

## Anti-varicella Zoster Virus IgG and hsCRP Levels Correlate with Progression of Coronary Artery Atherosclerosis

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### ABSTRACT

The relationship between high levels of anti-Varicella Zoster Virus (VZV) IgG in cerebrospinal fluid (CSF) and cerebrovascular atherosclerosis commends a possible similar association in other vessels. We aimed to investigate the association of VZV-seropositivity with coronary artery atherosclerosis.

We recruited 88 newly diagnosed patients with more than 50% stenosis in at least one of the main coronary arteries. As the control group, 99 age-matched individuals with normal/insignificant coronary artery findings were included. Clinical, paraclinical, and demographical data were gathered at the time of sampling. High-sensitivity C-reactive protein (hsCRP) levels were measured by nephelometry. VZV-seropositivity was determined by measuring of anti-VZV IgG level in plasma. Multivariable logistic regression was used to evaluate the correlation of data with coronary vascular atherosclerosis.

The frequency of VZV-seropositivity was significantly higher in the atherosclerosis group compared to the controls (OR=1.88; 95%CI=1.03-3.44). The plasma levels of anti-VZV IgG were significantly higher in patients with atherosclerosis (Median=2.70, IQR=1.53-4.30 AU/mL) than in the controls (Median=2.10, IQR=1.70-3.10 AU/mL,  $p=0.034$ ). The hsCRP levels in patients and controls were  $5.19\pm 2.00$  and  $1.51\pm 1.07$  mg/L, respectively. The correlation between hsCRP and anti-VZV IgG level in plasma was observed ( $r=0.40$ ,  $p<0.001$ ). The levels of hsCRP and anti-VZV IgG increased based on the number of diseased vessels but only the difference in hsCRP levels reached a significant level ( $p<0.001$  and  $p=0.168$ , respectively).

Our data suggest that VZV-seropositivity and hsCRP elevation jointly increase the risk of atherosclerosis. The multifactorial nature of atherosclerosis; however, leaves more options for the inflammatory milieu to be generated.

**Keywords:** Atherosclerosis; C-reactive protein; Immunoglobulin G; Varicella-zoster virus

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## INTRODUCTION

Varicella-Zoster Virus (VZV) is a member of herpes simplex virus family which can survive in the host body after the primary infection as a latent virus. VZV is responsible for chickenpox (primary infection) in the childhood and herpes zoster (re-exposure or latent reactivity) in adulthood.<sup>1,2</sup> Different molecular and serological studies have shown that VZV is one of the common infectious agents worldwide with an extensive range of clinical symptoms in the respiratory, skin, nervous, and vascular systems.<sup>3</sup> In addition, several studies have shown the association between VZV and cerebrovascular atherosclerosis (AS).<sup>4,5</sup> Atherosclerosis is a chronic inflammatory disease initiated by activation of endothelial cells (ECs), expression of adhesion molecules, secretion of pro-inflammatory chemokines and cytokines, migration of immune cells, and eventually foam cells formation, smooth muscle cells (SMCs) proliferation, and plaque formation.<sup>6,7</sup> Atherosclerotic lesion growth and rupture over time can cause obstruction or thrombosis in the lumen of the artery that reduces blood flow to the tissues. Depending on the affected arteries and tissues, this may lead to the cerebrovascular, coronary artery, and kidney artery diseases.<sup>8,9</sup>

Several studies showed the role of VZV in cerebrovascular atherosclerosis; using molecular, histological, seroepidemiological, and experimental examinations.<sup>10,11</sup> Primarily, by PCR method, VZV DNA in cerebrospinal fluid (CSF) and/or atherosclerotic plaques of patients with stroke was detected which suggested a correlation between the VZV and atherosclerosis. Afterward, the investigation of VZV or its antigens was used by histological methods to confirm the role of VZV in atherosclerosis.<sup>3</sup> Furthermore, seroepidemiological methods revealed high levels of anti-VZV IgG in the CSF of 93% of patients with cerebrovascular atherosclerosis.<sup>12</sup> On the other hand, several studies have suggested an increased risk of cardiovascular events following herpes zoster infection.<sup>9,13</sup> Contrarily, another study showed that VZV infection in childhood is negatively associated with coronary heart disease (CHD).<sup>14</sup> Accordingly, VZV infection in childhood was related to a 1.5 fold decreased the risk of CHD in adulthood. Most of the previous studies addressed VZV vasculopathy in cerebrovascular disease and information on the effect of VZV infection in cardiac

