Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/jptcp.v27i3.5444

EVALUATION OF HEAT SHOCK PROTEINS AND OXIDATIVE STRESS MARKERS IN SERUM IN PATIENTS WITH MYOCARDIAL INFRACTION

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Abstract

Background: Atherosclerosis, a chronic inflammatory disease, leads to arterial narrowing and cardiovascular events like myocardial infarction. Heat shock proteins (HSPs) are implicated in cellular response to stress, including oxidative damage. Understanding the roles of HSPs in atherosclerosis and myocardial infarction could offer insights into disease mechanisms and potential therapeutic targets.

Objectives: The objectives of this study are to investigate the association between heat shock proteins (HSPs) and cardiovascular diseases, particularly atherosclerosis and myocardial infarction. This includes examining the role of HSPs in oxidative stress, inflammation, and cellular protection, with potential implications for diagnosis and therapeutic interventions.

Material and methods: Patients diagnosed with myocardial infarction were selected from January 2018 to January 2019 at Bacha Khan Medical College Mardan's Biochemistry department. Inclusion criteria comprised individuals aged 35 to 75, diagnosed with myocardial infarction. Exclusion criteria included congenital heart disease and pregnancy. Blood samples were collected and analyzed using various techniques, including ELISA for HSPs and spectrophotometry for antioxidant enzymes.

Results: Demographic and hematological profiles of myocardial infarction patients and control subjects are presented. Circulating stress biomarkers and levels of heat shock proteins in myocardial infarction patients are also reported.

Conclusion: Heat shock proteins, including HSP27, HSP60, and HSP90, play critical roles in cardiovascular diseases such as atherosclerosis and myocardial infarction. Understanding their functions and levels in patients may provide valuable insights for diagnosis and treatment.

Keywords: Atherosclerosis, Myocardial infarction, Heat shock proteins, Cardiovascular diseases.

Introduction

Atherosclerosis, a complex and chronic inflammatory condition, stands as a predominant etiological factor in cardiovascular diseases, significantly impacting global health outcomes. As described by Ross in 1999, atherosclerosis is characterized by the gradual accumulation of lipid-rich plaques within arterial walls, leading to luminal narrowing and compromised blood flow, ultimately predisposing individuals to severe clinical manifestations such as myocardial infarction (MI) and stroke [1]. The multifaceted nature of atherosclerosis involves intricate interplay between various cellular and molecular mechanisms, contributing to its progression and clinical sequelae. Heat shock proteins (HSPs), a family of highly conserved intracellular proteins, have emerged as crucial players in the context of cardiovascular pathology, particularly atherosclerosis and MI. Initially discovered in Drosophila larvae by Ritossa and colleagues in 1965 [2], HSPs have since garnered significant attention for their diverse roles in cellular homeostasis, stress response, and disease pathogenesis. With numerous members identified, including HSP27, HSP60, and HSP90, these proteins exert profound effects on cellular function under physiological and pathological conditions [3]. The involvement of HSPs in atherosclerosis and MI pathophysiology stems from their ability to modulate key processes such as inflammation, oxidative stress, and protein folding. For instance, HSP27, also known as heat shock protein beta-1 (HSPB1), has been implicated in the regulation of apoptosis, endothelial function, and vascular remodeling, thus influencing atherosclerotic plaque stability and susceptibility to rupture [4]. Additionally, HSP60, predominantly localized within mitochondria, plays a pivotal role in protein folding and has been implicated in endothelial dysfunction and immune activation within atherosclerotic lesions [5]. Furthermore, HSP90, a molecular chaperone involved in protein folding and stabilization, has been shown to regulate vascular inflammation and endothelial nitric oxide synthase (eNOS) activity, thereby influencing vascular tone and atherogenesis [6]. Several studies have underscored the significance of HSPs in cardiovascular diseases, providing insights into their diagnostic and therapeutic potential. Lebherz-Eichinger et al. demonstrated elevated blood levels of HSP27 in MI patients compared to healthy controls, suggesting its potential utility as a diagnostic biomarker [7]. Moreover, experimental studies have explored the therapeutic implications of targeting HSPs in cardiovascular diseases. Der Sarkissian et al. elucidated the cardioprotective effects of HSP90 inhibition in ischemic injury, highlighting the therapeutic potential of targeting HSPs in cardiovascular pathologies [8]. In light of the growing body of evidence implicating HSPs in atherosclerosis and MI, there is a compelling need for comprehensive understanding of their roles and therapeutic implications. This review aims to elucidate the intricate interplay between HSPs and cardiovascular diseases, synthesizing current evidence and exploring avenues for future research and therapeutic interventions.

MATERIALS AND METHODS

From January 2018 to January 2019, all patients were chosen and tested at Bacha Khan Medical College Mardan's Biochemistry department. Subjects were chosen based on the diagnosis of myocardial infarction at the cardiology department. Based on their history of risk factors, all participants were further divided into risk groups and non-risk groups using the ACC/AHC 2018 guideline criteria for myocardial infarction diagnosis, which included cardiac enzymes, chest discomfort, and ECG findings.

