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ORIGINAL ARTICLE

Assessment of Serum Levels of Oxidative Stress Markers and Heat Shock Proteins in Myocardial Infraction Patients

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ABSTRACT

Introduction: Heat stress proteins also known as shock proteins are the molecular chaperones due to various stimuli response to heat and ischemic injury to the cells. They are important in folding and degradation of other proteins involved in genesis of cardiovascular pathologies.

Objectives: The main aim is to upgrade the role of oxidative markers and these stress proteins not only in development but also in evolution related to heart disease.

Material and methods: Subjects were selected as diagnosed myocardial infarction in Faisalabad institute of cardiology, Faisalabad Division, Punjab. All subjects were further categorized as risk group and non-risk group on the basis of their risk factors history using ACC/AHC 2017 guided criteria for detection of myocardial infarction included chest pain, ECG finding and cardiac enzymes.

Results: Data clearly depicts the demographical and hematological profile i-e complete blood picture of patients with diagnosed myocardial infraction and control subject. Hemoglobin as a highly advanced allosteric protein present in the form of HbA with two α and two β chains while globin chains are synthesized in erythrocytes cytosol.

Conclusion: Despite of increasing evidence from the recent data, it is recommended that functions of these heat shock proteins related to guard cells against programmed apoptosis from stressful aspects e.g. infection, mechanical stress, oxidized low density lipoprotein and oxidants are also known as stress proteins.

Keywords: Stress Proteins, Atherosclerosis, Apoptosis, Myocardial Infarction, Oxidative Markers

INTRODUCTION

Major cardiovascular disease, Atherosclerosis, is a chronic inflammatory disease which causes narrowing of vessels with advanced age. It is multifactorial disease and caused by the deposition of an extracellular matrix with fat plaques in the arteries. It was proposed first time in the 1850s as a gradually progressing disease throughout life and has globally increased mortality rate about 17% [1]. There are a lot of risk factors for AS included age, gender(male), obesity, hypertension, diabetes, smoking, hypercholesterolemia, family history, social lifestyle, nutritional deficiency, UV radiation, OxLDL, hypoxia and biomechanical as well surgical stress [2].

All these factors evoke HSP over-expression in endothelial cells as target cells through the initiation of heat shock transcription factors-1. The target endothelial cells cause detection of many adhesion molecules e.g. E-selectin, T- activated lymphocytes and mature monocytes resulting in the expression of HLA-II antigen class that causes release of several cytokines [3]. Stress proteins consist of more than two dozen proteins which are associated with numerous homologous species sequences e.g. from bacteria such as Escherichia coli to humans. The main stimuli of stress proteins are increased temperature, oxidized LDL (oxLDL), oxidants, free radicals, mechanical and surgical stress, cytokine stimulation and infection under the unfavorable condition. They are formed as auto-antigens in the development of this heart disease [4]. In this way they have a mechanical role in arterial physiology that is exponentially expanded in recent literature research on both the basic science and clinical level. During biochemical stress, HSPs expression is now up-regulated and has a significant role in formation and pathology of heart ischemia [5].

According to physiologically accumulating evidence relevant in vivo systems, Molecular chaperones are highly preserved homologous intracellular non-related proteins present in most cells that help in correct fold of other proteins but they don't participate in final assemblage of other proteins and are called heat shock proteins (HSPs). Ritossa and his colleagues in 1962 discovered them from Drosophila larval salivary gland. They have specific gene proteins in their giant chromosomes that range in size from 150-10 kDa present in most compartments of the cell [6]. They are as follows according to their weight: small HSPs (15-30 kDa), HSP10, HSP60, HSP40, HSP70, HSP110, HSP100 and HSP90 with one member in its family in vitro [7]. The distinction between cognate members or constitutively expressed members (e.g., Hsc70 and HSP90β) of HSP family and their isoforms are subjective due to cell tissue-restricted expression. The small HSP family comprises of HSP27 (15-42kDa), p 20, HSP-B3, MKBP/HSP-A crystalline and α-B-Crystalline [8]. All these HSPs play an essential role in protein folding, protein unfolding and cell survival from damage in response to environmental, physical and chemical stress stimuli. They consist of different multigene groups of functionally associated classes of heat responsive cell proteins depending upon their molecular interaction on tissue and cell regulated expression. Some members of HSP75, HSP60, and HSP10 are mostly present in mitochondria, while others are located in nucleus and cytoplasm under suitable functional settings [9].