vasculopathy is scarce.<sup>15</sup>

C-reactive protein (CRP) is an acute inflammatory protein that is produced by liver hepatocytes, macrophages, lymphocytes, ECs, SMCs, and adipocytes in inflammation.<sup>16</sup> Age, gender, obesity, hypertension, dyslipidemia, diabetes mellitus, smoking state, and acute and chronic inflammatory diseases (such as infections and atherosclerosis) are factors that can alter CRP levels in individuals.<sup>17,18</sup> CRP is a direct risk factor for the induction and progression of atherosclerotic plaques.<sup>19,20</sup> High sensitive CRP (hsCRP) levels have clinical value for the detection of inflammation and predictive value for asymptomatic atherosclerosis in the presence of other risk factors. In this regard, hsCRP levels of 1-3 mg/L have intermediate and hsCRP levels greater than 3 mg/L have high-risk values for atherosclerosis.<sup>16,21</sup> CRP acts through induction of complement activation, phagocytosis, nitric oxide (NO) release, and the secretion of cytokines, such as interleukin (IL)-1, -6, and tumor necrosis factor (TNF)- $\alpha$ .<sup>16</sup> Mean levels of CRP are elevated in infectious diseases, but previous studies reported no association between CRP levels and anti-HIV or anti-H. pylori IgG in atherosclerosis.<sup>22,23</sup>

It is shown that plasma levels of CRP are associated with clinical manifestations of VZV infection,<sup>24</sup> however, a percentage of patients with VZV infection do not develop high levels of CRP.<sup>25</sup> Therefore, low and high-grade inflammation attributed to different levels of infection activity (acute, latent or chronic phases) may have different effects in terms of inflammatory diseases progression such as coronary artery disease (CAD). The immune response to VZV infection develops over time and is boosted by intermittent activation of a latent virus which may rather induce a low grade chronic inflammation. Therefore, we asked if there is a correlation between anti-VZV IgG as a correlate of the immune response to VZV, hsCRP levels, and CAD.

## PATIENTS AND METHODS

### Patients and Controls

A total of 88 non-smoker patients with coronary vascular atherosclerosis (37 females and 51 males, mean $\pm$ SD age of 61.75 $\pm$ 9.05 years) and 99 age-matched non-smoker individuals (54 females and 45 males, mean $\pm$ SD age of 59.67 $\pm$ 6.70 years) were enrolled in this case-control study. Patients with

atherosclerosis were defined with more than 50% stenosis in at least one of the main coronary arteries. The distribution of patients according to the number of diseased vessels was as follows: 15 patients had a single-vessel disease (SVD), 24 patients had the two-vessel disease (2VD), and 49 patients had the three-vessel disease (3VD). Whereas controls were individuals who had insignificant/normal coronary vascular findings confirmed by coronary angiography. All subjects were selected from individuals who were referred to hospitals affiliated to the Shiraz University of Medical Sciences, Shiraz, Iran between January and September 2018 for diagnostic angiography. Subjects were older than 48 years and were all admitted as new cases. Demographic characteristics, medical history, medication, disease history, smoking habits, and physical signs were collected during admission. The exclusion criteria were the positive history of previous coronary vascular disease, peripheral vascular disease, cancer, allergy, smoking history, a family history of cardiac disease, lipid-lowering therapy, and inflammatory or infectious diseases in the last 3 months (CRP>10 mg/L). The control group consisted of individuals who were suspected of coronary artery problems and were referred to the angiography ward for diagnostic procedures by physicians. All individuals who participated in this research consented to enter the study. All procedures were reviewed and approval was obtained from the Ethics Committee of Shiraz University of Medical Sciences, Shiraz, Iran. The code of ethical approval of this project is IR.SUMS.REC.1396.S652

#### Sample Size Calculation

Based on the CAD prevalence of 38% in middle aged adults in Iran<sup>26</sup>, power=90%,  $Z_{\alpha/2}=1.96$ ,  $Z_{\beta 90\%}=1.28$ ,  $p_1=38\%$  and  $p_2=62\%$ , and the formula of  $n=(Z_{\alpha/2}+Z_{\beta})^2 \times (p_1(1-p_1)+p_2(1-p_2))/(p_1-p_2)^2$ , the sample size was calculated as  $n=86$  for each group.

#### Serological Analysis

Peripheral blood samples were obtained from all participants during angiography, plasma was separated, and kept frozen at  $-80^{\circ}\text{C}$  until the experiment. Each specimen was labeled with a number, thus the investigators were blind to all personal data of subjects at the time of the experiment until statistical analysis of the results. Anti-VZV IgG plasma levels were measured; using a commercial ELISA assay (DIESSE

Diagnostica Senese, Italy) according to the manufacturer's instructions. The titer was expressed in arbitrary Units/Milliliter (AU/mL) and IgG concentrations  $\geq 2.1$  AU/mL were considered as seropositive (anti-VZV<sup>+</sup>). The sensitivity and specificity of the assay were 89% and 100%, respectively. The minimum detection limit of the assay was  $\sim 0.1$  AU/mL. The hs-CRP levels were measured by a high sensitive nephelometry assay (Biorex, UK).

