Correlation between Blood Levels of CRP, TNF-a (as Inflammatory Factors), and IL-10 (as Anti-Inflammatory Factor) and Coronary Artery Disease

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Abstract

Background- The results of a great amount of research done the world over in recent years have indicated that atherosclerosis is an inflammatory disease. Most of these investigations were conducted on the correlation between inflammatory factors such as CRP, IL-2, and IL-1 and atherosclerosis. In this study, we evaluated inflammatory factors such as CRP and TNF-α as well as anti-inflammatory factor IL-10 and analyzed the correlation between the balance of these factors with atherosclerosis.

Methods- In total, 135 patients between the ages of 45 and 70 years who were admitted for coronary angiography were selected. All of the selected patients met the inclusion criteria for the research. After recording personal information, medical history, and any previous treatment in the questionnaire, blood samples were collected and levels of CRP (high-sensitive quantitative test), TNF-α, and IL-10 were measured in all the samples. We entered the acquired results, the routine blood examination, and the angiography results in the patients' charts and analyzed the results using statistical methods.

Results- The angiography results in the 135 patients were as follows: 19 (14.1%) cases had normal coronary arteries, 6 (4.4%) had minimal CAD, 43 (31.8%) had single-vessel disease, 29 (21.5%) two-vessel, and 38 (28.1%) had three-vessel disease. In the laboratory tests, the mean CRP level in patients with normal coronary arteries was 6±4 mg/l; however in patients with CAD it was 17±9 mg/l. Also, the mean IL-10 level in cases with normal coronary arteries was 4.4 pgr/mL, while in patients with CAD it was 2.6 pgr/mL; and serum level of TNF-α in patients with CAD was 6.3±3.8 pgr/mL, whereas in cases with normal coronary arteries, the average serum level of TNF-α was 4.5 ± 2.2 pgr/mL.

Conclusion- The obtained results in this research showed a direct correlation between the blood levels of CRP and TNF-α with the existence and intensity of coronary artery disease. In addition, we found a reverse significant correlation between blood levels of IL-10 and existence of coronary artery disease. Although we found a correlation between reduced levels of IL-10 and intensity of coronary artery disease, it was not statistically significant. Furthermore, in patients with elevated blood levels of inflammatory and anti-inflammatory factors, the intensity of the coronary artery disease was far less than that in patients with high levels of inflammatory factors and reduced levels of anti-inflammatory factors. Therefore, we concluded that high levels of CRP and TNF-α and low levels of IL-10 had a significant correlation with the intensity of coronary artery disease and also the balance between these factors had a significant correlation with the intensity of the coronary artery disease ***(Iranian Heart Journal 2009; 10 (3):6-11).***

Key words: coronary artery disease ■ C-reactive protein ■ tumor necrosis factor-α ■ interleukin-10 (IL-10) ■ inflammation

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Studies done on atherosclerosis have confirmed that this disease is an inflammatory process accompanied by deposition of lipid particles in body vasculature. Deposition of lipoprotein type LDL in vasculature causes the stimulation of endothelial cells in the region. This stimulation causes the expression of molecules on the endothelial cell's surface, which are called adhesion molecules.[1-4] These molecules are identified by ligands, which are located on the surface of phagocytes and lymphocytes consequently causing attraction of these cells into the region. The most important cells that enter the region are monocyte/macrophages. The importance of monocyte/macrophages includes phagocytosis and presentation of antigen to the lymphocytes. Additionally, they secrete several cytokines and thus play a central role in the inflammatory process.[5] The entry of macrophages to the inflamed region occurs in 2 stages: the first is attaching to the endothelium of vasculature that is mainly caused by the binding of adhesion molecules which are expressed on the surface of endothelial cells to their specific ligand on the surface of macrophages; the second is passing through endothelial cells and settling in the intima of the vascular wall. In this stage, messenger molecules called chemotactic factors play an important role. Endothelial cells secrete these chemotactic factors. Additionally, studies on humans and also on animals have demonstrated that the complement cascade has been activated in the subendothelium and the units produced from this activation such as C5a are released in the region, which is regarded as the most powerful chemotactic factor for monocytes.[6]

After macrophages settle in the intima, they act as phagocyte cells and start endocytosis of lipoprotein molecules which have been deposited in the area. After the endocytosis process, these cells die and they create the nucleus of the atheroma.[7] Before dying, these cells secrete several cytokines which cause an increase in the intensity of inflammation. One of the most important cytokines secreted is TNF-α. The physiological effects of TNF-α are attracting neutrophils and monocytes to the inflammation area, stimulating the endothelial cells to express adhesion molecules, and stimulating endothelial cells and macrophages to release chemotactic factors. In the meantime, TNF-α affects a number of cells causing a process called apoptosis (programmed death). An additional effect of TNF-α is the activation of T-lymphocytes. Also, by inhibiting lipoprotein lipase, it causes an increase in the systemic VLDL and triglyceride level. If TNF-α is over-secreted, it can cause major systemic affects and can be fatal.

