1.68
	Negative	77	79.90 ± 24.64	5.41 ± 8.00	6.07 ± 3.02	6.52 ± 2.16	6.34 ± 1.86

[#]Healthy group

The serum levels of autoantibodies according to medication have been summarized in Table 4. The administration of statin or aspirin had no significant effects on the serum levels of antibodies.

Table 4. Serum levels of RF, ANA, anti-Sm, anti-PS and anti-CL antibodies in patients with IHD according to medication.

Medication	Administration	No.	RF levels (IU/MI) (mean ± SD)	ANA (U/mL) (mean ± SD)	Anti-Sm (RU/mL) (mean ± SD)	Anti-PS (RU/mL) (mean ± SD)	Anti-CL (GPLU/mL) (mean ± SD)
Statin	Positive	43	82.84 ± 27.67	4.45 ± 2.25	6.07 ± 2.99	6.72 ± 2.13	6.33 ± 1.92
	Negative	77	79.70 ± 27.31	4.81 ± 7.75	5.04 ± 3.17	6.45 ± 2.09	6.22 ± 1.74
Aspirin	Positive	51	82.85 ± 26.65	6.00 ± 10.03	6.25 ± 3.30	6.31 ± 2.17	6.53 ± 1.78
	Negative	69	79.26 ± 28.18	3.82 ± 1.62	5.91 ± 2.98	6.69 ± 2.05	6.08 ± 1.79
No Medication	-----	60	60.71 ± 15.94	4.36 ± 2.77	5.59 ± 1.98	3.73 ± 2.40	6.16 ± 5.15

ANA: anti-nuclear antibodies, Anti-CL: anti-cardiolipin, Anti-PS: anti-phosphatidylserine, Anti-Sm: anti-small nuclear ribonucleoprotein, IHD: Ischemic heart disease, No: Number of subjects, SD: Standard deviation.

DISCUSSION

The results of the present study showed that the mean serum levels of RF and anti-PS antibodies in patients with IHD (including AMI and UA) were significantly higher than that observed in healthy control group. These associations were independent of traditional risk factors. These observations indicate that RF and anti-PS antibody may be independent risk factors for IHD. Although the incidence of cardiovascular disease is higher in patients with RA in comparison to healthy subjects, the prevalence of traditional risk factors has been reported to be similar in patients with or without RA (9). The precise mechanisms of involvement of RF and anti-PS antibody in the pathogenesis of IHD remain to be determined.

RF is an important test for diagnosis and prognosis of RA (26). It has been reported that patients who were positive for IgA and IgM RF showed significantly progressive disease and joint damage (27). In other words, the disease activity is more severe in the RF seropositive than the seronegative patients and more forceful treatments are needed for seropositive patients (27,28). The association of RF with IHD provides further evidence of the importance of inflammation as an important risk factor for vascular disease. RA is a chronic inflammatory disease and it has been reported that RA and atherosclerosis have similar pathological mechanisms (9, 10). The expression of adhesion molecules on the endothelial cells, elevation of inflammatory mediators such as TNF- α , IL-1, IL-6 and CRP, endothelial dysfunction and oxidative stress are major symptoms of RA and cardiovascular disease (10,13). Recently, studies have shown that CRP possesses proatherogenic properties (29,30). Moreover, the inflammatory mediator TNF- α has an important role in the initiation and progression of atherosclerosis in RA (31).

RF is strongly associated with RA and RA is associated with increased cardiovascular morbidity and mortality (12) but in our study the association between RF and IHD is not due to clinically active RA. However, RF-related inflammatory reactions, even in sub-clinical forms, may contribute in IHD pathogenesis. RF appears to cause direct tissue damage in RA as a component of immune complexes via activating the complement system (11). It may cause damage to the endothelium in a similar manner in IHD. The presence of immunoglobulins and complement components has been demonstrated in atherosclerotic plaques providing evidence for immune complex reactions (32).

Atherosclerosis is also an inflammatory response to the deposition of lipoproteins (cholesterol and triglycerides) in the arterial walls (32). It has been reported that oxidized low-density lipoprotein (oxLDL) which is present in lesions, induces antibody production (33,34). The specific antibodies were found to promote clearance of oxLDL in some studies and predict coronary events in the patients (34). There are also data supporting an association between anti-PL antibodies and atherosclerosis (18). Anti-PL antibodies are a diverse group of antibodies directed to negatively charged phospholipids (such as cardiolipin, phosphatidylserine, phosphatidylinositol), phospholipid-protein complexes or plasma proteins (such as β 2-glycoprotein I) (16). These antibodies have been associated with an increased risk for recurrent arterial and venous thrombosis, thrombocytopenia and fetal loss (35). One of the anti-PL antibodies is anti-PS antibody type (19). Phosphatidylserine (PS) is a membrane aminophospholipid that is usually expressed only on the inner plasma membrane layer. Under conditions of cell stress, PS is quickly translocated to the outer surface of the plasma

membrane (36). After exposure on the plasma membrane outer surface, PS contributes in membrane signaling processes that result in assembling of coagulation factors or leading to apoptosis (37). The PS expression establishes the membrane binding of plasma coagulation proteins. Many of these plasma proteins undergo conformational changes as a consequence of binding PS and afterwards express new antigenic epitopes, which can induce autoimmune responses in particular susceptible subjects (37).