#### Statistical Analyses

Chi-square test ( $X^2$ ) was used for comparison of qualitative variables such as distributions of demographic, clinical, and laboratory data between patients and controls. A simple (crude) logistic regression was performed on all known risk factors that were recorded at the time of sampling. Then, for those variables with a  $p$ -value less than 0.2 and one of the major risk factors (i.e. BMI), multivariable logistic regression (Enter method) was performed. Variables considered in the multivariate analysis included sex, age, obesity (BMI  $\geq 30$  kg/m<sup>2</sup>), hypertension, hyperlipidemia, diabetes mellitus, and VZV-seropositivity. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated; using a multivariable logistic regression model to examine associations between risk factors and the prevalence of coronary vascular atherosclerosis. Kolmogorov-Smirnov (K-S) test was carried out for the assessment of data normality. Student  $t$ -test was used for comparison of quantitative variables with a normal distribution such as age, systolic and diastolic blood pressure, lipids level, Fasting Blood Sugar (FBS) and hsCRP between patients with atherosclerosis and controls. Mann-Whitney  $U$  test was used for the assessment of quantitative variables without normal distribution such as the plasma levels of anti-VZV IgG between patients with atherosclerosis and controls. Also, the Kruskal-Wallis test was carried out for the assessment of hsCRP and anti-VZV IgG between more than two groups. The results for the quantitative variables with normal and non-normal distribution were reported as Mean $\pm$ SD and Median (IQR), respectively. Statistical analyses were performed using SPSS statistical software (version 18, SPSS Inc, Chicago, IL, USA) and for comparisons between two groups two-tailed  $p$ -value<0.05 was considered as significant. In the Kruskal-Wallis test, after Bonferroni correction, two-tailed  $p$ -value<0.0125 was considered significant.

## RESULTS

**Demographic, Clinical, and Laboratory Data of Patients with Coronary Artery Atherosclerosis and Controls**

The distribution of the patients with coronary vascular atherosclerosis and the controls according to demographic, clinical, and laboratory data are demonstrated in Table 1.

Whereas, there was no significant difference in the frequencies of males and females between patients and controls ( $X^2$  test,  $p=0.089$ ), men had a significantly higher odds for coronary artery atherosclerosis compared to women after adjusting for all potential confounding variables (OR=1.95; 95%CI=1.10-3.44). In addition, individuals above the age of 65 years had increased odds of atherosclerosis (OR=2.00; 95%CI=0.86-4.63). The frequencies of hypertension ( $X^2$  test,  $p<0.001$ ), hyperlipidemia ( $X^2$  test,  $p=0.061$ ), and diabetes mellitus ( $X^2$  test,  $p=0.006$ ) were higher in patients than controls. After adjusting for various confounding factors, the existence of hypertension

(OR=5.58; 95%CI=2.52-12.34), hyperlipidemia (OR=2.19; 95%CI=0.99-4.80), and diabetes mellitus (OR=2.29; 95%CI=1.09-4.80) were also correlated with a higher risk for atherosclerosis (Table 2).

**The Plasma Levels of Anti-VZV IgG**

Of the 88 patients in the atherosclerosis group, 61 (69.3%) had titers equal to or higher than 2.1 AU/mL (positive titer) and 27 (30.7%) had titers lower than 2.1 AU/mL (negative titers) for anti-VZV IgG antibody. In the control group, 50 individuals (50.5%) had positive titers and 49 (49.5%) had negative titers. The frequencies of individuals with positive titers for anti-VZV IgG antibody were significantly higher in patients with atherosclerosis than the control group ( $X^2$  test,  $p=0.009$ ; Table 2). After adjusting for all confounding variables, this difference remained significant (OR=1.88; 95%CI=1.03-3.44; Table 2).

Among the quantitative variables, the mean of systolic blood pressure on admission was significantly higher in atherosclerosis group than controls (Student  $t$ -test,  $p=0.001$ ; Table 1). The plasma levels of blood