C-reactive protein (CRP) is an acute-phase protein. Due to the ability to bind to protein C on the pneumococcal capsule, it is called C-reactive protein. This molecule is soluble in serum and its level in healthy people is low; however, in inflammation the amount increases enormously and occasionally reaches a thousand times the normal value. CRP enhances phagocytosis (opsonization) and causes the activation of the complement system, which helps to increase the inflammation process with multiple factors.[8-10] Not only is CRP an inflammatory marker, but it also leads to the deposition of leucocytes on vascular walls.

IL-10 is a cytokine with multiple effects that can lead to the inhibition of the inflammatory process. One of the major effects of IL-10 is to inhibit the production of IL-12 by macrophages. IL-12 causes the secretion of gamma interferon (IFN-γ). IL-10 inhibits the expression of MHC class II molecules on macrophages that cause the inhibition of cell-mediated immunity and the inflammation process.[11-13]

Based on the given information above, we decided to measure these factors in patients with atherosclerosis to find the relation between these factors and atherosclerosis, and also the effect of interactions between these

factors in patients with coronary artery disease.

Methods

In total, 135 patients admitted for coronary angiography between January 2008 and May 2008 and who had the following conditions were selected for the study:

- In the previous two months the patients did not have any acute heart events and also did not have prior history of angiography.
- The patients did not have any history of infection, inflammation, or cancer.
- They did not use any immunosuppressive drugs and also did not use lipid-lowering agents.
- The patients did not have a fever for at least two weeks before admission.
- The patients had to be between the ages of 45-70 years old and did not use any anti-inflammatory drugs for at least two weeks prior to admission and also did not have any history of cardiac surgery.

After the selection of the patients, a questionnaire about personal information, medical history, history of any kind of treatment, and presence of any known cardiac risk factors was completed. In addition, 24 to 48 hours prior to angiography, a blood sample was taken and then the serum and plasma were separated and the samples were kept in a freezer at -20°C until they were tested. Aside from routine laboratory tests, the blood levels of CRP, TNF-α, and IL-10 were measured as follows:

High Sensitive Quantitative CRP: Using the method of immunoturbidometry with the kit from Pars Azemoon Company with the help of Cobas Mayra autoanalyzer, we were able to measure CRP. With this method, the CRP present in the sample was mixed with polycolonal antibodies, which were coated on latex. This complex was turbid. The turbidity of the complex has a direct relation with the quantity of CRP in the sample. The measured wavelengths were 500 nanometers and the measured range was between 0.1 to 20 mg/mL. If the concentration of CRP was higher than this range, by diluting the serum, we were able to measure the exact amount of CRP. The normal range of CRP in adults is less than 10 mg/mL.

Tumor Necrosis Factor α: TNF-α, also known as cachectin, was measured by using the ELISA technique (enzyme-linked immunosorbent assay) with the help of Bendermed Systems Company kits. For this test, we used the ELISA washer and ELISA reader, from Awerness Company. The optical density was measured at a wavelength of 450 nanometers.

IL-10: This cytokine was also measured by the ELISA system with the use of R and D Company kit with the sensitivity of 0.5 pg/mL. The optical density was measured at a wavelength of 450 manometers. The normal amount of this factor in serum is 3.5 pgr/mL. It is important to mention that all of the tests were done in triplicate. All of kits were provided by Behsan Teb Azmaye Iranian for this research. For recording the laboratory test results and also the results of coronary angiography in the questionnaires and analyzing the results, we used SPSS software. Results are presented as mean ±SD or number (%). Comparisons were done with the statistical t-test, K2 test, and regression analysis and statistical significance was defined at the level of $p<0.05$.

Results

In this study, from the 135 patients that were examined, 52 (38.5%) were females and 83 (61.5%) were males. Additionally, 54 (40%) patients were smokers, 64 (47.5%) had elevated lipid levels, 65 (48%) had hypertension, 39 (28.9%) were diabetics, and 21 (15.5%) had a positive family history for coronary artery disease (Table I).

The angiography results revealed that 19 (14.1%) cases had normal coronary arteries, 6 (4.4%) had minimal coronary artery disease, 43 (31.9%) had single-vessel disease, 29 (21.5%) had two-vessel disease, and 38 (28.1%) had three-vessel disease.

