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The Role of Tumor Necrosis Factor α (TNF-α) and Intracellular Adhesion Molecules-1 (ICAM-1) in Atherosclerotic Coronary Heart Disease

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Abstract

Background Tumor necrosis factor-α elaborated soon after myocardial ischemic injury. The intracellular adhesion molecule-1 is required for polymorphonuclear emigration, the primary cause of inflammatory tissue damage due to ischemia-reperfusion.

Objective Detect the serum level of tumor necrosis factor-α and to look for the percentage of expression of intracellular adhesion molecule-1 in atherosclerotic coronary heart disease.

Methods Fifty patients (40 males and 10 females) were enrolled in this study with age range (42-80) years, and fifteen, age and sex matched, apparently healthy individuals. The patients group was further classified into acute and chronic cases. Blood sample was taken from each subject and divided into 2 parts. One part used for lymphocyte separation by using immunocytochmistry to detect intracellular adhesion molecule-1 and the other one for serum separation by using ELISA technique to detect tumor necrosis alpha-α.

Results Significant difference in the concentrations of tumor necrosis factor-α was found between patients and control groups and it was elevated in acute cases compared to chronic cases. Similarly, intracellular adhesion molecule-1 was elevated in patients compared o control groups and more in acute than chronic cases.

Conclusions TNF-α is an important marker that acts on coronary arteries which may contribute to the development of congestive heart disease. Elevation of intracellular adhesion molecule-1 level correlates well with the development of acute events in the disease.

Keywords Atherosclerotic coronary heart disease, TNF-α, ICAM-1, ELISA, immunocytochemistry technique.

List of abbreviation: **CHD** = Coronary heart disease, TNF-α = tumor necrosis factor-α, ICAM-1 = intra cellular adhesion molecules-1, PBLs = peripheral blood lymphocytes, ELISA = enzyme linked immuno sorbent assay.

Introduction

Atherosclerosis is a progressive inflammation disorder of the arterial wall (large and medium size arteries) that is characterized by focal lipid-rich deposits of atheroma that remain clinically silent until they become large enough to impair arterial perfusion or until ulceration or disruption of the lesion results in thrombotic occlusion or embolization of the affected vessel [(1)]. Accumulating experimental evidences support a key role for inflammation as a link between risk factors for atherosclerosis and the biology that underlies the complications of this disease [(2)].

Coronary heart disease (CHD) is the most common form of heart disease and the single most important cause of early death in all regions of the world. In United Kingdom 1 in 3

men and 1 in 4 women die from CHD, an estimated 300000 people have a myocardial infarct each year and approximately 1.3 million people have angina [(1)].

Atherosclerosis might, at least partly, be an inflammatory condition. Inflammation which is an immune response to injury characterized by swelling and redness which involves the production of proteins called "cytokines," that attract cells of the immune system to the site of injury. In atherosclerosis, damage to the artery walls seems to trigger inflammation, which helps the atherosclerotic plaques grow. Because of the potential involvement of inflammation in atherosclerosis, increased levels of circulating cytokines might be associated with an increased risk of CHD.

Tumor necrosis factor-α (TNF-α), a pivotal cytokine in the inflammatory cascade, is directly involved in vascular pathophysiology [(3)]. The inflammatory phenomenon and immunological mediators have been identified to positively correlate with the underlying disease load. They regulate every stage of the inflammatory cascade, from endothelial activation to the adhesion of inflammatory cells and platelets, and subsequent remodeling of the internal vascular environment [(4)]. Inflammation is thus moving from a theoretical concept to a tool that provides practical clinical utility in risk assessment and targeting of therapy.

Cytokines are key regulatory glycoproteins allied to inflammatory/immunological processes which modulate all aspects of vascular inflammation by altering the proliferation, differentiation and function of an extensive array of cell types. They are intimately associated with atherogenesis and modulate plaque morphology and stabilization [(5)].

Cytokines act by binding to specific receptors an interaction which has clarified their involvement in atherosclerosis, and highlighted potential new ways for therapeutic intervention [(6)].

TNF-α increases the risk of coronary artery disease by interfering with the thrombotic process by enhancing procoagulant activity (PAI-1, von Willebrand factor) and suppressing the antithrombotic protein C pathway in endothelial cells. The impact of TNF-α on vascular injury in both acute and chronic inflammatory conditions has made it is an important therapeutic target [(7)].

Hence this study tries to investigate the expression of activation markers, i.e., intracellular adhesion molecules-1 (ICAM-1) on peripheral blood lymphocytes (PBLs), and estimation of TNF-α serum levels in atherosclerotic patients.

Methods

Fifty patients with CHD (40 males and 10 females), their age ranges from 42-80 years old, were included in this study. Eleven patients (7 males and 4 females) with acute myocardial infarction were admitted to the Cardiac Care Unit at Al-Imamain Al-Kadhimain Medical City and 39 patients (33 males and 6 females) were attendant as outpatient clinic of Ibn Al-Baitar Hospital in Baghdad with history of atherosclerotic chronic coronary insufficiency. The period of sample collection was from May to July 2008. Consent for the participation in the research was obtained from each patient.

The diagnosis was done by cardiologist based on clinical presentation and history of ischemic heart disease, which was confirmed by electrocardiography, cardiac enzymes and coronary artery catheterization. Fifteen, age and sex matched, apparently healthy individuals, were included in this study as healthy control group.

Sample collection: From each patient and control, five ml venous blood was aspirated from a suitable vein after efficient disinfecting over the injection site.

Blood samples were divided into two parts, three ml were immediately transferred to sterile heparinized vacutainer tube for lymphocyte separation, and two ml of blood

immediately transferred to a sterile plain tube for serology.

