Short Communication

Iranian Journal of Immunology

https://iji.sums.ac.ir

Elevated HSP70 and HSP90 as Predictive Markers of Immune Activation and Lung Injury in SARS-COV-2 Disease

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ABSTRACT

Background: Heat shock proteins (HSPs) are involved in innate and adaptive immune responses, especially inflammatory responses due to immune cell activation. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was one of the most important causes of death in the recent pandemic. Increased cellular stress and excessive inflammation are common in coronavirus disease-19 (COVID-19), although the underlying mechanisms are still poorly understood.

Objective: To evaluate the relationship between HSP and the pathological effects of COVID-19.

Methods: A group of 107 patients was categorized to two populations (mild and severe) based on their chest high-resolution computed tomography (HRCT) results. The HSP70, HSP90 alpha, and serum levels of C-reactive protein (CRP) were measured by enzyme-linked immunosorbent assay (ELISA). Lactate dehydrogenase (LDH), and creatine phosphokinase (CPK) were measured by the automated analyzer.

Results: Our data showed increased levels of HSP70 and HSP90 in patients with COVID-19. The HSPs levels were elevated in the severe group compared to the mild group. This study demonstrated a positive correlation between both elevated levels of HSP70, HSP90, and HRCT grade and also a positive correlation with CRP and CPK in the severe group.

Conclusion: HSP90 and HSP70 contribute to excessive immune responses and cytokine storms. They may serve as prognostic serum markers for COVID-19 lung injury. Additionally, they are candidates for anti-inflammatory therapy.

Keywords: COVID-19, C-Reactive Protein, Heat Shock Proteins, Inflammation, Pulmonary

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Cite this article as:
Zangeneh Z, Khamisipour GR. Elevated HSP70 and HSP90 as Predictive Markers of Immune Activation and Lung Injury in SARS-COV-2 Disease. *Iran J Immunol.* 2023; 20(3):368-373, doi: 10.22034/iji.2023.97202.2488.

Received: 2022-11-07
Revised: 2023-01-24
Accepted: 2023-02-13

INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a transformative airborne respiratory virus identified as coronavirus disease 2019 (COVID-19). The rapid spread of the novel coronavirus from its outbreak in China to a life-threatening pandemic is a serious public health alarm around the world (1). The most important clinical manifestation of this disease is pneumonia, but there is a spectrum of asymptomatic, mild, and moderate-to-severe pneumonia (2, 3). An immune response is activated to fight this viral infection, but an exaggerated immune response, including a cytokine storm, associated with interleukin-1 (IL-1), IL-6, and tumor necrosis factor-α (TNF-α) leads to the rapid onset of acute respiratory distress syndrome (ARDS) and multiple organ failure (4). Recent studies in humans have displayed that heat shock proteins (HSPs) contribute to anti-inflammatory immune responses to infectious agents (5). HSPs are a conserved set of proteins known as stress response proteins and are expressed in all humans and microorganisms. (6). HSPs are categorized into six main families, including small HSPs (sHSP27), HSP40, HSP60, HSP70, HSP90, and large HSPs of different molecular weights (6, 7). Exposure to a range of factors, including microbial stimuli, oxidants, nutrients, trauma, and host immune reactions, induces host-specific HSP expression and activity (5-7). The intracellular functions of HSPs are the maintenance of modified proteins, controlling the folding and unfolding process of proteins, and regulation of apoptosis by preventing the activation of caspase cascades into the cell (6). HSPs are involved in the response to pathogens and play roles in cellular homeostasis as well as in the viral capsid protein folding process (8). In addition to their intracellular role, HSPs are released from cells upon trauma, anxiety, or infection and serve as potent mediators of the immune response (9). Recent studies have revealed that some HSPs play important parts in beginning innate and adaptive immune reactions. These proteins contribute to the inflammatory response by activating natural killer cells (NK cells), dendritic cells (DC), and macrophages (10). HSP70 and HSP90 play important roles in stimulating immune responses to viral infections. This is because viruses do not have HSP and depend on the host HSPs for viral protein arrangement (7). Extracellular heat shock protein 70 (HSP70) can be overexpressed upon exposure to cellular stress, inflammation, infection, and cancer and is known to induce immune responses. In innate immunity, HSP70 plays a role as a damage-associated molecular pattern (DAMP), binds toll-like receptor 4 (TLR-4), and leads to release the pro-inflammatory cytokines such as intrleukin-6, TNF-α, and IL-1 from macrophages and Dcs (11). High levels of HSP70 are associated with poor prognosis in some cancers, as well as inflammatory diseases. (11). In addition, serum HSP90, involved in central cellular procedures such as DNA repair, development, and immune response, is known to contribute to the severity of infections and cancer development (12). Therefore, in this study, we aimed to identify the relationship between serum levels of HSP70 and HSP90 and lung injury based on the results of chest high-resolution computed tomography (HRCT) in COVID-19 patients.

