The role of HSP60 in Atherosclerotic Coronary Heart disease

Wurood A.S. Kadhim* **BSc, MSc**
Nidhal A. Mohaymen* **BSc, PhD**

Summary:

Background: Heat shock proteins have a general role in the response of the arterial wall to stress and may serve as a mediator/inducer of atherosclerosis in particular circumstances when HSPs specifically bind to the Toll-like receptor 4/CD14 complex, initiating an innate immune response, including the production of pro-inflammatory cytokines, this also followed by cytokine amplification through transmigration of macrophages and neutrophils.

Objective: To investigate the percentage of expression of HSP60 by peripheral blood lymphocyte (PBL) in atherosclerotic coronary heart disease (CHD) patients using immunocytochemistry technique.

Method: A total of fifty patient (40 males and 10 females), ranged from the mean age (59.12±8.54) years with a range from 42-80 years and fifteen, age and sex matched, apparently healthy individuals, taken as a healthy control group, were enrolled in the current study and the patients group were further classified into acute cases (n=11 patients) ″7 males and 4 females″ and chronic cases (n=39 patients) ″33 males and 6 females″. Three ml of blood sample had been taken from each subject, and immediately transferred to sterile heparinised vacutainer tubes for lymphocyte separation. Immunocytochemistry was used to detect HSP60 by using monoclonal antibody to HSP60 protein.

Result: Results revealed a statistically significant elevation levels between patients and control groups (P=0.000), the expression of HSP60 was elevated in all patients with no significant difference between acute and chronic cases (P>0.001).

Conclusions: HSP60 plays an important role in induction and development of atherosclerosis by stimulating different mechanisms which are the triggers for the process of atherosclerosis development. It is found in higher levels in both acute and chronic cases of atherosclerotic CHD in comparison with control cases.

Keywords: Atherosclerotic coronary heart disease, Heat shock proteins(HSP60) , immunocytochemistry technique.

Fac Med Baghdad 2011; Vol. 53, No. 3
Received Jan. 2011
Accepted Feb. 2011

Introduction:

Atherosclerosis is a progressive inflammation disorder of the arterial wall (Large+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 embolisation of the affected vessel (1). Heat shock proteins (HSPs) are present in most cells, serving as molecular chaperones, and they play a role in cell protection from damage in response to stress stimuli. However, accumulating data indicate the involvement of HSPs in the pathogenesis of diseases, concerning the role of HSPs in atherosclerosis, it has been demonstrated that HSPs are highly expressed in the atherosclerotic lesions of humans, rabbits, and apolipoprotein E–deficient mice (2). Risk factors for atherosclerosis, e.g. infections, oxidized low density lipoprotein, oxidative stress, hypertension, and biomechanical stress, evoke HSP over expression in endothelial cells, macrophages, and smooth muscle cells via activation of heat shock transcription factor 1. Interestingly, HSPs, normally localized within the cell, have been found as a soluble form in the blood, which is positively correlated with atherosclerosis in humans. Several groups have reported that soluble HSPs specifically bind to the Toll-like receptor 4/CD14 complex, initiating an innate immune response, including the production of proinflammatory cytokines by macrophages and adhesion molecules in endothelial cells via nuclear factor-κB activation. Furthermore, the titers of autoantibodies against HSPs are significantly elevated in patients with atherosclerosis, and T lymphocytes specifically responding to HSPs have been found in atherosclerotic plaques. These pro-inflammatory responses and autoimmune reactions to HSPs in the vessel wall can contribute to the initiation and perpetuation of atherosclerosis. Thus, HSPs have a general role in the response of the arterial wall to stress and may serve as a mediator/inducer of atherosclerosis in particular circumstances (2). All risk factors, e.g. infections, biomechanical stress, oxLDL, and free radicals, directly stimulate cells of the arterial wall and/or other tissues to express high levels of HSPs. The physiological functions of these HSPs are to protect cells against apoptosis. Pathologically (ie, from stimulation by the risk factors), the cells are dying; this releases intracellular HSPs into

* *Clinical Immunology, Dept. of Microbiology, College of Medicine, Al-Nahrain University.*

intercellular spaces to form small Heat shock proteins (3, 4). Heat shock proteins (HSPs) bind to TLR4/CD14 receptors, resulting in endothelial cells expressing adhesion molecules, in smooth muscle cells leading to proliferation, and in macrophages inducing a range of proinflammatory cytokines. Simultaneously, macrophages present antigens to T and B cells, which produce autoantibodies and autoreactive cells against HSPs. All contribute to the development of atherosclerosis (5, 6).

