Correlation between Blood Levels of Adhesion Molecules ICAM-1 and VCAM-1 and Thrombomodulin with Coronary Artery Disease

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Abstract

Background- The results of studies on coronary artery disease risk factors have demonstrated that some adhesion molecules could be risk factors for coronary artery disease. ICAM-1 and VCAM-1 are the most important adhesion molecules. On the other hand, thrombomodulin is an anti-inflammatory factor and can reduce the risk of coronary artery disease. In this study, as well as evaluating these factors, we also studied the effect of the interaction between these factors on coronary artery disease.

Methods- One hundred twenty-three patients between the ages of 45 and 70 years old who were admitted for coronary angiography in the cardiovascular center and met the inclusion criteria for the research, were selected in the first half of 2008. After recording their personal information and medical history in the questionnaires, blood samples were collected and after routine examination, the blood levels of these factors were measured. We then entered the acquired results of the blood examination and the angiography in the patient's charts and analyzed the results using statistical methods.

Results- The angiography results in patients showed that 18 (14.7%) had normal coronary arteries, 5 (4%) had minimal coronary artery disease, 40 (32.5%) had single-vessel disease, 25 (20.3%) had two-vessel disease, and 35 (28.5%) had three-vessel disease. In laboratory tests, the mean soluble ICAM-1 level in patients with normal coronary arteries was 236 ngr/mL;[1] however, in patients with coronary artery disease, the mean level was 275 ngr/mL. The average amount of VCAM-1 in patients with normal coronary arteries was 697 ngr/mL, whereas patients with coronary artery disease had an average of 108 ngr/mL. Thrombomodulin in the normal coronary artery group was 42 ngr/mL, but in patients with coronary artery disease the average level was 30 ngr/mL.

Conclusion- The results in this research showed that increased levels of soluble ICAM-1 and also decreased levels of soluble thrombomodulin increased the risk and intensity of coronary artery disease, with statistical significance. The increase in soluble VCMA-1 also increased the risk of coronary artery disease; this was, however, not statistically significant. The important point is that increased levels of soluble ICAM-1 is a risk factor when the level of thrombomodulin is normal or below normal. When the levels of thrombomodulin and ICAM-1 have both increased, the increased risk and intensity of coronary artery disease is not statistically important ***(Iranian Heart Journal 2010; 11 (1):24-29).***

Key words: intracellular adhesion molecule-1 ■ inflammation ■ thrombomodulin ■ coronary artery disease ■ vascular cell adhesion molecule-1

Coronary artery disease (CAD) is one of the most important causes of death in the world and therefore there have been many researches on predisposing factors of CAD.

The results of these studies have demonstrated that this disease is an inflammatory process.[1,2]

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Inflammation is a response that attracts leukocytes and soluble molecules in the plasma to the lesion site.[1,3]

For migration to the site of inflammation, leukocytes must bind to endothelial cell surfaces and then pass through these cells.[4,5] In a normal situation, leukocytes are moving all over the body but in the inflammatory site, adhesion molecules expressed on the surface of endothelial cells are identified by ligands, which are located on the leukocytes, and consequently cause leukocytes to bond to endothelial cells. Also, some cytokines have an important role in migration. One group of these cytokines stimulates the endothelial cells to express more adhesion molecules. Another group of cytokines is called chemokines, which cause chemotaxis of the cell to the region. Another group of cytokines causes proliferation and maturation of inflammatory cells.[6-8]

The number of adhesion molecules plays a role in the migration of leukocytes to the region. ICAM-1 and VCAM-1 are the most important adhesion molecules which express on endothelial cells. LFA-1 and VLA-4 are ligands of these adhesion molecules which express on leukocytes.[9,10] During atherosclerosis, deposition of lipid particles inside endothelial cells causes the stimulation of the cells to express adhesion molecules and also secrete cytokines that initiate the inflammatory process in the region. The entrance of cells to the region and stimulation of them to secrete cytokines intensifies the inflammatory process. After the entrance of macrophages to the region, the cells start phagocytosis of the lipid particle. After a period of time, these macrophages will die and convert to foam cells that are the central nucleus of the atheroma. However, in addition to these cells and different cytokines, we should consider the activity of platelets, mast cells, complement system, coagulation system, fibrinolytic system (plasmin), and kinin system.[11-16]

