Association of Severity of Coronary Lesion with Markers of Acute Infection and Inflammation in Patients with Acute Coronary Syndrome

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Abstract

Aim: Inflammation and some infectious agents play a key role in acute coronary syndrome (ACS) caused by atherosclerosis. The purpose of this study was to assess the effects of inflammatory markers and the positivity of Chlamydia pneumoniae (CP), Helicobacter pylori (HP), and Cytomegalovirus (CMV) on the level of atherosclerosis in patients with ACS.

Materials and Methods: Patients (57) that were referred to the emergency unit with classic angina symptoms or angina equivalent symptoms and were determined to have critical lesions in the coronary angiography (>70% stenosis, coronary artery disease (CAD) severity assessed by the Gensini score) were compared with 27 ACS patients who had no critical lesions in terms of procalcitonin (PCT), tumor necrosis factor-alpha (TNF-α), interleukin-2 receptor (IL-2r), interleukin-6 (IL-6), and interleukin-10 (IL-10) levels and positivity of CP, HP, and CMV. Also, the two groups of ACS patients were compared in terms of cytokine levels measured at hours 0 and 48.

Results: No significant association was found between the degree of the coronary lesion and the inflammatory and infectious agents. However, in patients with critical coronary lesions, as markers of inflammatory agents, the levels of IL-6 were significantly lower and levels of IL-10 were significantly higher (p<0.001 and p=0.030, respectively) at hour 48 than originally found at hour 0.

Conclusion: There is no association between the severity of coronary lesions and cytokine levels and positivity of infectious agents in ACS since the levels of proinflammatory cytokines in ACS are higher than those in atherosclerosis. The changes in cytokine levels at hour 48 were found to be significant.

Keywords: Coronary lesion, biomarkers, infection, inflammation, acute coronary syndrome

Introduction

Inflammation plays a key role in atherosclerosis, which leads to acute coronary syndrome (ACS) (1-3). The pro-inflammatory markers interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) along with anti-inflammatory marker interleukin-10 (IL-10) are described as part of the inflammatory process in atherosclerosis (4). IL-2 and interleukin-2 receptor (IL-2R), which is expressed on the surface of lymphocytes, have key roles in the beginning of inflammation via their direct effects on T cells (5). Various studies have shown that the diffuseness of atherosclerosis and then the probability of coronary artery disease (CAD) can be predicted by studying the levels of inflammatory markers in healthy individuals (6, 7).

A series of studies have reported that infectious agents of low virulence, such as Chlamydia pneumoniae (CP), Helicobacter pylori (HP), and Cytomegalovirus (CMV), may play a role in the pathogenesis of atherosclerosis by affecting the coronary artery walls. These studies have claimed that these agents may directly induce inflammation in the arterial walls by mechanisms such as innate immunity, molecular imitation, and autoimmunity (8-11).

The purpose of this prospective study was to assess the association of inflammatory markers and the positivity of CP, HP, and CMV with the severity of atherosclerosis in patients with ACS.

The study was presented partially on 1st International Emergency Medicine Symposium, 13-15 April 2012, Baku, Azerbaijan.

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Materials and Methods

Study population
The study included patients referring with angina symptoms and angina equivalent symptoms to the emergency department of a university hospital in the 6 month period between July 2011 and January 2012. The patients who had clinical, electrocardiographic (ECG) (concordant ST elevation >1 mm in leads with a positive QRS complex, concordant ST depression >1 mm in V1-V3, excessively discordant ST elevation >5 mm in leads with a negative QRS complex; Sgarbossa criteria), and echocardiographic (ECHO) findings (wall motion disorder), changes in cardiac markers, and critical lesions determined by coronary angiography comprised the test group; and patients with no critical lesions found in coronary angiography comprised the control group.

Exclusion criteria
The cases with the following disorders, which might affect the obtained data (related to the infection and inflammation process), were excluded from the study: chronic renal insufficiency, hepatic insufficiency, advanced cardiac failure, respiratory insufficiency, rheumatic disease, sepsis, and cancer or undergoing cancer therapy.