MATERIALS AND METHODS

This cross-sectional study was approved by the University's Ethics Board (IR.BPUMS.REC.1399.095) and conducted by informed agreement in hospitalized COVID-19 patients. The group of 107 patients hospitalized with the confirmed disease was determined after validation by (viral nucleic acid extraction kit, Begen, Iran, COVID-19 one-step real-time PCR kit, Genova) from July to September 2021. The patients were separated into two collections based on HRCT scans of the chest (lung lesions percent): the mild group (1-50%, n=57) and the severe group (51-100%,

n=50). Chest HRCT examination results are derived from patients' documents, reviewed, and evaluated by experienced radiologists. Thanks to Jiang et al. for their contributions in the above classification (13).

The inclusion criteria for this project were the first-time disease with no metabolic disorders, COVID-19 vaccination, and therapies. A blood sample was obtained from the patient and the following laboratory tests were performed: C-reactive protein (CRP), lactate dehydrogenase (LDH), HSP-70, HSP-90, creatine phosphokinase (CPK) in serum, and complete blood count (CBC).

This study was performed in line with the agreement of the University's Ethics Board (IR.BPUMS.REC.1399/095), and with an established agreement procedure. The results from the patients are anonymous for us and the publication.

ELISA Method

Serum amounts of HSP70 (Human HSP70: Catalog Number: DY1663), and HSP90 (Human HSP90: Catalog No. SKT-107) were assayed by ELISA for all the patients. Serum CRP, CPK, and LDH levels were measured by quantifiable approaches (Biorex Fars, Iran) with an automated analyzer (Biotecnica BT3000) and CBC was measured with a hematology instrument (Sysmex, XP300). For a better explanation, blood factors, lymphocytes percentage, and neutrophils count are calculated and converted to absolute counts for statistical purposes. White blood cell (WBC) count, except the lymphocyte population, are considered neutrophils for easy interpretation in this study.

Statistical Analysis

The SPSS statistics software was used analyze the collected data. The normality was checked and evaluated by the Shapiro–Wilk test of normality. An independent t-test was used to compare the mean between the groups. The Spearman correlation coefficient test was used to evaluate the possible correlation. Significant differences were considered for the tests with a p value<0.05.

RESULTS

WBC Counts Significantly Reduced in the Severe Population Compared with the Mild One

The percentage of lymphocytes in the severe population (p<0.001) decreased compared with the mild one. The increased neutrophil (p=0.04) and lymphocyte count (p=0.1) were not statistically supported between these groups. The mean platelet count was lower in the severe population, but the difference was not statistically significant compared with the mild group (p=0.62). In addition, comparing the percentage of neutrophil to lymphocyte count (N/L ratio), the severe population presented a higher N/L ratio (p=0.005). The levels of serum LDH and CRP were augmented in the severe populations (LDH p<0.001, CRP p=0.003, Table 1).

Serum Levels of HSP90 and HSP70 in COVID-19 Patients in Mild and Severe Populations

The serum levels of HSP70 and HSP90 were significantly elevated in the severe group compared to the mild group (p=0.008, p=0.020, respectively) (Table 2).

Positive Correlations between HSP90 and HSP70 Levels Associated with Acute Inflammation and Impaired Lung Function

In the severe groups of COVID-19 patients, serum HSP90 and HSP70 positively correlated with CRP and serum CPK concentrations (Table 3). In the severe group, serum HSP90 and 70 positively correlated with the severity of lung involvement based on chest HRCT whereas in the mild group no significant correlation was found. Although serum LDH concentration increased in both groups, there was not a significantly positive correlation in HSP90 and HSP70 with serum levels of LDH.

Table 1. The CBC test results in COVID-19 patients obtained from a mild and severe group at the University Hospital in July-September 2021.

	Mild group (n=54 Female: 23, Male: 31)	Severe group (n=50 Female: 24, Male: 26)	*p*-value
Age	48.4±7.7	51.82±7.9	0.18
WBC $\times 10^3/\mu L$	9.14±4.3	7.36±3.6	0.048
Lymphocyte percentage	21.51±10	13.33±8.8	<0.001
Lymphocyte $\times 10^3/\mu L$	1.38±0.74	1.08±0.73	0.1
Neutrophil $\times 10^3/\mu L$	5.94±3.4	7.84±4.2	0.04
Platelet $\times 10^3/\mu L$	268.8±169.7	243.8±81.5	0.62
Neutrophil/lymphocyte ratio	4.3±1.28	7.25±2.3	0.005
CRP (mg/l)	35.96±21.4	72.2±56.9	0.003
LDH (IU/L)	365.6±110.3	639.5±275.7	<0.001

The data are shown as the mean±SD. White Blood Cell (WBC), Lactate Dehydrogenase (LDH), C-Reactive Protein (CRP)