Inclusion criteria: Patients with a diagnosis of myocardial infarction, both male and female, and ages 35 to 75, met the inclusive criteria.

Exclusion criteria: Patients with congenital cardiac disease under the age of thirty and expectant women met the exclusion criteria..

Methods for Sampling and Testing in the Laboratory: fifty individuals with a diagnosis of myocardial infarction were chosen.

When these patients were admitted to the hospital on a regular basis, the on-call cardiologist looked over them because they had certain risk factors, a certain lifestyle, a family history of heart disease, type 1 diabetes, or other autoimmune disorders, and a history of using any other medications. Every patient had a five milliliter (liter) blood sample drawn from the antecubital vein. Blood ampules were collected and placed in EDTA vials. For eight minutes, blood was centrifuged at 3850 rpm. Before being examined, serum was isolated and kept at -78^{o}C. Using an ELISA diagnostic kit, the concentration of heat shock proteins 60, 27, and 90 was found. The variability coefficient value (CV) across and within assays was determined.

Superoxide Dismutase Enzyme (SOD) measurement:
SOD was evaluated by spectrophotometric technique (Zhu et al 2018.) Superoxide dismutase (SOD) activity was measured in a test tube with 100µl of serum. About 1.2 milliliters of a 0.052M sodium phosphate buffer solution with a pH of 8.3 was added. The test tube was then filled with 200µl of NADH (750µm), 300µl of nitro blue tetrazolium (300µm), and 100µl of phenazine methosulphate (186µm). As soon as NADH was introduced, the reaction began, and it ran at 300C for at least 90 seconds. After adding 100µl of glacial acetic acid, the reaction was stopped. The reaction mixture was then moved dynamically with the addition of 4.0 ml of n-butanol. Centrifugation was used to separate the butanol layer from the reaction mixture after it had rested for 10 minutes. The concentration of SOD in this sample is shown in units/g, and the absorbance data is compared to a standard curve made by this original SOD. Chromogen, which is present in the butanol layer, was quantified at 560 nm in relation to 4 milliliters of n-butanol.

Analyzing Malanodialdehyde (MDA): Malanodialdehyde (MDA) analysis was done using a spectrophotometric method (Fang et al., 2021), which measured absorbance at 532 nm. A 200µl sample and 1.5 mL of a 20% acetic acid solution were added to the test tubes. Next, around 200 µl of 8.1% sodium dodecyl sulfate and 1.5 mL of 0.8% TBA were added, and the mixture was then topped up with 4.0 mL of distilled water. This combination was cooked in a water bath for 60 minutes at 90°C. This combination was chilled with the aid of tap water, and then about 1.0 mL of distilled water and 5.0 mL of n-butanol were added. It was then vigorously agitated and centrifuged for 10 minutes at 3850 rpm to produce its upper butanol. After pouring this layer into the tube, the absorbance at 532 nm was measured.

Calculating the Catalase (CAT) level: Its antioxidant value was calculated using Wang et al.'s 2019 spectrophotometric approach. The absorption of $H2O_2$ was measured at 240 nm because the catalase enzyme reduced the absorbance of catalase. When the enzyme's absorption dropped, its activity was estimated. A test tube containing 100µl serum and 1.9mL of 50mM phosphate buffer with a pH of 7.0 was filled. H2O2 broke down in response to the addition of 1 mL of freshly made 30 mM H2O2. Using the previously mentioned method, this value was determined at 240 nm and given the label U/mg.

Calculating the Gsh-Reduced: When glutathione combines with 5, 5'-dithiobis nitro benzoic acid, a yellow-colored product is produced with an absorbance of 412 nm, and this reaction is used to assess GSH-Red. To create 0.1ml of supernatant, around 1ml of 0.2M sodium phosphate buffer with a pH of 8.0 was obtained. The concentration of Standard GSH, which ranges from 2 to 10 nmoles, has to be prepared. About 2 milliliters of the freshly made acid solution mentioned above was added.A yellow color solution was created, and its intensity was measured for ten minutes at 412 nm using the spectrophotometric method. This approach uses the oxidized (GSSG) and reduced (GSH) forms of glutathione to compute GSH, which is expressed as n-moles GSH/g of material. The direct equation derived from this GSH might be used to estimate the quantity or concentration of the unknown sample (Valerio et al., 2019).

Calculating the Products of Advanced Oxidation Proteins: The methodology used to estimate and quantify the stress marker AOPP included diluting the samples with 300 µL of 0-100 µM chloramine T,

waiting two minutes after adding 30 µL of glacial acetic acid, then centrifuging the precipitate at 5800 g for five minutes. After being taken in a different tube, the supernatant's absorbance was measured at 595 nm, the wavelength at which it does not absorb. (Et al., Rasool, 2019)

HSP-27, HSP-60, and HSP90 Estimation Using Elisa Kit: Anti-HSP27, 60, and 90 ELISA assays were conducted in accordance with Muhammad T et al., 2021. In a nutshell, the ELISA kit (bio-vendor) was used to assess the concentration of these antibodies in a human serum sample in accordance with the manufacturer's instructions. Blood was drawn at room temperature from individuals with confirmed myocardial infractions as well as a control group. To extract serum, all samples were centrifuged at 3000 g for about 10 min at 4°C. The serum was then stored at -80°C for the test that followed. Three duplicate measurements of the antiHSP27, 60, and 90 concentrations were made. The kit's formula was used to determine the amount of antibodies. Analytical Statistics: Data was gathered and SPSS version 19 was used for analysis. Each and every value was stated using the mean and standard deviation.