Other types of cytokines and molecules exist in the body, which cause the inhibition of the inflammatory process. Two of these are IL-10 and thrombomodulin. Thrombomodulin is one of the most important protective factors in the vascular bed. The endothelial cells continuously express this protein. This protein has an extracellular part which binds to thrombin. After thrombin binds to the protein, it is activated. This activation causes another reaction, which activates protein C. Activated protein C digests coagulation factors 5 and 8, which reduces clot formation.[17-20]

In most studies in the field of atherosclerosis, only the relation between inflammatory factors and risk of the disease has been evaluated. The role of anti-inflammatory factors and interaction between inflammatory and anti-inflammatory factors have been far less evaluated. In this study, not only did we measure soluble ICAM-1 and VCAM-1 as inflammatory factors, we also measured the soluble thrombomodulin as an anti-inflammatory factor and also the interaction between these 2 groups of factors in the initiation and intensity of coronary artery disease.

Methods

In total, 123 patients admitted for coronary angiography from January 2008 until May 2008 at our center who had the following conditions were selected for the study: no acute heart events in the past two months, no history of angiography, no history of infection, inflammation, or cancer, no use of immunosuppressive or lipid-lowering agents, no fever since two weeks before admission, age between 45 and 70 years old, no use of any anti-inflammatory drugs two weeks prior to admission, and no history of cardiac surgery.

After selection of the patients, a questionnaire was filled out about personal information, medical history, history of any kind of treatment, and presence of any known cardiac risk factors. Additionally, 24 to 48 hours prior to angiography, a blood sample was taken and then the serum and the plasma were separated and the samples were kept in a freezer at -20°C until they were tested. Aside from the routine laboratory tests, the blood levels of soluble intercellular adhesion molecule-1 (S-ICAM-1) and soluble vascular adhesion molecule-1 (S-VCAM-1) were tested, and also the soluble thrombomodulin was tested in the following way:

The blood levels of all three mentioned proteins were measured by ELISA (enzyme-linked immunosorbant assay) (R and D Co.). With this method, by having the standard antibodies we were able to find and measure the specific antigens in the plasma and therefore by having the standard antigen we were able to measure the amount of specific antibody in the serum. For this purpose, there are different techniques that increase the sensitivity of the tests every day. For the reduction of error in the measurements, we measured the samples in triplicate. We used the ELISA washer and ELISA reader (Awerness Co.), and the optical densities of the samples were measured at a wavelength of 450 nanometers. Behsan Teb Azmaye Company Iran provided the above-mentioned kits. The normal level of S-ICAM-1 in blood is 240 ngr/ml, the normal level of S-VCAM-1 in blood is 700 ngr/ml, and the normal level of soluble thrombomodulin in blood is 37 nanogram/ml.

Results are presented as mean ±SD or number (%). Comparisons were done with the statistical T test, K2 and regression analyzing and statistical significance was defined at the level of $P<0.05$.

Results

From the 123 patients studied, 47 were female (38.2%), 76 were male (61.8%), mean age of patients was 58.8 years old, 50 were smokers (40.6%), 58 had hyperlipidemia (47.2%), 59 had hypertension (48%), 36 were diabetic (29.2%), and 19 had positive family history for coronary artery disease (15.5%, Table I).
The results of the angiography done on the patients were as follows: 18 had normal coronary arteries (14.7%), 5 had minimal coronary artery disease (4%), 40 had single-vessel disease (32.5%), 25 had two-vessel disease (20.3%), and 35 had three-vessel disease (28.5%, Table II).