Table 2. Comparison of serum HSP70 and serum HSP90 in the mild and severe group

Group	Serum HSP70		Serum HSP90	
	Mean±SE	*p*-value	Mean±SE	*p*-value
Mild	0.57±0.13	0.008	4.57±0.53	0.020
Severe	1.43±0.24		6.68±0.50	

Heat Shock Protein (HSP)

Table 3. Correlations of HSP90 and HSP70 with CRP and CPK in patients with COVID-19 in the severe group

Correlation	HSP90	HSP70
CRP	r=0.68 *p*<0.001	r=0.65 *p*<0.001
CPK	r=0.52 *p*<0.003	r=0.41 *p*<0.023

Heat Shock Protein (HSP); Creatine Phosphokinase (CPK); C-Reactive Protein (CRP)

DISCUSSION

Patients suffering from SARS-CoV-2 infection develop pulmonary inflammation and sometimes devastating multiple organ failure (1). The activation of innate and adaptive immune responses, including neutrophil, macrophage, and lymphocyte function, and their pro-inflammatory cytokines are involved in lung tissue destruction (14). Enhanced stimulation of inflammatory signals such as viral infections and increased cell turnover leads to the upregulation and production of intracellular HSPs into the extracellular environment (6, 15). Significant increases in HSP70 and HSP90 levels were shown in the sera of the patients suffering from COVID-19. Moreover, the serum levels of HSP70 and HSP90 significantly increased in the severe group compared with the mild group. Elevated serum levels of HSP70 and HSP90 expressively correlated with pathological conditions such as HRCT results (lung lesions), the length of hospital stay, serum CRP, LDH, and CPK concentrations. The main finding of this work is the association between HSP70 and HSP90 with the severity of the COVID-19. Recent studies have shown that extracellular HSP70 initiates inflammatory responses with a significant pro-inflammatory cytokine induction primarily through toll-like

receptor binding to the immune cells such as macrophages, neutrophils, and DCs (16). Several recent studies have reported that HSPs, mainly HSP70 and HSP90, contribute to the overproduction of pro-inflammatory cytokines (IL-6 and TNF-α) by immune cells, and are distinguished predictors of cancer and inflammatory disease severity (17). In this regard, previous studies on COVID-19 showed higher amounts of serum TNF-α and induced nitric oxide synthase (iNOS) in intensive care unit (ICU) patients compared with those outside the ICU (18). HSP90 is revealed to be overexpressed in the injured lungs of patients with COVID-19 (19). HSP90 inhibitors could prevent repairing pulmonary microvascular endothelial dysfunction induced by SARS-CoV-2 spike protein. The recent works on SARS-CoV-2 showed that inflammatory responses reduced due to chemical inhibition of HSP90 activity (20). In patients with COVID-19, HSP90 inhibitor drug caused reduction in coronavirus replication (21, 22). Extracellular HSP70 has inflammatory properties, a favored effect in COVID-19 leading to cytokine storm. Works by Barone et al. reported that HSP60 and HSP90 have an active role in the development of the thromboembolic phenomenon that led to the death of a limited number of patients affected by COVID-19 (19). These data illustrate that raised serum levels of HSP70 and HSP90 have an important role in mediating the immune reaction and excessive inflammation in COVID-19 patients. Furthermore, these HSPs may be valuable markers of disease progression and response to therapy (9, 23). Studies on SARS-COV-2 have found that the inhibitors of HSP90 may help control SARS-COV-2 disease (12, 20). Upregulation of HSPs due to cell injuries can lead to autoimmune and inflammatory diseases in humans (24).

CONCLUSION

In summary, our results indicated that higher levels of HSP90 and HSP70 could worsen the severity of disease in patients with COVID-19. Pharmacological inhibition of HSP70 and HSP90 may be of interest as it may alleviate inappropriate inflammatory responses in severe COVID-19 patients. Further studies with more participants are needed to clarify the character of HSPs and inflammatory cytokines during this emerging viral disease.

ACKNOWLEDGMENTS

The authors would like to express their appreciation for the University's support and the Hospital staff for their kind cooperation. We should thank the Clinical Research Development Center, The Persian Gulf Martyrs Hospital, and Bushehr University of Medical Sciences, Bushehr, Iran for editorial statistical assistance. This work was supported by the Research and Technology Council of BPUMS [Grant number: 1617-2020]. Gholamreza Khamisipour has received research support from the Research and Technology Council of Bushehr University of Medical Sciences, Iran.

AUTHORS' CONTRIBUTION

All authors contributed to this study's conception and design. Material preparation, data collection, and analysis were performed by Zivar Zangeneh and Gholamreza Khamisipour. The first draft of the manuscript was written by Zivar Zangeneh and Gholamreza Khamisipour. All authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

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