Objectives: The main aim is to upgrade the role of oxidative markers and these stress proteins not only in development but also in evolution related to heart disease.

MATERIALS AND METHODS

Ethical consent: All patients were selected and screened at Faisalabad institute of Cardiology, Faisalabad. This research work was permitted by the "Research and Ethics Committee" at the Institute of Molecular Biology and Biotechnology (IMBB), The University of Lahore, Lahore, Pakistan.

Subjects: Subjects were selected as diagnosed myocardial infarction in Faisalabad institute of cardiology, Faisalabad Division, Punjab. All subjects were further categorized as risk group and non-risk group on the basis of their risk factors history using ACC/AHC 2017 guided criteria for detection of myocardial infarction included chest pain, ECG finding and cardiac enzymes.

Inclusion criteria: Inclusive criteria were diagnosed myocardial infarction patients both male and females, age between 40-80 years.

Exclusion criteria: Exclusion criteria was included congenital heart disease patients age less than 35 years and pregnant females.

Laboratory Techniques for Sampling And Testing: A total of 50 diagnosed myocardial infarction patients were selected.

Undergoing routine admission in hospital, on duty cardiologist was examined these selected patients having specific risk factors, life style, previous and family history of any heart disease, type 1 diabetes or other autoimmune diseases, history of any other medication. Five (5) ml blood sample was taken from antecubital vein of each patient. Blood ampules were collected into EDTA vial. Blood was centrifuged at 4000rpm for 10 minutes. Serum was separated and stored at -80ºC until tested. The concentration of heat shock proteins 60,27,90 were detected by an ELISA diagnostic kit. Intra and inter-assay variability coefficient value (CV) was obtained.

Measurement of Sod (Superoxide Dismutase Enzymes): SOD was estimated by spectrophotometric technique (Zhu et al 2020.) Took 100µl serum to measure Superoxide dismutase (SOD) activity in test tube. About 1.2ml of 0.052M solution of sodium phosphate buffer having pH 8.3 was added. Then Added 100µl of phenazine methosulphate (186µm), 300 µl of nitro blue tetrazolium (300µm) and 200µl of NADH (750µm) in the test tube. When NADH was added, the reaction occur immediately which is cultivation at 30^0C for at least 90 sec. As we added 100µl of glacial acetic acid, the reaction was stopped and this reaction mixture was moved dynamically with 4.0ml of n-butanol. Kept this reaction mixture to stand for 10 min then centrifuged to separate the butanol layer. The butanol layer has chromogen with color intensity was measured at 560 nm against 4ml of n-butanol and units/g is used to labelled the concentration of SOD in this sample and thus compare absorbance values with a standard curve generated by this original SOD.

Estimation of Malanodialdehyde (MDA): MDA was determined with the help of spectrophotometric process (Fang et al., 2021) in which absorbance was taken at 532nm. 200µl sample was poured in the test tubes with 1.5 mL of 20% acetic acid solution. Then added about 200 µl of 8.1% sodium dodecyl sulfate and 1.5 mL of 0.8% TBA to make a mixture up to 4.0mL with distilled water. This mixture was heated at 90◦C for 60 min in a water bath. With the help of tap water, this mixture was cooled and was added about 1.0mL of distilled water and 5.0 mL of n-butanol in it and stirred dynamically and centrifuged for 10-minute at 4000 rpm to make its upper butanol. This layer was poured in the tube and noted its absorbance at 532 nm.

Measurement of Catalase (CAT): It is an antioxidant and was estimated with the help of spectrophotometric technique of Wang.et al., 2019. At 240nm the absorption of H_2O_2 was determined as enzyme catalase degraded its absorbance of catalase. The enzyme activity was calculated when its absorption decreased. 100µl serum and 50mM phosphate buffer about 1.9mL having pH 7.0 was added into test tube. When 1mL of newly prepared 30mM H_2O_2 added, the response was produced with the breakdown of H_2O_2. This value was calculated at 240nm by above mention technique and was labelled as U/mg.