Study protocol
The following features of the patients were noted: age, sex, pulse rate, respiratory rate, average blood pressure, body temperature, history of alcohol consumption and smoking, hyperlipidemia (HL), diabetes mellitus (DM), hypertension (HT); presence of CAD, cerebrovascular event (CVE) or coronary artery bypass graft operation (CABGO) surgery in the anamnesis; and presence of unstable angina pectoris (USAP) or acute myocardial infarction (AMI) at the time of referral. At the time of patients’ referral to the emergency unit, the following were tested and their levels were noted: white blood cells (WBC), creatine-kinase-MB fraction (CK-MB), troponin-I (Tnl), procalcitonin (PCT), TNF-α, IL-2R, IL-6, IL-10, triglyceride (TG), cholesterol, low density lipoprotein (LDL), high density lipoprotein (HDL), international normalized ratio (INR), activated partial thromboplastin time (aPTT), CMV, HP, and CP. Likewise, after 48 hours, the following were tested and their levels were noted: WBC, CK-MB, troponin-I, TNF-α, IL-2R, IL-6, and IL-10.

The criteria for HT was a blood pressure over 140/90 mm Hg after resting for at least 10 minutes or the presence of HT or history of anti-hypertensive agent use; for DM, fasting blood glucose level over 126 mg/dL or use of antidiabetic agents; and for HL, lipoprotein level over 130 mg/dL or TG level over 200 mg/dL or use of hypolipidemic agents.

Venous blood specimens of 10 cc were taken from each patient at the time of referral and also after 48 hours. Complete blood count and cardiac marker tests were performed immediately after samples were taken; blood specimens to be tested for inflammatory markers were centrifuged and then kept at ~80°C until the time of testing. All patients included in the study were informed about the investigation, and their consents were obtained. The study was begun after obtaining approval from the local ethics committee of Meram Medical Faculty (approval date and no: 24.02.2011 - 056).

Angiographic assessment of coronary artery disease severity
The anatomic severity was determined with a high-quality cineangiogram. The severity of the lesions in the arteries was measured and noted. A critical lesion was defined as 70% stenosis in at least one artery. The number of lesions and severity and anatomic localization of the obstruction were assessed by the Gensini score (12). The angiographic examinations of the patients were performed by cardiologists who did not take part in the study.

Markers of inflammation
The inflammatory and cytokine profiles of the patients were determined by laboratory staff who did not know of the patients’ angiograms. The presence of CMV-DNA (Artus™, GmbH, Hamburg, Germany), HP-DNA (Artus™, GmbH, Hamburg, Germany), and CP-DNA (Artus™, GmbH, Hamburg, Germany) in the sera of both the test and control patients were assessed using the real-time polymerase chain reaction (PCR) method. The DNA material in the sera was extracted with an automated DNA extraction device (EZ1 Advanced-XL™, Qiagen Instruments, Hombrechtikon, Switzerland) using DNA extraction kits for C. pneumoniae and H. pylori (EZ1™ Bacteria Mini Kit V2.0, Qiagen GmbH, Hilden, Germany) and for CMV (EZ1™ Virus Mini Kit V2.0, Qiagen GmbH, Hilden, Germany). The DNAs were determined with real-time PCR using a Rotor-Gene Q™ cycler (Qiagen, Hilden, Germany).

The assays for IL-2, IL-6, IL-10, and TNF-α (Seimens Healthcare Diagnostics Products Ltd., Lianberis, Gwynedd, UK) levels were carried out with the chemiluminescence method (Immuno1000™ Immunoassay System, Siemens Healthcare Diagnostics Ltd., NJ, USA). The procalcitonin assays (Procalcitoin, Siemens Healthcare Diagnostics Inc., Tarrytown, NY, USA) were also performed with the chemiluminescence method (AdviaCentaur XP™ immunoassay System, Siemens Healthcare Diagnostics Inc., Tarrytown, NY, USA).

Statistical analysis
For statistical analysis, the Statistical Package for the Social Sciences™ version 15.0 (SPSS Inc.; Chicago, IL, USA) software package was used. The quantitative variables were expressed as mean ± standard deviation and categoric variables as number of cases (%). All data were subjected to normality analysis. For the comparison of the test group with the control group, quantitative variables with normal distribution were compared with a Student’s t-test and quantitative variables with non-normal distribution with a Mann-Whitney U test. The categoric variables of the two groups were compared with the chi-square test and Fisher’s exact test. The patients’ variables dependent on the change of markers with time were evaluated with t-tests (paired samples t-test).

Results
The study included 57 test and 27 control patients, a total of 84 patients. Of the test and control patients, 46 (80.7%) and 11 (40.7%) were males, respectively. Male patients were significantly higher in the test group (p=0.006). The respective mean ages of the test and control groups were 64.67±11.63 and 61.04±13.44 and there was no significant difference (p=0.208). The demographic data of the test and control groups and the vital findings determined on referral are shown in Table 1. The rates of the presence of DM and HL and past CABGO were significantly higher in the test group than in the control group (p=0.012, p=0.006, and p=0.020, respectively).

