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Comparison between HSP70 Levels in Acute and Chronic Coronary Artery Diseases

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Abstract

Background: Heat shock proteins are a family of endogenous proteins that act as molecular chaperon and increase in different stress situations like heart disease and atherosclerosis. Therefore, the aim of this study was to investigate whether there is a difference between Hsp70 level in the blood of myocardial infarction patients and apparently normal population; and between recent myocardial infarction patients and previous myocardial patients using immunocytochemistry technique. **Method:** Peripheral blood sample was taken from 50 patients with history of myocardial infarction divided into two groups (11 with acute or recent myocardial infarction and 39 with old or chronic myocardial infarction. Another 50 apparently healthy individuals were taken as a control group. Heat shock protein 70 level was measured by immunocytochemistry technique. **Results:** There was significant rise of heat shock protein 70 in myocardial infarction patients as compared with control group. Also, a significant decrease in heat shock protein 70 level in the chronic myocardial infarction patients group as compared with the acute myocardial infarction patients group. **Conclusion:** this study showed that heat shock protein 70 increases in acute myocardial infarction patients' but its level decreases in chronic myocardial infarction. So heat shock protein 70 can be used as a biomarker to differentiate acute, from chronic, myocardial infarction and may be helpful as an indicator of acute myocardial infarction.

Keywords: *acute myocardial infarction, heat shock protein 70, chronic myocardial infarction, immunocytochemistry.*

Introduction

Cardiovascular disease is one of the global public health problems contributing to 10% of the global disease burden and 30% of global mortality [(1)]. Myocardial infarction (MI) is one of the five main presentations of coronary heart disease, which include unstable angina pectoris, stable angina pectoris, MI, heart failure and sudden death, and is defined as myocardial cell necrosis due to remarkable and sustained ischemia. It is usually an acute manifestation of atherosclerosis-related coronary heart disease [(2)].

Atherosclerosis is a widely accepted risk factor for coronary heart disease and a well-known inflammatory disease [(3)]. In addition to inflammation in the coronary plaque, there may be myocardial inflammatory response after acute myocardial infarction (AMI) as part of the healing and scar formation processes [(4)].

Heat shock proteins (HSPs) are a family of endogenous proteins responsible for different types of stresses. They are classified according to their molecular weight into families, e.g. HSP27, HSP70, etc [(5)]. They have the capability to act as 'molecular chaperones, since they guide protein folding, stabilize macromolecules, carry out refolding and get rid of irreversibly denatured proteins in the cell [(6)]. In addition, HSPs can be overexpressed in various stressful conditions such as hyperthermia [(7)], hemodynamic stress caused by heart diseases [(6)], physical exercise [(8)] and atherosclerosis [(9)]. After cell death or stress, HSP peptide complexes may be presented on the cell surface or released to the

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circulation leading to activation of the adaptive immune system cells in addition to activating innate immune system, thus; signaling cell stress or damage to the immune system [10]. Among HSPs, HSP70 is a powerful endogenous activator of the innate immune system working as a putative Toll-like receptor (TLR) ligand and is capable of stimulating the production of cytokines by macrophages (Figure 1) [11].

Multiple previous studies indicated and confirmed the protective role of HSP especially in myocardial ischemia [9, 12]. Hsp70 may suggest important mechanisms of ischemic injury including misfolding, unfolding or pathological changes of critical proteins [13]. Therefore, the aim of this study was to investigate whether there is a difference between Hsp70 level in the blood of myocardial infarction patients and apparently normal population; and between recent myocardial infarction patients and previous myocardial patients using immunocytochemistry technique.