Estimation of Gsh-Reduced: GSH-Red was measured when glutathione reacts with 5, 5'-dithiobis nitro benzoic acid which gave a yellow colored product at absorbance of 412nm. About 1ml with 0.2M sodium phosphate buffer having pH 8.0 was taken to make 0.1ml supernatant. We had to prepare the concentration of Standard GSH in between 2 and 10nmoles. Freshly prepared above mention acid solution was added about 2ml.A yellow color solution was produced which intensity was measured with the help of spectrophotometric technique for 10 minutes at 412nm. By this method the calculated GSH is the sum of oxidized (GSSG) and reduced (GSH) form of glutathione was taken as n-moles GSH/g sample.). The amount or concentration of unknown sample could be measured by using the direct equation produced from this GSH (Valerio et al.,2019)

Estimation of Advanced Oxidation Protein Products: This stress marker AOPP was estimated and determined by the protocol in which samples were diluted with 300 µL; 0–100 µM chloramine T followed by 2 min after the addition of 30 µL glacial acetic acid the precipitate was centrifuged at 5800 g for 5 minutes. The supernatant was taken in separate tube and its absorbance noted was at 595 nm at which wavelength does not absorb. (Rasool et al.,2019)

Estimation of HSP-27, HSP60 and HSP90 Via Elisa Kit: ELISA of anti HSP27, 60 and 90 were performed according to Muhammad. T et al.,2021. Briefly the concentration of these antibodies was measured in human serum sample by following the manufacturer's instructions using ELISA kit (bio-vendor). The blood of diagnosed myocardial infraction patients and control were collected at room temperature. All the samples were undergoing in centrifugation at 3000 g for about 10 min at 4ºC to collect their serum and kept at -80ºC for subsequent assay. The concentration of antiHSP27, 60 and 90 was determined in triplicate. The antibodies content was calculated using the formula mention in kit.

Statistical Analysis: The data was collected and analyzed using SPSS version 19. All the values were expressed in mean and standard deviation.

RESULTS

Table 1 clearly depicts the demographical and hematological profile i-e complete blood picture of patients with diagnosed myocardial infraction and control subject. Hemoglobin as a highly advanced allosteric protein present in the form of HbA with two α and two β chains while globin chains are synthesized in erythrocytes cytosol. It helps to transport oxygen and carbon dioxide between the lungs and tissues. Low concentration of Hb <10mg/dl indicates anemia and were present in myocardial infraction patients as compared to control subjects.

Table 1: Demographic and Hematological Profile of Myocardial Infraction Patients

Variable	Control cases	Patients
Hb (g/dl)	15.2±1.26	10.4±2.28
Hematocrit (%)	37±6.33	26±4.25
MCV (fl)	81±9.65	87.9±16.35
MCH (Pg)	33±8.259	35.1±6.35
RBCs (million/mm³)	4.5±1.09	2.95±0.265
WBCs (million/mm³)	9.4±2.09	8.3±3.29
PLTs (10^9/L)	240±23.10	218±15.26
Neutrophils (%)	75±14.26	70±8.59
Lymphocytes (%)	21±3.26	25±3.29
Monocytes (%)	4±0.266	1±1.05
Eosinophils (%)	2±0.226	1±0.002
MCHC (g/dl)	40±4.59	31.9±3.29

The data compiled in table 02 suggested that the oxidative stress profile in the myocardial infraction patients. These oxidative stress markers have an important role in the pathogenesis of this disease. MDA is the oxidative stress marker that occur in myocytes. Superoxide dismutase SOD are catalyzed the dismutation of superoxide to convert it into oxygen and hydrogen peroxide which are important antioxidant in nearly all cells. Glutathione causes the conversion of oxidized glutathione into its reduced form using substrate as NADPH which is direct measure of enzyme activity. The values of GRx and GPx were also reduced in myocardial infarction patients in contrary to healthy individuals.

Table 2: Profile of Circulating Stress Biomarkers in the Myocardial Infraction Patients

Variable	Control cases	Patients
MDA (nmol/ml)	0.96±0.012	6.35±1.127
SOD (U/gHb)	1.292±0.033	0.0659±0.01255
GSH-PX (µmol/ml)	0.625±0.23	0.0125±0.00125
Red GSH (µmol/L)	9.356±2.29	3.0223±1.056
CAT(U/gHb)	3.2658±0.951	1.056±1.0325
NO (µmol/L)	15.29±3.29	39.35±5.29
OPP (nmol/ml)	0.696±0.156	0.1265±0.0019

The data compiled in table 03 suggested that heat shock proteins profile in the patients suffering from myocardial infraction. HSP 27 is a large oligomer in the cell and tissues. HSP60 forms a heter-oligomeric protein TCPI complex takes part in mononuclear

cell infiltration and are expressed by T-lymphocytes, endothelial cells, smooth muscle cells and mature macrophages. HSP90 as a component of receptor significantly glucocorticoids bind to the protein kinase and steroid receptors is assembled in a complex of several proteins which help them to protect against apoptosis caused by oxidative stress markers. Significantly a high level of HSP90 were also observed in myocardial infraction patients in comparison with control healthy individuals.