**Table 1. Demographic, clinical, and laboratory data of patients with coronary artery atherosclerosis and controls**

Variable	Atherosclerosis Group (n=88)	Control Group (n=99)	Statistical Test	p-value
<b>Demography data</b>				
Gender, n <sup>a</sup> (%)				
Female	37 (42)	54 (54.5)	<i>A</i>	0.089
Male	51 (58)	45 (45.5)		
Age (years)	61.75±9.05	59.67±6.70	<i>B</i>	0.073
BMI <sup>b</sup>	25.20±3.50	25.32±3.59	<i>B</i>	0.825
<b>Clinical data</b>				
Blood pressure (mmHg) <sup>c</sup>				
Systolic	125.67±15.36	119.02±11.06	<i>B</i>	<b>0.001*</b>
Diastolic	80.00±10.77	77.71±7.71	<i>B</i>	0.093
<b>Laboratory data</b>				
FBS <sup>d</sup> (mg/dL)	108.89±39.44	103.12±24.86	<i>B</i>	0.228
TG <sup>e</sup> (mg/dL)	204.82±73.04	180.55±64.84	<i>B</i>	0.074
Cholesterol (mg/dL)	186.60±42.39	171.73±45.69	<i>B</i>	0.082
LDL <sup>f</sup> (mg/dL)	126.83±19.68	119.02±13.11	<i>B</i>	<b>0.019*</b>
HDL <sup>g</sup> (mg/dL)	38.83±9.78	40.45±9.54	<i>B</i>	0.391
hsCRP <sup>k</sup> (mg/L)	5.19±2.00	1.51±1.07	<i>B</i>	<b>&lt;0.001*</b>
Anti-VZV IgG Ab (AU/mL)	2.27 (1.53-4.30)	2.1 (1.70-3.10)	<i>C</i>	<b>0.034*</b>

<sup>a</sup>n, number; <sup>b</sup>BMI: body mass index; <sup>c</sup>mmHg: millimeter of mercury; <sup>d</sup>FBS: fasting blood sugar; <sup>e</sup>TG: triglycerides; <sup>f</sup>LDL: low-density lipoprotein; <sup>g</sup>HDL: high-density lipoprotein; <sup>k</sup>hsCRP: high sensitive c-reactive protein; \*Bold text indicates statistical significance ( $p<0.05$ ).

*A*: Chi-square or Fisher's exact test (expected cells value less than 5) was used for comparison of discontinuous variants. *B*: Student  $t$ -test (Mean±SD) was used for comparison of parametric continuous variants. *C*: Mann-Whitney  $U$  test (Median (IQR)) was used for comparison of non-parametric continuous variants.

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**Table 2. Association of different risk factors and VZV-seropositivity with atherosclerosis in the patient group compared to the control group based on single and multivariable logistic regression.**

Risk factors			Crude Logistic Regression		Multivariable Logistic Regression	
	Atherosclerosis Group (n=88)	Control Group (n=99)	OR <sup>d</sup> crude (95% CI <sup>f</sup> )	p-value crude	OR Regression (95% CI)	p-value Regression
Gender, n <sup>a</sup> (%)						
Female	37 (42)	54 (54.5)	1 [ref]		1 [ref]	
Male	51 (58)	45 (45.5)	1.65 (0.93-2.95)	<b>0.089</b>	1.95 (1.10 -3.44)	<b>0.022*</b>
Age (years), n (%)						
≤55	19 (21.6)	32 (32.3)	1 [ref]		1 [ref]	
56-65	47 (53.4)	50 (50.5)	1.58 (0.79-3.17)	0.194	1.30 (0.66-2.57)	0.449
≥66	22 (25.0)	17 (17.2)	2.18 (0.93-5.10)	<b>0.072</b>	2.00 (0.86-4.63)	0.106
BMI <sup>b</sup> ≥30 kg/m <sup>2</sup> , n (%)						
No	79 (89.8)	91 (91.9)	1 [ref]		1 [ref]	
Yes	9 (10.2)	8 (8.1)	1.30 (0.48-3.52)	0.610	1.28 (0.47-3.47)	0.628
Hypertension, n (%)						
No	50 (56.8)	89 (89.9)	1 [ref]		1 [ref]	
Yes	38 (43.2)	10 (10.1)	6.76 (3.11-14.73)	<b>&lt;0.001*</b>	5.58 (2.52-12.34)	<b>&lt;0.001*</b>
Hyperlipidemia, n (%)						
No	34 (38.7)	39 (39.4)	1 [ref]		1 [ref]	
Yes	26 (29.5)	14 (14.1)	2.13 (0.96-4.72)	<b>0.061</b>	2.19 (0.99-4.80)	<b>0.054</b>
Missing	28 (31.8)	46 (46.5)				
Diabetes mellitus, n (%)						
No	61 (69.3)	85 (85.9)	1 [ref]		1 [ref]	
Yes	27 (30.7)	14 (14.1)	2.69 (1.30-5.55)	<b>0.006*</b>	2.29 (1.09-4.80)	<b>0.029*</b>
hsCRP <sup>c</sup> , n (%)						
Low risk (<1 mg/L)	1 (1.1)	31 (31.3)	1 [ref]		1 [ref]	
Intermediate risk (1-3mg/L)	11 (12.5)	57 (57.6)	5.98 (0.74-48.51)	0.094	4.89 (0.59-40.45)	0.141
High risk (>3mg/L)	76 (86.4)	11 (11.1)	214.18 (26.51-1730.42)	<b>&lt;0.001*</b>	180.36 (22.27-1460.56)	<b>&lt;0.001*</b>
VZV serological status, n (%)						
Seronegative (<2.1 AU/mL)	27 (30.7)	49 (49.5)	1 [ref]		1 [ref]	
Seropositive (≥2.1 AU/mL)	61 (69.3)	50 (40.5)	2.21 (1.22-4.04)	<b>0.009*</b>	1.88 (1.03-3.44)	<b>0.040*</b>