The unheparinized blood in plain tube was left to clot and then centrifuged at 1000 rpm for 5 minutes to separate the serum and dispensed into tightly closed Eppendorf tubes in 0.1 ml aliquots and stored at -20^{o}C until assayed.

Detection of serum TNF-α

The procedure of Enzyme Linked Immuno Sorbent Assay (ELISA) was done according to the manufacturer instructions, as it was written in the kit guideline, product code: KAC1751; while the standardization of the kit to produce a standard curve was done by using a duplicate sample of each standard and the mean of them was used.

Detection of PBLs ICAM-1

Primary monoclonal antibody (USBiological$^{(R)}$) specific for Human ICAM-1 protein were applied on slides; then immunoperoxidase Secondary Detection system that were used from Dako Cytomation Company, USA, (Ref K0673). Positive results were identified by presence of brown colored precipitate.

Statistical analysis

Statistical analysis was performed with the SPSS 15.01 Statistical Package for Social Sciences and also Excel 2003. Data analysis was done using independent sample t-test for tables with means and standard deviations. *P* value of ≤ 0.05 was used as the level of significance and *P* value of ≤ 0.001 was used as the level of highly significance. Descriptive statistics for the clinical and laboratory results were formulated as mean and standard deviation.

Results

Serum levels of TNF-α of all acute and chronic cases was 43.659±6.374 which is significantly higher (*P* = 0.000) than 16.340±3.645 of the control group. Moreover, significant difference (*P* < 0.025) was found between the acute and chronic cases (44.934±12.421 and 40.847±10.872, respectively) as seen in table 1.

Table 1. Serum tumor necrosis factor-α in the coronary heart disease patients and control group

		Tumor necrosis factor-α	*P* value
Group	Patients (N=50) Control (N=15)	43.65±6.37 16.34±3.64	< 0.001
Disease phase	Acute (N=11) Chronic (N=39)	44.93±12.42 40.85±10.87	0.025

The ICAM-1 expression on PBLs was equal to 56.571 ±16.434 which is significantly higher (*P* = 0.000) in the patients as compared to 24.500±7.623 of the control group. Similarly, ICAM-1 expression on PBLs was statistically higher (*P* < 0.043) in the acute cases compared to the chronic cases (65.364±14.583 and 54.026±16.226, respectively) as shown in table 2.

Table 2. Expression of intracellular adhesion molecule-1 on peripheral blood lymphocytes in the coronary heart disease patients and control group

		Intracellular adhesion molecule-1	*P* value
Group	Patients (N=50) Control (N=15)	56.57±16.43 24.50±7.62	<0.001
Disease phase	Acute (N=11) Chronic (N=39)	65.36±14.58 54.03±16.22	0.043

Discussion

The current study showed that serum level of TNF-α in CHD patient was higher than healthy control group; such findings come in agreement with Cybulsky et al [6] who found that many cytokines, including, IL-1, TNF-α, and IFN-γ, have been implicated in the induction of an array of adhesion molecules and chemokines in the vascular wall and Natanson *et al* [7] and Ridker *et al* [8]. The difference between acute and chronic cases was in agreement with Deten et al [9]; although they did their study on experimental rats. IL-1 and TNF-α stimulate membrane expression of leukocyte adhesion molecules ICAM-1, ICAM-2, VCAM-1, E-selectin, and P-selectin by endothelial cell. These molecules interact with specific ligands expressed by neutrophils, lymphocytes, and circulating monocytes.

Unfortunately, results done by Chung et al [10]; Muller-Ehmsen and Schwinger [11] allied to TNF neutralization therapies have been inconsistent thus far. Trials using TNF-α antagonist, such as infliximab (ATTACH trial), failed to show any improvement in cardiac failure, while the ATTACH trial was associated with an increased all and cause mortality. Although there have been no studies focusing on anti-atherogenic therapeutic interventions in peripheral arterial disease, a study by dePalma et al workers [12] indicated that there were no marked differences in TNF-α levels in peripheral arterial disease patients receiving/not receiving statin therapy.

Our results showed the expression of ICAM-1 on PBLs was higher in patients than in controls and there was a significant difference between acute and chronic cases of patients; a findings which is in agreement with studies done by Blann and McCollum [13], Hwang *et al* [14] who reported significantly higher values of circulating ICAM-1 in patients with peripheral vascular disease and ischemic heart disease than in healthy control subjects. Caroline *et al* [15] showed that patient with cardiovascular events during follow-up had higher ICAM-I and VCAM-I than those without events.

Squadrito *et al* [16] reported significantly higher levels of circulating ICAM-1 and E-selectin in patients with acute myocardial infarction than in patients with chronic stable angina and healthy control subjects.

There was a consistent relationship between the levels of circulating ICAM-1 and incident CHD. One possible explanation for these data is that levels of circulating ICAM-1 are more closely related to the activity of atherosclerosis. Increased levels of ICAM-1 may be important in migration of increased numbers of T lymphocytes into active lesions [17]. Other possibility is that patients with higher circulating levels of ICAM-1 have an increased number of plaques prone to rupture, thrombi, or other events leading to clinical CHD. The interaction between fibrinogen and ICAM-1 observed in *in vitro* study provides evidence suggesting an association between ICAM-1 and thrombosis/ischemic events [18].

This study revealed that TNF-α is considered as an important marker on coronary arteries which may contribute to the development of (CHD), and ICAM-1 levels correlates well with the development of acute events in CHD.

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Conflict of Interest

There were no personal or financial conflicts or problems raised during the performance of this work.

Author contribution

All authors participated in performing this work worked as one team.

We shared nearly the same responsibilities regarding data collection preparing and performing the lab work, writing and printing and other steps were necessary to complete this work.

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