Table 3: Level of Heat Shock Proteins in Myocardial Infraction Patients

Variable	Control cases	Patients
HSP27 (pg/ml)	92.65±8.29	256.26±15.26
HSP60 (pg/ml)	2.156±0.2526	7.598±1.255
HSP90 (pg/ml)	16.35±3.29	47.2599±4.25

DISCUSSION

HSPs have a large family of intracellular proteins which are name after their discovery in responds to heat to protect against certain alarming condition. They are known as molecular chaperones. Some of the HSPs have secondary functions. Traditional their nomenclature designated by their respective molecular weight [10]. Atherosclerosis is a serious slow but steady progressing disease that occurs due to various chronic inflammatory processes in the intima of the arteries stimulated by different stressed factors and cause narrowing of the vessel with life threatening offence to the heart [11]. The chronic inflammation generally terminates in the formation of a plaque depriving the heart muscle of adequate blood flow and sufficient oxygen resulting cardiac ischemia [12].

However, coronary artery occlusion can occur by sudden rupture of the plaque, causing a myocardial infarction. HSP27 is a relevant inhibitor of the apoptotic intracellular pathway and interact with pro-apoptotic components such as the caspase pathway [13]. It also exerts antioxidant activity to lower ROS levels by increasing the intracellular glutathione level and by decreasing the intracellular level of iron [14]. Additionally, it contributes to decreased low-density lipoprotein (LDL) oxidative modification to compete with oxLDL uptake by macrophages that show their protective role in this disease. Keezer et al. demonstrated that this protein inhibits proliferation and migration of endothelial cell stimulated via endostatin and thrombospondin-1 [15]. Furthermore, this protein promotes the production of anti-inflammatory cytokines by target cells and inhibits TLR4 expression and their differentiation into dendritic cells [16]. Park et al. observed their expression in human atherosclerotic plaques occurs in myocardial infarction patients compared to that in control vessels [17]. Lebherz-Eichinger et al. performed a study which revealed increased serum levels of HSP27 in patients of myocardial infarction as compared to the levels in healthy controls. A high concentration of serum HSP27 was positively correlated with age and C reactive protein (CRP) [18]. Jaroszynski et al. demonstrated that lower serum levels of HSP27 in cardiovascular patients with carotid atherosclerosis and oxidative stress is independent with sudden cardiac death (SCD). Based on these results, this protein acts as linking molecules inducing cardiovascular mortality [19].

The bacterial and human HSP60 is present in a wide collection of organisms, including fungi, plants, bacteria, and mammals because they have many homologies between species exists. In order to study the function of HSP60 in cell biology, their structure similarities are very helpful. Several prototypes used give a knowledge about HSP60 which include bacterial homologues such as E. coli GroEL, Chlamydial HSP60GroEL, Mycobacterium HSP65, Aspergillus, Candidal and Histoplasma of fungi. HSP60 is mainly situated inside the mitochondria help in protein-folding and preventing the aggregation of misfolded polypeptide chains while supporting their refolding [20-21]. Some are located within the organelle while other are extramitochondrial like cytosol, endoplasmic reticulum, and nucleus where it offers its chaperonin activity. Kampinga et al. designated HSP60 with the name of HSPD1but not popularly used. Due to this exceptional feature of exosomal HSP60, it is used as a prognostic marker for many other diseases. However, the fate of these vesicles is still fully understood [22-23].

CONCLUSION

Despite of increasing evidence from the recent data, it is recommended that functions of these heat shock proteins related to guard cells against programmed apoptosis from stressful aspects e.g. infection, mechanical stress, oxidized low density lipoprotein and oxidants are also known as stress proteins. They result in the release of these proteins into intracellular space to make them soluble heat proteins to which bind TLR4/CD receptors. So, proliferation of endothelial adhesion molecules and cardiac smooth muscle cell causes the release of cytokines but macrophages presenting antigens attach to T and B lymphocytes. In this way, they produce auto-antibodies and auto reactive cells against these proteins that contribute in development and progression of myocardial infarction. HSP27 has diagnostic and multidimensional therapeutic capacities.

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