The diagnostic distribution of all patients included in the study is shown in Table 2. The angiographic examination of the 84 patients showed the presence of stenosis varying between 20% and 70% in
the 27 cases that were not accepted as critical. The distribution of artery pathologies in the 57 patients that were found to have critical lesions in the angiography is also shown in Table 2.

The results of the biochemical and inflammatory markers of the test and control groups measured at the time of referral to the emergency department are shown in Table 3. The CK-MB and Tn-I levels were statistically higher in the test group than those in the control group (p<0.001 and p=0.001, respectively). There was no significant difference between the test group and control group in terms of WBC and PCT values and lipid profile.

When the two groups were compared, no statistically significant correlation was found between the levels of inflammatory markers IL-6, TNF-α and IL-2R and the level of anti-inflammatory marker IL-10.
Also, there was no statistically significant difference between the two groups in terms of serological positivity of CMV, HP, and CP.

Comparison of inflammatory and cardiac markers of the patient group on referral to the emergency department and 48 hours later are shown in Table 4. In the patient group, the IL-6 levels measured at hour 48 were statistically lower than those measured at the time of referral, whereas the levels of IL-10 were found statistically higher (p<0.001 and p=0.030, respectively). There was no statistically significant change in the levels of IL-2R and TNF-α in terms of initial and hour 48 measurements.

Discussion

It has been shown that various inflammatory markers used to determine cardiovascular risk are useful in the classification of risks, and can also be used in the determination of patients to benefit from interventional therapy (13). Moreover, it has been found that the significant rise in the levels of inflammatory markers in ACS helps in prediction of future cardiovascular risk (14). For instance, it has been claimed that monocyte procoagulant activity stimulated by IL-6 can cause an association between inflammation and thrombosis in patients with CAD (15). But in our study, we did not find such an association when we assessed the infection seropositivity and acute inflammatory response in the acute stages in patients referring with suspected ACS.

Also, we found no significant difference between patients with critical artery lesions and patients with no critical artery lesions in terms of inflammatory cytokine levels (IL-2R, IL-6, IL-10, and TNF-α).

Sukhija et al. (16), in their study of 249 ACS patients referring with chest pain and undergoing angiography, found no significant differences between the levels of IL-6 and TNF-α and the severity of atherosclerosis.

Gotsman et al. (4), in their study of 119 patients undergoing angiography in a consecutive order, determined a significant association between the markers IL-6 and TNF-α and the severity of CAD. They observed that, with increasing severity of the arterial lesion, the levels of IL-6 and TNF-α show a significant rise. The authors determined a strong association between high cytokine levels and the degree of atherosclerosis in the subgroup of patients with a stable coronary; but in the ACS subgroup, a weak association between TNF-α and atherosclerosis was found with no association between IL-6 and atherosclerosis. This situation is caused by the rise in proinflammatory cytokine levels during acute coronary events, which leads to the rise in cytokine levels; and for this reason, an association between cytokine levels and severity of CAD cannot be established (4).

Heinisch et al. (17) compared 20 ACS patients (AMI and USAP) with 20 stable angina pectoris (SAP) patients in terms of IL-6 and TNF-α levels and determined a significant rise in IL-6 and TNF-α levels in the ACS group. Although they observed the IL-6 levels in ACS patients to be indeterminate after 15 to 30 days, they found a significant rise in the TNF-α levels of ACS patients after 30 days (17). When this situation was interpreted in light of former studies (7), it was claimed that TNF-α levels rise in the months following AMI and this rise increases the risk of coronary event recurrences (17).