Materials and method:

Fifty patients with coronary heart disease (40 males and 10 females), their age ranges from 42-80 years old, were included in this study. 11 patients (7 males and 4 females) with acute myocardial infarction were admitted to the Cardiac Care Unit (CCU) at Al-Kadhumyia Teaching Hospital in Baghdad, 39 patients (33 males and 6 females) were attendant as outpatient clinic of Ibn –Albetar Hospital in Baghdad with history of atherosclerotic chronic coronary insufficiency. The period of sample collection was from May to July 2008. The diagnosis in every patient 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. Fifteen, age & sex matched, apparently healthy individuals, were included in this study as healthy control group.

Sample collection: From each patient and control, Three ml venous blood was aspirated, and immediately transferred to sterile heparinised vacutainer tubes for lymphocyte separation. Immunocytochemistry staining technique for the detection of HSP60 in PBLs: Immunoperoxidase Secondary Detection system that were used from Dako Cytomation, USA, Ref K0673, monoclonal antibody to HSP60 protein (UsBiological) with specificity to human HSP protein, and the biotinylated secondary antibody was anti-mouse Ab.

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 $\leq 0.001$was used as the level of highly significance. Descriptive statistics for the clinical and laboratory results were formulated as mean and standard deviation.

Results:

In this study result of HSP60 expression on PBLs was detected by immunocytochemistry technique. A comparison between patients and control groups and among patients groups (acute and chronic cases) 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=0.000). And there was no significant difference between acute and chronic cases patients group (P>0.001) in expression of HSP60 on PBLs.

Table-1: Comparison between CHD patients and control group in expression of HSP60 in PBLs.

	Grouping	Mean	Std. Dev.	Std. Error	Sig. (2-tailed)
HSP-60	Patients	62.816	16.822	2.403	0.000
	Control	24.429	4.603	1.230	

Highly significant difference (P≤0.001).

Table-2: Comparison between acute and chronic cases group of CHD patients in expression of HSP60 in PBLs.

	Disease phase	Mean	Std. Dev.	Std. Error	Sig. (2-tailed)
HSP-60	Acute	73.000	12.075	3.641	0.009
	Chronic	59.868	16.969	2.753	

HSP-60 highly significant difference (P≤0.001).

Discussion:

Our study revealed a high statistically significant difference between patients and control groups (P=0.000) and the expression of HSP60 in PBLs was higher in patients than in controls. And there was no significant difference between acute and chronic cases patients group (P>0.001) in expression of HSP60 in PBLs. These results of our study in line with a study done by Zhang *et al.* (7) who proved that elevated HSP60 levels are associated with an increased risk for CHD and HSP60 and anti-HSP60 antibody levels combine to increase this risk. In addition, acute myocardial infarction induces Hsp60 release. Thus, HSPs appear to be important in preventing damage and in cellular repair processes after injury. Indeed, increased production of HSPs has been shown to protect cells against apoptosis induced by oxidative stress, toxins, heat shock, and cellular damage after ischemia or sepsis-induced injury (3, 4). Given the high degree of amino acid sequence homology between HSPs of different species, the immune response to HSPs derived from pathogens may cross-react with host HSPs.Thus, HSPs may be auto antigens in some circumstances, and has been shown to be involved in the pathogenesis of atherosclerosis (8, 9). HSP60 are the antigens recognized by T cells in atherosclerotic lesions, They are possibly a notion that regarding anti-human HSP60 antibodies in a study done by Burian *et al.*(10) and Zhu *et al.* (11), 2 independent groups demonstrated that >70% of the study subjects had anti-human HSP60 antibodies. The prevalence of coronary artery disease was significantly increased in seropositive compared with seronegative patients. Importantly, HSP60 antibodies were related

to disease severity, which persisted after adjustment for traditional risk factors, ie, age, race, sex, smoking, diabetes, hypercholesterolemia, hypertension, and C-reactive protein levels. Moreover, Huittinen *et al*. (12) reported that human HSP60 antibodies were a significant risk factor for coronary events. Circulating antibodies to HSPs may be induced or maintained by several different mechanisms. First, infection with agents that contain HSP60 proteins could induce an anti–self-response through molecular mimicry in susceptible individuals (13). Second, the protein could become immunogenic because of structural alteration or posttranslational modification resulting from oxidation or metabolic alterations (14). Third, other foreign or self-antigens could interact with HSP60 to form immunogenic complexes in which B cells recognize HSP60 and T cells direct their response at the associated antigen (15). Fourth, soluble HSP might be not recognized as a self-protein by a population of T and B lymphocytes, in as much as HSPs being leaked are intracellularly localized in physiological conditions (16). Finally, genetic auto antibodies, suggesting that cell-mediated immune responses to HSP60 are involved in the pathogenesis of this disease. In further support of autoimmunity are findings that rabbits and mice develop atherosclerosis after immunization with HSPs (17, 18). Therefore, serum auto antibodies and T cells react not only with bacterial HSP65 but also with human HSP60 in vascular cells.