Table I. Sex distribution and frequency of risk factors in the study population

Information	Number	Percent
Males	76	61.8%
Females	47	38.2%
Smoker	50	40.6%
Hyperlipidemia	58	47.2%
Hypertension	59	48%
Diabetics	36	29.2%
Family History	19	15.5%

Table II. The results of coronary angiography and soluble ICAM-1, VCAM-1 and thrombomodulin in the study population

Result*	No.	%	ICAM-1 ngr/ml	VCAM-1 ngr/ml	Thrombo-modulin ngr/ml
Normal	18	14.	236	697	42
Min.CAD	5	4	248	712	37
SVD	40	32.5	267	702	33
2VD	25	20.3	273	706	30
3VD	35	28.5	289	715	25
Average	105	85.3	275	708	30

* Min. CAD = minimal coronary artery disease; SVD = single-vessel coronary artery disease; 2VD = two-vessel coronary artery disease; 3VD = three-vessel coronary artery disease.

The results of the laboratory data were as follows: patients with normal coronary arteries had an average of 236 ngr/ml of soluble ICAM-1; however, in patients with coronary artery disease this was 275 ngr/ml. The average amount of soluble VCAM-1 was 697 ngr/ml in patients with normal coronary arteries, while in patients with coronary artery disease this was 708 ngr/ml. The average amount of thrombomodulin in patients with normal coronary arteries was 42 ngr/ml but in patients with coronary artery disease the average amount was 30 ngr/ml.
With respect to the analysis and the interaction between the other risk factors, this result demonstrated that there was a significant relation between increased SICAM-1 and coronary artery disease, and there was also another significant relation between decreased soluble thrombomodulin and coronary artery disease ($P=0.001$ and $P<0.0001$, respectively). Also, the results demonstrated that if SICAM-1 and thrombomodulin were both more than normal, the patient either had normal coronary or minimal coronary artery disease or single-vessel disease. This result is statistically significant ($P=0.005$). Nevertheless, in patients with high SICAM-1 and low soluble thrombomodulin, the intensity of the disease increased. This result, however, is not statistically significant. No significant relation was found between SVCAM-1 and existence of coronary artery disease. Be that as it may, there was a relation between increased SVCAM-1 level and existence of coronary artery disease (Table II).

Conclusion

The results obtained from this research can be divided into the following groups:

1. These results demonstrated that atherosclerosis was an inflammatory process, as is the case in other studies that tested inflammatory factors, pro-inflammatory factors, and anti-inflammatory factors (Paul M, et al.,[6] Ridker et al.,[21] Kenneth et al.[9]).
2. There was a direct and significant relation between soluble ICAM-1 and coronary artery disease. This result was also obtained by the studies done by Kenneth et al.,[9] Libby P. [22] and Esmon et al.,[23] although this result was not statistically significant in some other studies.
3. There was a significant relation between the decreased level of thrombomodulin and coronary artery disease. The researches done by Saloma et al.[24] and Esmon et al.[23] also confirmed this result.
4. Measuring soluble ICAM-1 and thrombomodulin simultaneously was more precise than measuring each of them separately. Therefore, in patients with high SICAM-1, the level of thrombomodulin will be important.

If the amount of soluble thrombomodulin is more than 37 nanogram/ml, either coronary artery disease does not exist or the patient has minimal coronary artery disease or single-vessel disease. This result was also obtained by Kenneth et al.[9]

5. Although the level of soluble VCAM-1 was higher in patients than healthy people, it was not statistically significant.

More research is necessary to identify:

a. Which factors influence the amount of soluble VCAM-1
b. Does soluble VCAM-1 increase in the specific period of the disease or in a specific age?
c. Does its increase affect the patient's general condition?

The relation between soluble VCMA-1 and coronary artery disease was also demonstrated in other studies such as the study done by Blankberg et al.[25] and the study done by Malik et al.[26]

There were many restrictions in this study, such as the existence of subclinical inflammatory disease, the use of narcotic drugs by some patients not being mentioned (narcotic drugs affect the levels of inflammatory factors and also the vascular permeability), and the wide range of the patients' age; these limitations can affect the results of our study. Furthermore, even though the soluble ICAM-1 and soluble VCAM-1 and soluble thrombomodulin were measured from the peripheral circulation, the results would be far more accurate if taken from the lesion site.

To find the exact correlation of these factors with the existence and intensity of coronary artery disease, more studies are required with the exclusion of the above-mentioned restrictions.

Conflict of Interest

No conflicts of interest have been claimed by the authors.

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