EVALUATION OF PRO-INFLAMMATORY INTERLEUKINS (IL-1, TNF& AND IFNγ) IN ISCHAEMIC DISEASES PATIENTS IN SOUTH OF IRAQ

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ABSTRACT

Ischaemic heart disease (IHD) or called coronary heart disease (CHD), It is the end result of the accumulation of atheromatous plaques within the walls of arteries that supply the myocardium. There is growing evidence for a pathogenic role of cytokines in atherogenesis, the presence of a certain cytokines have been documented in human atherosclerotic blood vessels. This study aimed to evaluate the presence of pro-inflammatory interleukins (IL-1, TNFα and IFNγ) in the serum of subjects whom submitted to this study. In this study we found great difference in means between subjects with ischaemia and subjects without ischaemia towards subjects with ischaemia for three interleukins, but statistically this difference did not reach to significance.

INTRODUCTION

The heart is an aerobic organ that is dependent for its oxygen supply entirely on coronary perfusion (Munther &Homoud, 2008). Myocardial ischemia is an imbalance between the supply (perfusion) and demand of the heart for oxygenated blood. In most cases there is a long period (up to decades) of silent, slow progression of coronary lesions before As a consequence of the chronic inflammatory state, activated leukocytes and vascular wall cells release growth factors that promote smooth muscle cell proliferation and ECM synthesis (Vinay et al. , 2010 ).
symptoms appear, thus, the syndromes of IHD are only the late manifestations of coronary atherosclerosis that may have started during childhood or adolescence (Vinay et al., 2010). Ischemic heart disease is caused by narrowing of one or more of the three major coronary artery branches. These are functional end-arteries, and sudden occlusion of any one leads to infarction in the area of supply (Parakrama and Clive, 1998). Two variants of clinical presentation of ischaemic heart diseases: angina pectoris (which is subdivided into: typical or stable angina, prinzmetal or variant angina and unstable angina or called crescendo angina) (Vinay et al., 2007). and myocardial infarction (MI) (Boon et al., 2007).

Although normal vessels do not bind inflammatory cells, early in atherogenesis, dysfunctional arterial endothelial cells express adhesion molecules that encourage leukocyte adhesion; vascular cell adhesion molecule 1 (VCAM-1), in particular, binds monocytes and T cells. After these cells adhere to the endothelium, they migrate into the intima under the influence of locally produced chemokines. Monocytes transform into macrophages and avidly engulf lipoproteins including oxidized LDL. Monocyte recruitment and differentiation into macrophages (and ultimately into foam cells) is theoretically protective, because these cells remove potentially harmful lipid particles. However, the oxidized LDL augments macrophage activation and cytokine production (e.g. tumor necrosis factor-alpha (TNF-α)). This further increases leukocyte adhesion and production of chemokines (e.g., monocyte chemotactic protein 1 (MCP-1)), creating a stimulus for recruitment of additional mononuclear inflammatory cells. Activated macrophages also produce reactive oxygen species that aggravate LDL oxidation and elaborate growth factors that drive smooth muscle cell proliferation. T lymphocytes recruited to the intima interact with the macrophages and can generate a chronic inflammatory state. It is not clear whether the T cells are responding to specific antigens (e.g., bacterial or viral antigens, heat-shock proteins or modified arterial wall constituents and lipoproteins) or are nonspecifically activated by the local inflammatory milieu. Nevertheless, activated T cells in the growing intimal lesions elaborate inflammatory cytokines, (e.g. interferon-gamma (IFN-γ)), which can stimulate macrophages as well as endothelial cells and smooth muscle cells (Vinay et al., 2010). Consequently, there is increased production of inflammatory cytokines such as interleukin-1 (IL-1) (Crabtree, 1996).

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MATERIALS AND METHODS

The subjects of this study are (50) persons, whose ages ranged between 45 to 70 years, submitted to catheterization or percutaneous intervention (PCI) in AL-Nassierya center for cardiology from September 2012 to April 2013. Two divisions of subjects with ischemia and without ischemia, according to PCI and catheterization.

The 10 ml of venous blood was drawn from each subject aseptically then put in plain tube at room temperature for coagulation and then centrifuged at 3000 rpm to separate the serum that will be kept in sterile eppendorf tube in –20 C in deep freeze (Collee et al., 1996). The serum stored as 10-12 replicates to be used in evaluation of the cytokines.

The method used in evaluation the (IL-1, TNFα and IFNγ) was based on ELISA technique and the procedure was done according to instructions provided by manufacturer using commercial kits from peprotech company in USA and using reader and washer equipments manufactured by biotek company in USA. The backbone of obtaining estimated concentration of interleukins was done by standard curve that binds serial concentrations of interleukin and their absorbancies.

Statistical package for social science version 19 (SPSS 19) was used for data input and analysis. T test was used to test the significance of association between two continuous variables which were presented as means and standard deviation. P-value used was asymptotic and all tests were two sided. Findings of P-value at levels less or equal to 0.05 was considered significant.

RESULTS

In this study we estimated the level of pro-inflammatory interleukines (IL-1, TNFα & IFNγ) in the serum of persons with and without ischemia as pg/ml according to the manufacturer standard curve by ELISA technique, these parameters have no normal values and their values depend on the immunological status of subject, table (1) explain these results.