<sup>a</sup>n, number; <sup>b</sup>BMI: body mass index; <sup>c</sup>hsCRP: high sensitive c-reactive protein; <sup>d</sup>OR, odds ratio; <sup>f</sup>CI, confidence interval; \*Bold text indicates statistical significance ( $p < 0.05$ ). The logistic regression test was used.

LDL, hsCRP and anti-VZV IgG concentrations in patients with atherosclerosis (Mean±SD= 126.83±19.68 mg/dL; Mean±SD= 5.19±2.00 mg/L and Median (IQR)= 2.70 (1.53-4.30) AU/mL, respectively) were significantly higher than controls (Mean±SD = 119.02±13.11 mg/dL; Mean±SD 1.51±1.07 mg/L and Median (IQR)=2.10 (1.70-3.10) AU/mL, Student *t*-test,

$p=0.019$ , Student *t*-test,  $p < 0.001$  and Mann-Whitney *U* test,  $p=0.034$ , respectively; Table 1).

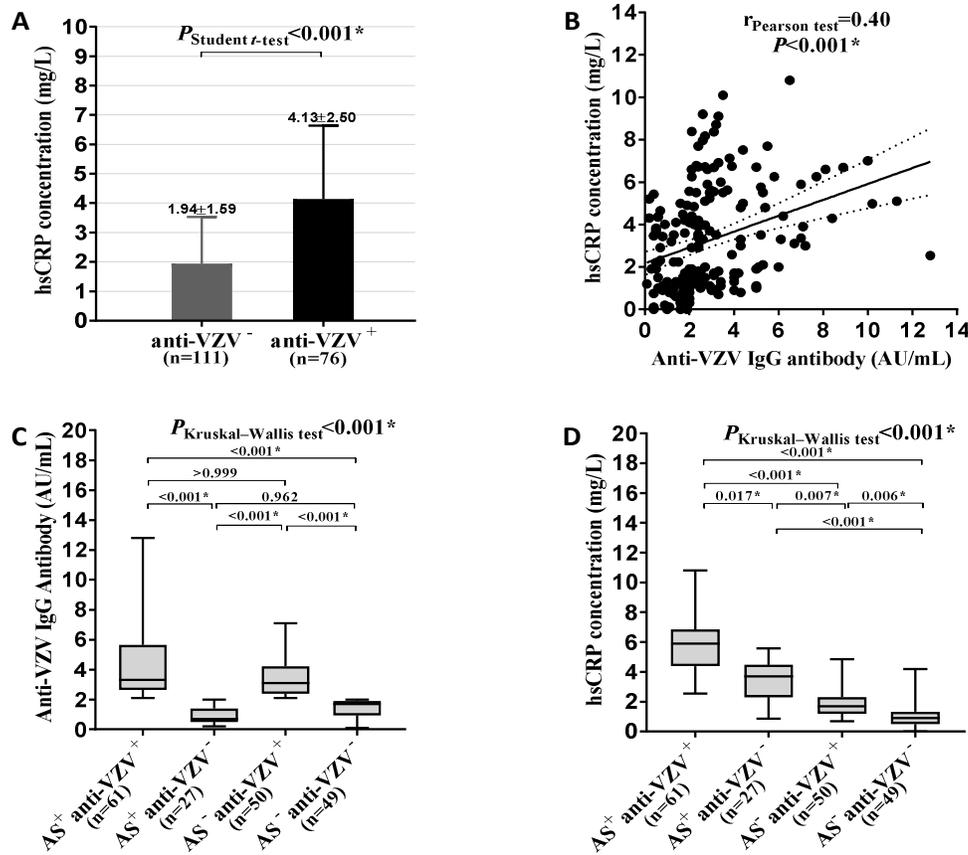
The mean levels of hsCRP concentration in patients with atherosclerosis were significantly higher than controls (Student *t*-test,  $p < 0.001$ ; Table 1). On the other hand, individuals with VZV-seropositivity had increased levels of hsCRP compared to subjects who

were VZV-seronegative (Student *t*-test,  $p < 0.001$ ; Figure 1A). We also found a statistically significant correlation between hsCRP concentration and plasma level of anti-VZV IgG in (Spearman test,  $r = 0.40$ ;  $p < 0.001$ ; Figure 1B). The highest level of anti-VZV IgG antibody was seen in “AS<sup>+</sup> anti-VZV<sup>+</sup>” group followed by “AS<sup>-</sup> anti-VZV<sup>+</sup>” group (Figure 1C). Interestingly, the plasma levels of hsCRP were significantly higher in “AS<sup>+</sup> anti-VZV<sup>+</sup>”, “AS<sup>+</sup> anti-VZV<sup>-</sup>” and “AS<sup>-</sup> anti-VZV<sup>+</sup>” groups as compared to the “AS<sup>-</sup> anti-VZV<sup>-</sup>” group (Dunn's multiple comparisons test,  $p < 0.001$ ,  $p < 0.001$  and  $p = 0.006$ , respectively; Figure 1D).