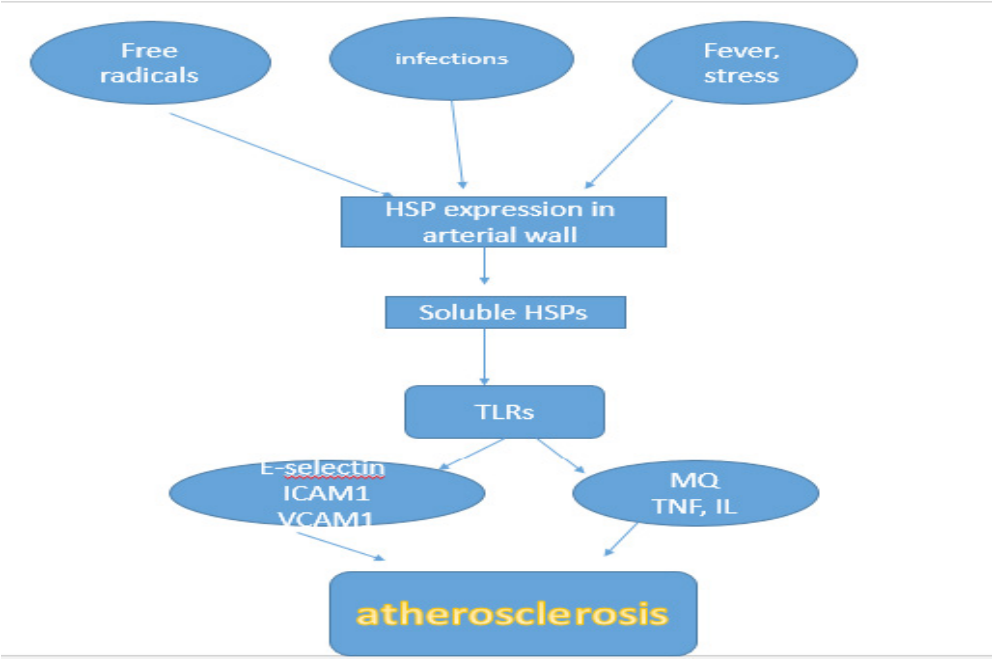

Figure 1 Schematic representation of potential role of HSP in atherogenesis. Various stressors induce HSP production in the arterial wall, soluble HSPs bind to TLR complex resulting in proinflammatory responses and autoimmune reactions which contribute to atherosclerosis. TLR: Toll-like receptor). MQ: macrophages [9].

Materials and Method

This cross-sectional study was approved by Al-Nahrain College of Medicine Ethical committee. All participants gave their informed consents prior to their inclusion in the study. The study was carried out from September 2018 till 31st, December 2018. There were three groups of participants, their age ranged from 42-80 years, first group (group A) included 11 patients (7 males and 4 females) with acute myocardial infarction who were admitted to the Cardiac Care Unit (CCU) at Al-Imamain Al-kadhymain Teaching Hospital in Baghdad. The second group (group B) included 39 patients (33 males and 6 females) who were attending the outpatient clinic of Ibn –Albitar Hospital in Baghdad with chronic MI (more than 4 weeks from diagnosis of MI) [14]. Third group (group C) of 50 apparently healthy individuals, who were age-, and sex-, matched to patients participants, were included as control group.

The diagnosis of patients was done by a specialist in cardiology based on clinical presentation and history of ischemic heart disease, which was confirmed by ECG, cardiac enzymes and coronary artery catheterization.

Blood samples collection: A 3-ml venous blood sample was drawn from each participant, for group A, within less than 24 hours from the myocardial infarction, for group B, after 4 weeks from MI diagnosis. The blood sample was immediately transferred to sterile heparinized

vacutainer tubes for lymphocytes separation.

After rehydration of smears, peroxidase block and protein block were added respectively. Primary antibody (US biological company) was added on smears (20ml of diluted mouse monoclonal Ab specific human CD marker) and incubated for 1 hour at 37°C, washed then incubated with secondary antibody and peroxidase conjugate and substrate-DAB chromogen complex were added respectively with buffer washing between each addition. Finally, the slides were counter-stained with hematoxylin, and then slides were covered with cover slide using mounting media. The slides were examined under 400x magnification power of light microscope (Olympus). The dark brown cytoplasmic or membranous staining of cells were considered positive (Figure 2), the percentage of positive cells was calculated as follows:

Percentage of positive cells = (No. of positive cells / total No. of cells)*100%.