In their study, Sakamoto et al. (18) divided 286 patients angiographically assessed into two groups based on arterial lesions as the CAD group and a healthy control group. They found significantly higher levels of IL-2R in the CAD group than in the control group. Nijm et al. (19) compared a total of 65 CAD patients, which included 20 patients diagnosed with ACS upon their referral and 45 patients of SAP determined to have significant stenosis in the angiography performed at the time of referral, with 45 healthy controls in terms of IL-2R and IL-10 levels and found no significant difference between the two groups. Hu and Hwang (20), in their study of AMI, USAP, SAP, and control groups (20 subjects in each), found significantly high levels of IL-6 and IL-10 only in the AMI group when compared with the control group. They determined no significant difference in TNF-α levels between AMI, USAP, and SAP groups, but significant levels of TNF-α in all these groups when compared with the control group. After one week, they found a significant decrease in IL-6 levels in the AMI group, but no changes in the levels of IL-10 and TNF-α (20). In our study, after 48 hours, we found a statistically significant decrease in IL-6 and significant increase IL-10 levels, but an insignificant increase in TNF-α level. In the literature, heterogeneity among the compared groups, the difference in test repetition times, small study populations, the fact that they are single-centered studies, and inconsistency between the results obtained are insufficient to make concrete claims in this respect. Whenever possible, there is a need for multicentered studies with large populations among homogenous groups.

The non-specific and non-infectious stimulation of PCT is far lower when compared with the other markers of inflammatory response. In local and systemic inflammation, successive monocyte activation is a prerequisite for PCT production (21-24). PCT serves as a chemo-attractant. Primarily, it is produced in coherent monocytes, but for more production, in the case of inflammation, it is produced by parenchymal cells in the inflammatory tissues. Peripheral blood mononuclear cells express PCT both on mRNA and protein levels. Lipopolysaccharides and various proinflammatory cytokines, such as IL-1β, IL-2, IL-6 and TNF-α, directly play an important role and indirectly have pronounced stimulatory effects on the expression of PCT mRNA (24-27). In our study, we found no significant association between PCT levels and the severity of ACS. Likewise, Sinning et al. (28) reported a significant difference in PCT between patients with AMI and USAP and patients with SAP, but no such difference between AMI and USAP.

In our study, we assessed the test group and control group in terms of HP, CMV, and CP antibodies, but found no difference between the two groups. Likewise, Padmavati et al. (29) compared a group of CAD patients with the control group in terms of HP, CMV, and CP antibodies, and found no difference between the two groups. In a similar study, Mundkur et al. (30) found no difference between the CAD group and control group in terms of HP and CP antibodies, but a significant increase in CMV antibodies in the patient group. In a recent study, Nikitskaya et al. (31) showed that the amount of CMV viral DNA copies in the plasma of patients with ACS was higher than in healthy volunteers. Tabata et al. (32) found that HP-seropositivity and interleukin-1 polymorphisms were associated with higher levels of high-sensitivity C-reactive protein and elevated 5G-segment elevation myocardial infarction risk.

When summarized, in the comparison of ACS patients with stable patients or healthy controls, there was a significant difference in terms of inflammatory markers, but no difference when AMI and USAP were compared. In the comparison of blood parameters determined after 48 hours, there was no apparent difference in the stable patients whereas there were important changes in the parameters of the ACS patients. Although the results of our study are compatible...
with some of the other studies, we think that the number of patients in our study groups, the characteristics of the patients; no distinct separation in AMI, USAP, SAP, and healthy controls or uniting the mentioned disorder groups to form heterogeneous groups for comparison; and regional differences caused difficulty in drawing definite conclusions from the results obtained.

Study limitations

This study included a limited number of patients. For this reason, the data obtained cannot be generalized. Also, since the test patients and control patients in the study were consecutive patients referring to our emergency department, there were differences between these two groups in terms of anamnese and sex. How this situation affected the results is unknown.

Conclusion

The concept of inflammation in atherosclerosis has been increasingly accepted and the proven role of inflammation in atherogenesis has attracted a lot of interest from investigators. At present, it is clear andingly accepted and the proven role of inflammation in atherogenesis has increased much in the last few years, the roles of inflammatory cytokines and the load of infectious pathogens in determining the severity of coronary disease in acute coronary events have not been fully clarified due to different results obtained in various studies. An association could not be established between the severity of the coronary lesion and the cytokine levels because of the rise in inflammatory cytokine levels, independent of atherosclerosis in acute coronary events. On the other hand, in stable patients who were not in acute stress due to angina, an association between the cytokine level and severity of coronary lesion could not be determined. To determine the association between control cytokine levels and coronary risk, further investigations with control coronary angiography and assessment of cytokine levels performed over a long period of time after acute coronary syndrome are recommended.

Ethics Committee Approval: Ethics committee approval was received for this study from the Ethics Committee of Meram School of Medicine (24.02.2011, Decision No: 056).

Informed Consent: Written informed consent was obtained from patients who participated in this study.

Peer-review: Externally peer-reviewed.

Conflict of Interest: No conflict of interest was declared by the authors.

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