In patients, the plasma levels of anti-VZV IgG

increased with advancing of the disease, however, the difference in the plasma levels of anti-VZV IgG between groups based on the number of diseased vessels was not statistically significant ( $p = 0.168$ ; Figure 2A). No significant difference regarding the plasma levels of anti-VZV IgG was observed in comparisons between groups (Dunn's multiple comparisons test,  $p > 0.05$ ; Figure 2A).

Kruskal-Wallis test showed a significant difference in the plasma levels of hsCRP between groups based on the number of diseased vessels ( $p < 0.001$ ; Figure 2B). In patients, the plasma levels of hsCRP increased with advancing of the disease, i.e. a significant difference in the plasma levels of hsCRP between groups based on



**Figure 1.** Comparison of the plasma levels of anti-VZV IgG and hsCRP levels. **A.** hsCRP levels were higher in individuals who were anti-VZV<sup>+</sup>. **B.** There was a correlation between hsCRP and anti-VZV IgG antibody levels in all subjects. **C.** The highest level of anti-VZV IgG was seen in “AS<sup>+</sup> anti-VZV<sup>+</sup>” group. **D.** The highest level of hsCRP was seen in “AS<sup>+</sup> anti-VZV<sup>+</sup>” group and decreased gradually in subjects based on their status of atherosclerosis and seropositivity for anti-VZV.

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the number of diseased vessels was seen. The blood levels of hsCRP in patients having SVD, 2VD, 3VD were significantly higher as compared with the control group (Dunn's multiple comparisons test,  $p < 0.001$ ,  $p < 0.001$  and  $p < 0.001$ , respectively; Figure 2B).

### The Relationship between Anti-VZV IgG and Risk Factors

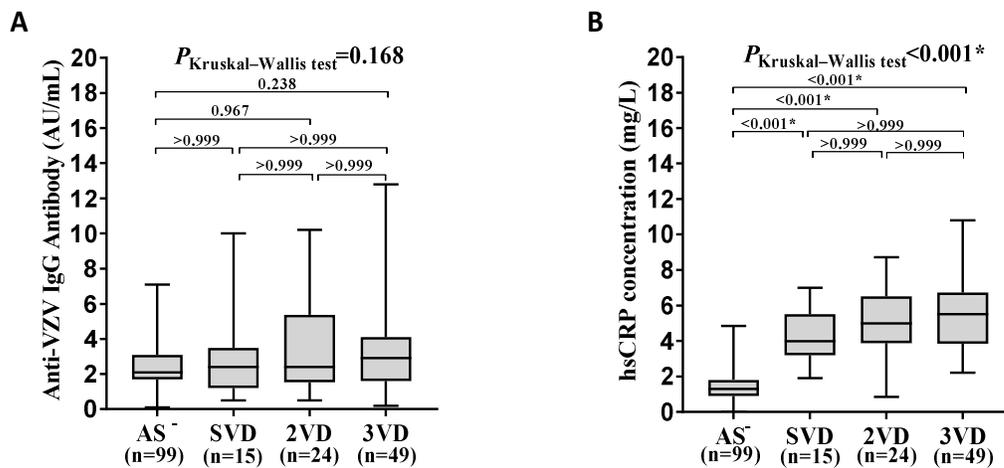
To determine the relationship between risk factors and anti-VZV IgG in the induction of atherosclerosis, patients were divided into VZV-seronegative and seropositive subgroups. Our results showed that patients with VZV-seropositivity exhibited a higher prevalence of diabetes mellitus than patients with

VZV-seronegativity ( $X^2$  test,  $p = 0.039$ ). This difference remained significant after adjusting for all confounding variables. Multivariable logistic regression indicated that the presence of VZV-seropositivity was associated with an increased risk for coronary artery atherosclerosis (OR=3.33; 95%CI=1.02-10.88; Table 3). Interestingly, "AS<sup>+</sup> anti-VZV<sup>+</sup>" patients were less likely to have BMI higher than 30 kg/m<sup>2</sup> compared to "AS<sup>+</sup> anti-VZV<sup>-</sup>" patients ( $X^2$  test,  $p = 0.022$ ), which after adjusting for all potential confounding variables remained significant (OR=0.17; 95%CI=0.04-0.76). Individuals between ages 56-65 years had higher odds of being anti-VZV seropositive (OR=2.86; 95%CI=0.93-8.84; Table 3).