As mentioned, PS participates also be in apoptosis. It is now demonstrated that various cells such as smooth muscle cells and macrophages undergo apoptosis within unstable atherosclerotic plaques (36). Accordingly, PS-related apoptosis process may contribute to the pathogenesis of cardiovascular system including atherosclerotic vascular disease. Physiologically, PS also serves as a marker for macrophages to phagocytose and delete apoptotic cells (37).

Anti-PS antibody may also involve directly and/or indirectly in the pathogenesis of IHD. It has been reported that anti-PL antibodies bind to the phospholipid/plasma protein complex on endothelial cells and/or the monocyte surface and then increase the expression of the tissue factor on monocytes and vascular endothelial cells (38,39). The raised tissue factor expression on monocytes and vascular endothelial cells in response to anti-PL antibodies may be a mechanism for development of atherosclerosis (38,30). Moreover, anti-PL antibodies promote atherosclerosis by recruitment of monocytes toward endothelial cells and/or increasing the influx of oxLDL into macrophages (40, 41). Furthermore, recent experimental evidence suggests that anti-PL antibodies induce pro-inflammatory cytokines such as TNF- α and IL-1 and this process also contributes to the development of atherosclerosis (41,42).

Our study cannot determine whether RF and anti-PS antibody are nonspecific mediators of inflammation or are involved directly in the pathogenesis of IHD. However, the lack of association between IHD and ANA, and anti-Sm and anti-CL antibodies suggests that the association between RF and anti-PS antibody with IHD was not due to non-specific polyclonal B-cell activation secondary to inflammation but that RF and anti-PS antibody may have a particular role in the pathogenesis of IHD. Moreover, RF positivity has been presented as predictors of cardiovascular events and mortality in both those with or without rheumatic diseases (43).

The possible mechanisms responsible for the elevation of RF and anti-PS antibody in IHD remain to be clarified. It has been reported that RF production is associated with many infectious diseases (such as *Chlamydia pneumoniae*, Cytomegalovirus, Herpes simplex virus, Epstein-Barr virus, Hepatitis- A, B and C) and may also be induced by some infectious-derived components such as lipopolysaccharides (44). It has been also demonstrated that *Chlamydia trachomatis* and *Chlamydia pneumoniae* infections elicit the externalization of PS on the surface of human epithelial, endothelial, granulocytic, and monocytic cells (45). An association between IHD and some infectious agents including *Chlamydia pneumoniae*, *Helicobacter pylori*, herpes simplex virus, cytomegalovirus, hepatitis A, respiratory tract and dental infections has been reported in some epidemiological studies (2,46). In our previous study, higher seroprevalence of *Chlamydia pneumoniae* was also observed in patients with IHD in comparison to the healthy control group (3). Therefore, the infectious agents may directly and/or indirectly trigger RF production and the expression of PS, which could also provide a potential link between infections and cardiovascular events.

Our results showed that the mean serum levels of RF in men with IHD were significantly higher as compared to healthy male group. Although in female patients the mean serum levels of RF was higher than that of the control group, the differences were not statistically significant. These results represent an association between RF and IHD in men. Similar findings have also been reported by Edwards et al. (12). The reason for these associations remains unclear. However, differential IHD pathogenesis and/or differential hormonal pattern may account for these observations.

The results of the present study also showed that the differences of the mean serum levels of ANA, anti-Sm and anti-CL antibodies between patients with IHD and healthy subjects were not statistically significant. The results of some studies have demonstrated that ANA and anti-CL antibody are among risk factors for coronary artery disease (15, 47) but in other investigations no association was found between coronary artery disease with ANA and anti-CL antibodies (48,49). These discrepancies may be attributed largely to differences in age, race and ethnic background of the subjects. Moreover, different inclusion criteria of patients and controls used in various studies and differences in the distribution of traditional risk factors of IHD may also account for some of the differences.

It should be noted that our study has several limitations. First, measurement of autoantibodies was performed at one time only. These results could not provide information on the time of and the duration of elevation of serum RF and anti-PS antibodies. It should also be noted that the early rise in serum autoantibody levels may be a part of immune response against the protein released from the necrotic heart tissue. Thus, the autoantibodies may play a direct role in the pathogenesis of ischemic heart diseases or may be formed during immunopathological responses after events. Second, the effects of circulating autoantibodies on long-term clinical outcomes were not part of the protocol. However, as mentioned, RF positivity has been presented as predictor of cardiovascular events and mortality in both those with and without rheumatic diseases (39). Estimating the time course of autoantibodies during the cardiovascular event might also improve their prognostic value. Third, measurement of autoantibodies was performed on samples that were stored at -20°C. We cannot exclude the possibility of protein degradation. However, this should affect both cases and controls in a similar way. Fourth, it should be also noted that the medication including statins and aspirin may have influenced autoantibody patterns and accordingly the pre-treatment of some patients with these drugs may produce confusing results.

The results of the present study showed higher serum levels of RF and anti-PS antibodies in patients with IHD. Therefore, RF and anti-PS antibodies may be independent risk factors for IHD. The differences of the serum levels of ANA, anti-Sm and anti-CL antibodies were not statistically significant between IHD and healthy control groups. The serum levels of autoantibodies were not influenced by traditional risk factors. Examining the time course of autoantibodies before or during the cardiovascular event may improve their predictive or prognostic values. The results of our study suggest that the manipulation of autoantibodies production or their effects may be considered in designing novel therapeutic approaches.

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