References:

1. *Boon NA, Fox KAA, Bloomfield P and Bradbury A. Cardiovascular disease.In:Davidson's principle and practice of medicine 19th ed./edited by Haslett C,Chivers ER, Boon NA, Colledge NR and Hunter JA. 12:360-480, (2002).*
2. *Xu Q. (2002) Role of Heat Shock Protein in Atherosclerosis. Arterioscler Thromb Vasc Biol. 22:1547-1559.*
3. *Buzzard KA, Giaccia AJ, Killender M and Anderson RL. (1998) Heat shock protein 72 modulates pathways of stress-induced apoptosis. J Biol Chem. 273:17147–17153.*
4. *Yaglom JA, Gabai VL, Meriin AB, Mosser DD and Sherman MY. (1999)The function of HSP72 in suppression of c-Jun N-terminal kinase activation can be dissociated from its role in prevention of protein damage. J Biol Chem. 274:20223–20228.*
5. *Wick G, Romen M, Amberger A, Metzler B, Mayr M, Falkensammer G and Xu Q. (1997) Atherosclerosis, autoimmunity, and vascular-associated lymphoid tissue. FASEB J. 11:1199–1207.*
6. *Pockley AG. (2002) Heat shock proteins, inflammation, and cardiovascular disease. Circulation 105: 1012–1017.*
7. *Zhang X, He M, Cheng L, Chen Y, Zhou L, Zeng H, Pockley AG, Hu FB and Wu T. (2008) Elevated Heat Shock Protein 60 levels are associated with higher risk of coronary heart disease in Chinese.* Circulation *118:2687-2693.*
8. *Xu Q and Wick G. (1996) The role of heat shock proteins in protection and pathophysiology of the arterial wall. Mol Med Today. 2:372–379.*
9. *Roma P and Catapano AL. (1996) Stress proteins and atherosclerosis. Atherosclerosis. 127:147–154.*
10. *Burian K, Kis Z, Virok D, Endresz V, Prohaszka Z, Duba J, Berencsi K, Boda K, Horvath L, Romics L, Fust G and Gonczol E. (2001) Independent and joint effects of antibodies to human heat-shock protein 60 and Chlamydia pneumoniae infection in the development of coronary atherosclerosis. Circulation. 103:1503–1508.*
11. *Zhu J, Quyyumi AA, Rott D, Csako G, Wu H, Halcox J and Epstein SE. (2001)Antibodies to human heat-shock protein 60 are associated with the presence and severity of coronary artery disease: evidence for an autoimmune component of atherogenesis. Circulation 103: 1071–1075.*
12. *Huittinen T, Leinonen M, Tenkanen L, Manttari M, Virkkunen H, Pitkanen T, Wahlstrom E, Palosuo T, Manninen V and Saikku P. (2002) Autoimmunity to human heat shock protein 60, Chlamydia pneumoniae infection, and inflammation in predicting coronary risk. Arterioscler Thromb Vasc Biol. 22:431–437.*
13. *Mollenhauer J and Schulmeister A. (1992) The humoral immune response to heat shock proteins. Experientia. 48:644–649.*
14. *Schattner A and Rager-Zisman B. (1990) Virus-induced autoimmunity. Rev Infect Dis. 12:204–222.*
15. *Feige U and van Eden W. (1996) Infection, autoimmunity and autoimmune disease. EXS. 77:359–373.*
16. *Multhoff G and Botzler C. (1998) Heat-shock proteins and the immune response. Ann N Y Acad Sci. 851:86–93.*
17. *Xu Q, Dietrich H, Steiner HJ, Gown AM, Schoel B, Mikuz G, Kaufmann SH and Wick G. (1992) Induction of arteriosclerosis in normocholesterolemic rabbits by immunization with heat shock protein 65. Arterioscler Thromb 12: 789–799.*
18. *George J, Shoenfeld Y, Afek A, Gilburd B, Keren P, Shaish A, Kopolovic J, Wick G and Harats D. (1999) Enhanced fatty streak formation in C57BL/6J mice by immunization with heat shock protein-65. Arterioscler Thromb Vasc Biol 19: 505–510.*