**Table 3. Association of different risk factors of atherosclerosis with VZV-seropositivity in the patient group based on single and multivariable logistic regression**

Risk factors	Patient with atherosclerosis (n=88)		Crude Logistic Regression		Multivariable Logistic Regression	
	Anti-VZV <sup>+</sup> (n=61)	Anti-VZV <sup>-</sup> (n=27)	OR <sup>d</sup> <sub>crude</sub> (95% CI <sup>f</sup> )	p-value <sup>g</sup> crude	OR <sub>Regression</sub> (95% CI)	p-value Regression
Gender, n <sup>a</sup> (%)						
Female	25 (41.0)	12 (44.4)	1 [ref]		1 [ref]	
Male	36 (59.0)	15 (55.6)	1.15 (0.46-2.88)	0.762	1.11 (0.45-2.76)	0.826
Age (years), n (%)						
≤55	10 (16.4)	9 (33.3)	1 [ref]		1 [ref]	
56-65	36 (59.0)	11 (40.7)	2.95 (0.96-9.09)	<b>0.060</b>	2.86 (0.93-8.84)	<b>0.067</b>
≥66	15 (24.6)	7 (25.9)	1.93 (0.54-6.90)	0.311	1.80 (0.50-6.46)	0.68
BMI <sup>b</sup> ≥30 kg/m <sup>2</sup> , n (%)						
No	58 (95.1)	21 (77.8)	1 [ref]		1 [ref]	
Yes	3 (4.9)	6 (22.2)	0.18 (0.04-0.79)	<b>0.022*</b>	0.17 (0.04-0.76)	<b>0.020*</b>
Hypertension, n (%)						
No	32 (52.5)	18 (66.7)	1 [ref]		1 [ref]	
Yes	29 (47.5)	9 (33.3)	1.81 (0.70-4.66)	0.217	1.56 (0.60-4.06)	0.360
Hyperlipidemia, n (%)						
No	19 (31.1)	15 (55.6)	1 [ref]		1 [ref]	
Yes	17 (27.9)	9 (33.3)	1.49 (0.52-4.28)	0.458	1.40 (0.49-4.05)	0.531
Miss	25 (41.0)	3 (11.1)				
Diabetes mellitus, n (%)						
No	38 (62.3)	23 (85.2)	1 [ref]		1 [ref]	
Yes	23 (37.7)	4 (14.8)	3.48 (1.07-11.34)	<b>0.039*</b>	3.33 (1.020-10.88)	<b>0.047*</b>
hsCRP <sup>c</sup> , n (%)						
Low risk (<1 mg/L)	0 (0.0)	1 (3.7)	1 [ref]		1 [ref]	
Intermediate risk (1-3 mg/L)	4 (6.6)	7 (25.9)	NA <sup>h</sup>	NA	NA	NA
High risk (>3mg/L)	57 (93.4)	19 (70.4)	NA	NA	NA	NA

<sup>a</sup>n, number; <sup>b</sup>BMI: body mass index; <sup>c</sup>hsCRP: high sensitive c-reactive protein; <sup>d</sup>OR, odds ratio; <sup>e</sup>CI, confidence interval; <sup>f</sup>NA, not applicable because of the presence of a zero in the denominator; <sup>g</sup>Bold text indicates statistical significance ( $p < 0.05$ ). The logistic regression test was used.



**Figure 2. Comparison of anti-VZV IgG and CRP levels in individuals based on the number of diseased vessels. A. The level of anti-IgG antibodies non-significantly increased along with the number of involved vessels. B The level of hsCRP significantly increased along with the number of involved vessels.**

## DISCUSSION

Our study demonstrated that VZV-seropositivity is associated with higher odds of atherosclerosis after controlling for sex, age, BMI, hypertension, hyperlipidemia, and diabetes mellitus. In support of these results, Wang et al. showed the relationship between VZV infection and CAD.<sup>13</sup> The information on the correlation between VZV and coronary vascular atherosclerosis is scarce.<sup>27</sup> Most studies, however, have examined the relationship between VZV infection and atherosclerosis in the peripheral and cerebral vascular system.<sup>15,28,29</sup> Previously, higher anti-VZV IgG titers in CSF of patients with cerebrovascular atherosclerosis was shown.<sup>12</sup> In most of the recent studies, increased CSF levels and reduced serum/CSF ratio of anti-VZV IgG in patients with cerebrovascular atherosclerosis confirmed intrathecal production of anti-VZV IgG.<sup>12,30</sup> Although, there was no previous study on the association of serum or plasma level of anti-VZV IgG and coronary vascular atherosclerosis, it is known that anti-VZV IgG increases in serum after primary or re-exposure with the virus (natural or vaccination), as well as at the time of reactivation of the latent virus.<sup>31,32</sup> Uthman et al.<sup>33</sup> and Massano et al.<sup>34</sup> suggested that anti-VZV IgG induce the formation of immune complexes (antigen-antibody) due to cross-reaction with different lipid types such as phospholipids. The sedimentation of immune complexes can induce systemic inflammatory damage in various vascular tissues.<sup>35</sup> In addition, VZV