Statistical analysis was performed with Statistical Package for Social Sciences (SPSS, version 15.01). Data analysis was done using independent sample *t*-test for tables with means and standard deviations. *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

HSP70 expression on PBLs was detected by immunocytochemistry technique. A comparison between patients (groups A and B) and control groups and between group A and B were summarized in Table 1 and Table 2, respectively. Independent sample *t*-test revealed a high statistically significant difference between patients and control groups (*P*=0.001). In addition, there was a high statistically significant difference between acute and chronic MI patients group (*P*=0.000) in expression of HSP70 on PBLs.

Table 1 Expression of HSP70 in PBLs by MI patients and control group

	Group	Mean	Std. Dev.	Std. Error	Sig. (2-tailed)
HSP-70	Control (group C)	19.571	8.751	2.339	0.001
	Patients (groups A+B)	31.327	17.376	2.482	

Highly significant difference (P≤0.001)

Table 2 Expression of HSP70 in PBLs by recent and old MI patients groups

	Patients group	Mean	Std. Dev.	Std. Error	Sig. (2-tailed)
HSP-70	Group A	57.909	10.134	3.055	0.000
	Group B	23.632	9.646	1.565	

Highly significant difference (P≤0.001)

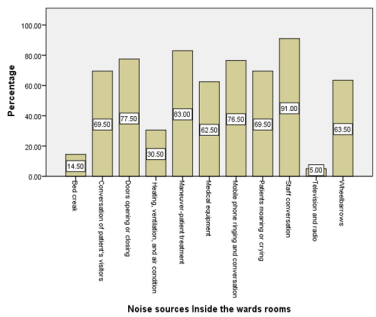

Figure 2 Immunocytochemistry of HSP70 in blood of patients with acute MI showing brownish cytoplasmic staining of RBC (40x).

Discussion

HSP70 proteins are central components of the cellular chaperone system associated with folding, assembly, disassembly and degradation of proteins [16]. During the last decades, a number of studies had suggested that stress-induced overexpression of HSP70 may confer protection against ischemia and reperfusion injury of the heart [17].

Previous studies had evaluated the role of Hsp70 in heart failure [18,19], but few of them had investigated its

role in myocardial infarction and none had compared between recent and old MI.

Our study revealed that Hsp70 was significantly increased in peripheral blood of myocardial infarction patients in comparison with healthy control group, these findings were similar to [20] who had measured serum level of Hsp70 in 24 patients with acute MI at the arrival, 6 hours later and in the next morning using ELISA method and found that serum HSP70 in patients with acute MI were significantly higher than in the control group at all-time points and concluded that there was a relation between HSP70 and the extent of myocardial necrosis measured by the increase in cTnT and CK-MB (known markers of myocardial necrosis), as well as by typical echocardiographic findings. Our study agreed with [21] who had investigated synthesis of HSP70 in the human heart (*in vivo*) after CABG and found that the increase in HSP70 occurred after at least 2 hours of stress induction by CABG. Similarly [22] had examined the immunohistochemical expression and distribution of HSP72 after various periods of ischemia (from 30 minutes to 7 days) and reperfusion in the non-stress-pretreated rat heart. Moreover, [23] also studied the protective role of HSP70 by direct HSP70 gene delivery in rabbits hearts (*in vivo*) and inducing ischemic condition. They concluded that HSP70 reduced the size of infarcts and thus reducing the severity of ischemic injury. HSP70 was studied by [24] in atherosclerotic plaques in aorta of humans and rabbits through immunohistochemistry and they found that HSP70 staining was most pronounced in the central portions of advanced atherosclerotic plaques.

Our method of HSP70 detection in blood by immunocytochemistry was never been reported in previous studies. Also, no previous similar studies had compared the difference between recent or acute MI from old previous MI in humans.

Conclusion

HSP70 may be used as a biomarker for myocardial infarction and as a confirmatory test to differentiate between acute and chronic MI.

Ethical Clearance: The research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq.

Conflict of interest: The authors declare that they have no conflict of interest.

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