Table 1: The results of pro-inflammatory interleukines (IL-1, TNFα & IFNγ) in the serum of study groups subjects.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
<th>No.</th>
<th>Mean</th>
<th>Standard deviation</th>
<th>Standard error</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-1</td>
<td>With IHD</td>
<td>34</td>
<td>125.57</td>
<td>373.864</td>
<td>64.114</td>
<td>0.19</td>
</tr>
<tr>
<td></td>
<td>Without</td>
<td>16</td>
<td>0.81</td>
<td>1.355</td>
<td>0.339</td>
<td></td>
</tr>
</tbody>
</table>
As shown in table above there were differences in means between persons with and without ischemia for the three interleukines towards persons with IHD (125.57 versus 0.81 in IL-1, 61.18 versus 21.31 in TNF-α & 35.91 versus 9.75 in IFNγ), in spite of that, this could not form significant association between study groups, P-values = 0.19, 0.231 & 0.516 respectively. Figures (1), (2) and (3) show standard curves obtained practically for IL-1, TNFα and IFNγ respectively.

Figure 1: standard curve by which we calculate concentrations of IL-1 in the sera of persons with and without IHD as pg/ml on X-axis versus absorbancy on Y-axis.
Figure 2: standard curve by which we calculate concentrations of TNFα in the sera of persons with and without IHD as pg/ml on X-axis versus absorbancy on Y-axis.

Figure 3: standard curve by which we calculate concentrations of IFNγ in the sera of persons with and without IHD as pg/ml on X-axis versus absorbancy on Y-axis.

DISCUSSION
Cytokines are extracellular signaling proteins secreted by immunocompetent cells as well as nonimmune cells such as vascular endothelium (Dinarello, 1987). Although these intercellular peptide mediators have long been known to play an important role in the regulation of inflammation by controlling recruitment and activation of various responsive cell populations, it is only recently that cytokines have been proposed to have a role in the triggering and perpetuation of atherosclerosis (Libby, 1992). Among the different cytokines, some are believed to be more relevant than others in the context of atherosclerosis and it’s
complications, such as ischemic heart disease. Among these are interleukin-1, tumor necrosis factor alpha and interferon gamma (Schleerf et al., 1998). These cytokines promote angiogenesis and induce morphological and functional alterations in the endothelial cells (Schleerf et al., 1998). In addition, these cytokines cause expression of human leukocyte antigen (HLA) (Alvaro Garcia et al., 1990) and other leukocyte adhesion molecule (Pober et al., 1986).

Tumor necrosis factor promotes adhesion of neutrophils, basophils, eosinophils and lymphocytes on endothelial cells (Bivelacqua et al., 1987). Interferon-gamma acts synergistically with tumor necrosis factor to increase the expression of intracellular adhesion molecule as well as class 1 and class 2 MHC antigen (Collins et al., 1986). These cytokines increase the activity of plasminogen activator inhibitor as well as the synthesis of tissue plasminogen activator (Schleerf et al., 1998), but the overall effect of cytokine secretion appears to be procoagulant. IFN-γ is released locally in the arterial intima during the vascular response to injury and has been shown to regulate vascular smooth muscle proliferation (Pober and cotran, 1990). IFN-γ is generated by T cells (Bruserud et al., 1993), whereas TNFα is produced by several types of cells, including smooth muscle cells (Barah et al., 1990), lymphocytes (Bruserud et al., 1993) and macrophage (Trinchieri, 1991). Macrophage colony stimulating factor (MCSF) and IL-1 released by injured endothelium (Liao et al., 1995) promote the interaction of endothelial cells with circulating leukocytes (Shyy et al., 1993), and may thus contribute to the development and progression of atherosclerosis (Huh et al., 1996). MCSF induces the synthesis by endothelial cells of monocyte chemotactic protein 1, which enhances the migration of monocytes into the subendothelial layer (Shyy et al., 1993). MCSF additionally increases cholesterol uptake by macrophages (Huh et al., 1996), resulting in foam cell formation, the hallmark of atherogenesis. The MCSF, acting in synergy with IL-1, induces the activation and proliferation of monocytes/macrophages (Dinarello and Wolff, 1993). These 2 cytokines determine a further release of cytokines from vascular cells (Shyy et al., 1993), including IL-6 which may be involved in smooth muscle cell proliferation (Seino et al., 1994). MCSF and IL-1 upregulate the expression of leukocyte adhesion molecules on the endothelial surface and of specific integrins on monocytes, leading to enhanced adhesion of monocytes to the endothelium (Shyy et al., 1993). This study agreed with (Juvonen et al., 1997) in IL-1, TNFα and IFNγ.
In spite of that, statistically results could not reach to significance between persons with IHD and persons without IHD, but high difference in means (for the three interleukins) between persons with IHD and persons without IHD towards persons with IHD, must keep in mind and perform later studies with large No. of subjects depending on cohort study design with high sensitive statistical criteria and using more sensitive technique, to detect ischaemia, rather than catheterization such as intravascular ultrasound or angioscopy, in addition we prefer not to deal with ischaemia in general but dealing with ischaemia variants such as stable angina, unstable angina and myocardial infarction, also specimen collections done during attack or acute phase of disease not during chronicity.

By above recommendations in further studies we can conclude relatively an absolute fact in field of medicine.

REFERENCES
in vascular endothelial cells and dermal fibroblasts in vitro. Proc Natl Acad Sci USA, 1986; 83: 446-450.