RESULTS

The demographic and hematological profile, or whole blood profile, of patients with a diagnosis of myocardial infraction and the control subject are clearly shown in Table 1. Hemoglobin is a highly developed allosteric protein that is generated in the cytoplasm of erythrocytes and is present as HbA, which has two α and two β chains. It facilitates the movement of carbon dioxide and oxygen between the tissues and the lungs. Anaemia is indicated by a low concentration of Hb <10 mg/dl, which was seen in individuals with myocardial infraction compared to control participants.

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

Table 2: Circulating Stress Biomarker Profile in Patients with Myocardial Infraction

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

Table 3: Heat Shock Protein Level in Patients with Myocardial Infraction

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

The primary outcome of our research focused on elucidating the association between heat shock proteins (HSPs) and cardiovascular diseases, particularly atherosclerosis and myocardial infarction (MI). Through comprehensive analysis, we aimed to understand the roles of HSPs in disease pathogenesis, their diagnostic utility, and therapeutic potential. Our study involved evaluating circulating levels of HSPs, including HSP27, HSP60, and HSP90, in patients with myocardial infarction and comparing these levels with those of healthy controls. Our findings revealed significant alterations in HSP levels among patients with myocardial infarction compared to healthy controls. Specifically, we observed elevated levels of HSP27 in MI patients, consistent with previous studies suggesting its role as a diagnostic biomarker [9]. Additionally, our study demonstrated increased expression of HSP60 and HSP90 in MI patients, implicating these proteins in the pathophysiology of atherosclerosis and cardiovascular events [10]. Comparing our findings with previous studies, several key observations emerge. reported elevated blood levels of HSP27 in MI patients compared to healthy controls, corroborating our findings and underscoring the diagnostic potential of HSP27 in cardiovascular diseases [11]. Moreover, our study aligns with research which highlighted the role of HSP27 in regulating apoptosis and endothelial function, thus influencing atherosclerotic plaque stability [12]. Similarly elucidated the involvement of HSP60 and HSP90 in endothelial dysfunction and vascular inflammation, supporting our findings of increased expression of these proteins in MI patients [13].Furthermore, our study corroborates the therapeutic implications of targeting HSPs in cardiovascular diseases, as demonstrated Their research elucidated the cardio protective effects of HSP90 inhibition in ischemic injury, highlighting the therapeutic potential of modulating HSPs in cardiovascular pathologies [14]. These findings underscore the multifaceted roles of HSPs in cardiovascular diseases and emphasize the need for further research to explore their therapeutic applications. In addition to diagnostic and therapeutic implications, our study sheds light on the mechanistic pathways through which HSPs contribute to cardiovascular pathology. Specifically, our findings suggest that HSP27 may modulate apoptosis and endothelial function, while HSP60 and HSP90 may influence endothelial dysfunction and vascular inflammation [15]. These mechanistic insights provide a deeper understanding of the complex interplay between HSPs and cardiovascular diseases, paving the way for targeted therapeutic interventions. Moreover, our study underscores the potential of HSPs as biomarkers for cardiovascular risk stratification and prognosis. Elevated levels of HSPs, particularly HSP27, HSP60, and HSP90, may serve as indicators of underlying vascular pathology and disease severity. By integrating HSP measurements into clinical practice, healthcare providers can enhance risk assessment and tailor treatment strategies for patients with cardiovascular diseases[16]. However, despite the promising diagnostic and therapeutic potential of HSPs, several challenges remain. The complexity of HSP-mediated pathways and their interactions with other molecular pathways pose challenges for targeted therapeutic interventions. Additionally, standardization of HSP assays and interpretation of circulating levels require further refinement to ensure reproducibility and clinical utility[17].In conclusion, our study elucidates the significant association between HSPs and cardiovascular diseases, particularly atherosclerosis and myocardial infarction. By demonstrating altered levels of HSPs in MI patients and highlighting their diagnostic and therapeutic implications, our findings contribute to the growing body of evidence on the role of HSPs in cardiovascular pathology. Future research efforts should focus on unraveling the mechanistic pathways underlying HSP-mediated cardiovascular damage and exploring novel therapeutic strategies targeting HSPs[18].

CONCLUSION

this study sheds light on the intricate roles of heat shock proteins (HSPs) in cardiovascular diseases, emphasizing their potential as diagnostic biomarkers and therapeutic targets. Further research in this area holds promise for the development of novel strategies for the diagnosis, prevention, and treatment of cardiovascular diseases.

Acknowledgement: We would like to thank the hospitals administration and everyone who helped us complete this study.

Disclaimer: Nil

Conflict of Interest: There is no conflict of interest.

Funding Disclosure: Nil

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