displays pro-atherogenic roles through direct infection of the arterial wall, as well as provocation of pro-inflammatory, and pro-thrombotic responses.<sup>36</sup> On the other hand, it is shown that viruses induce secretion of chemokines and cytokines, recruitment of leukocytes, the formation of foam cells, the proliferation of VSMCs, and the progression of plaques in the atherosclerosis process.<sup>6,37</sup> Also, in the early phase of VZV induced vasculopathy, the presence of different immune cells in adventitia leads to intima thickening and vascular remodeling.<sup>29</sup>

Our finding of the correlation of hsCRP concentration with atherosclerosis irrespective of other factors is not new.<sup>38</sup> CRP is one of the main inflammatory factors produced by liver hepatocytes, SMCs, macrophages, ECs, lymphocytes, and adipocytes in the body.<sup>16</sup> We also found that hsCRP levels were significantly higher in individuals with higher anti-VZV IgG. This indicates that humoral immune response to VZV is generally associated with inflammation and hsCRP production depicted in the correlation analysis (Figure 1B). Further analysis indicated that the highest hsCRP level in the blood was found in the presence of both atherosclerosis and anti-VZV. Either one of atherosclerosis or anti-VZV alone was associated with lower levels of hsCRP production. The above findings once again point to the importance of hsCRP in inflammatory responses and also suggest that VZV infection is not the sole mechanism for hsCRP elevation.

We also showed that the plasma levels of anti-VZV IgG and hsCRP increase with advancing of atherosclerosis, which is suggestive of the relation between VZV infection, inflammation, and progression of atherosclerosis and is in accordance with a recent meta-analysis study.<sup>15</sup>

Our findings that patients between 56-65 years of age were more likely to have anti-VZV IgG, shows that seropositivity against VZV is not solely dependent on older age, as our group with ages greater than 65 did not have the highest level of antibody. These finding rules out the effect that older age of patients compared to controls may have had on the antibody levels. On the other hand, the higher level of hsCRP and a higher frequency of atherosclerosis in individuals older than 65 years were notable. Accordingly, when we considered VZV-seropositivity along with age, older subjects had a 5.62 fold odds of increased hsCRP and 1.80 folds increased risk of coronary artery atherosclerosis after adjusting for all confounding variables. This implied that atherosclerosis in older people is more likely to be associated with previous VZV infection as well as inflammatory response. One question that may arise is the correlation of hsCRP, anti-VZV and viral load between AS<sup>+</sup> patients with acute VZV reactivation and those without. Obviously this piece of informative data is missing in our study; however, due to the chronic nature of atherosclerosis progression, it is more likely that repeated reactivations in the past and even a combination of infection history may be more relevant to atherosclerosis progression than a single episode of reactivation. Although a more careful matching of age between cases and controls would add to the credibility of the results, previous data have shown that weakened immune responses in older individuals with hyperlipidemia and/or diabetes mellitus may increase their susceptibility to reactivation of VZV infection and increase the odds of atherosclerosis.<sup>39,40</sup> Interestingly, studies that were conducted in the Taiwanese, Canadian, and English populations, showed that VZV infection in patients over the age of 65 years was associated with poor prognosis.<sup>13,41</sup>

In the original analysis, we found that atherosclerosis was more frequent in men (Table 2), which is in accordance with previous reports.<sup>42,43</sup> However, since the frequency of VZV-seropositivity was higher among women, including the VZV factor in the comparison of atherosclerosis between males and

females counteracted the gender effect. This finding once again points to the multifactorial nature of atherosclerosis in general and also to the more cautious interpretation of gender differences in this disease. Previously, Giallorete et al. showed that the most important risk factor for reactivation of VZV in female and older subjects is immune compromise. Nevertheless, the role of gender is yet to be clarified.<sup>44</sup>

In conclusion, this study showed that VZV-seropositivity is prevalent among those who are at a higher risk of atherosclerosis. Thus, the higher titers of anti-VZV IgG antibody and hsCRP in plasma or serum may predict a higher risk of atherosclerosis and its progression. Higher titers of anti-VZV IgG may indicate a possible recent re-exposure or recent reactivation of a latent VZV infection, which may indirectly cause vascular change and remodeling through hsCRP generation. These results support the role of infection mediated immune inflammation in exacerbation of atherosclerosis.

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