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

	Numbers of Cases	Percentage
Males	52	38.5%
Females	83	61.5%
Smoker	54	40%
Hyperlipidemia	64	47.5%
Hypertension	65	48%
Diabetics	39	28.9%
Family History	21	15.5%

The mean CRP level in patients with normal coronary arteries was 6±4 mg/l; however, in patients with coronary artery disease it was 17±9 mg/l. Also, the mean IL-10 level in patients with normal coronary arteries was 4.4±2.5 pg/ml, whereas in patients with coronary artery disease it was 2.6±1.7 pg/ml. The average serum level of TNF-α in patients with normal coronary arteries was 4.5±2.2 pg/ml, but in patients with coronary artery disease it was 6.3±3.8 pg/ml (Tables II, III).

Table II. Results of angiography and mean CRP, IL-10 & TNF- α levels in the study population

Results of Coronary Angiography	Patients with normal coronary arteries	Patients with minimal coronary artery disease	Patients with single vessel disease	Patients with two vessel disease	Patients with three vessel disease	Total of Patients with CAD
No.	19	6	43	29	38	116
%	14.1%	4.4%	31.9 %	21.5%	28%	85.9%
Mean CRP (mg/l)	6 ± 4	10 ± 5	12 ± 6	18 ±9	23 ± 11	17 ± 9
Mean IL-10 (pg/ml)	4.4 ± 2.5	4.3 ± 2.5	2.7 ± 1.4	2.7 ± 1.4	2.3 ± 1.3	2.6 ± 1.7
Mean TNF-α (pg/ml)	4.5 ± 2.2	4.8 ± 2.3	5.1 ± 3.1	5.1 ± 3.1	8.2 ± 4.5	6.3 ± 3.8

The statistical analysis confirmed that there was a direct relation between serum CRP level and existence and severity of coronary artery disease, which had a statistical significance (p<0.001). Also, there was a direct relation between TNF-α serum level and existence of coronary artery disease (p=0.005). On the other hand, there was a reverse relation between plasma level of IL-10 and the existence of coronary artery disease, which had a statistical significance (p=0.003). Although there was a relation between decreased levels of IL-10 and severity of coronary artery disease, it was not statistically significant.

An important significant conclusion is that in patients with coronary artery disease in whom other risk factors were compatible and who had high levels of CRP and IL-10 more than 3.5 pg/ml, the severity of coronary artery disease was less than that in patients with elevated CRP (more than 10 mg/l) and less than 3.5 pg/ml of IL-10, and this was statistically significant (p=0.01). The results of this research showed that measuring both inflammatory and anti-inflammatory factors was more important than measuring each of them alone.

Discussion

The results obtained from this study showed that a measurement of inflammatory factors helped predict the existence of coronary artery disease. The same results were obtained in other studies, including the study done by Liuzzo et al., Chao-Hung[14] et al., and Franklin et al.[15] On the other hand, the study done by Morrow et al.[16] and the study of Heeschen and Ledue et al.[17] did not have the same results. The other important point is that this research demonstrated that simultaneous measurements of inflammatory factors such as CRP and TNF-α together with anti-inflammatory factors such as IL-10 could help to predict a more precise diagnosis of coronary artery disease and also the degree of intensity of the disease. This result was obtained from other studies done by other researchers such as Pinderski et al.,[18] Anguera et al.,[19] and Mallat et al.,[20] as well as the study of Christopher Heeschen, et al.[21] However, studies conducted by Smith DA, et al.[22] and also Von Der Thousen[23] did not achieve the same results.

Reasons for this discrepancy can be explained by the following:

1. Not measuring TNF-α receptors due to the reason that cytokines would link to the specific receptors and can cause biological effects; therefore, during the inflammatory process, the amount of this cytokine would increase. If we had measured the amount of

specific receptors, it would perhaps have given us a more precise result.
2. Other factors that can cause the secretion of this cytokine would interfere with the final results.
3. The presence of subclinical inflammatory diseases was not identified.
4. Patients who use narcotic drugs do not mention the use of them because of legal and social factors.

Most cytokines have autocrine effects. The paracrine effects of cytokines depend on the amount of increase, which could be local or systemic. If we obtain the samples near the lesion, a more precise result can be acquired. But because of the difficulty of this task and the stimulation of cells to secrete inflammatory factors during sampling, it can result in an increase in the intensity of the disease and may be dangerous.

Conclusion

In light of the above-mentioned factors, further research is required to provide more precise and definite results. Careful planning can reduce the effect of these factors in future studies.

Conflict of Interest

No conflicts of interest have been claimed by the authors.

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