International Journal of Medicine Sciences
www.medicinejournal.in
Online ISSN: 2664-889X, Print ISSN: 2664-8881
Received: 12-02-2022, Accepted: 27-02-2022, Published: 14-03-2022
Volume 4, Issue 1, 2022, Page No. 1-5

Heat shock protein 70 (HSP-70) levels in chronic spontaneous urticaria

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Abstract
Background and aim: Chronic urticaria (CU) whether spontaneous or induced is a common condition. The exact pathological mechanisms responsible for CU are not fully understood. Heat shock proteins (HSPs) are a large group of molecular chaperones widely involved in many aspects of cellular homeostasis. HSP70 has been linked to allergic and autoimmune conditions. The present study aimed to assess serum levels of HSP70 in patients with chronic spontaneous urticaria and to correlate these levels with patients' characteristics.
Subjects and Methods: The present case control study included 70 patients with chronic spontaneous urticaria (CSU). In addition, there were 70 age and sex-matched healthy control. The severity of their urticaria symptoms were assessed using Urticaria Activity Score (UAS). Symptoms were classified as mild, moderate or intense. Serum levels of HSP70 measured by ELISA method, using commercially available kits after withdrawal of oral antihistamines for 1week.
Results: Comparison between patients and controls regarding serum HSP70 revealed significantly higher levels in the patients' group [median (IQR): 0.615 (0.230 - 0.900) versus 0.260 (0.210 - 0.420) ng/ml, p=0.003]. Patients with intense disease had significantly higher HSP70 levels when compared with patients with moderate and mild disease [median (IQR): 2.280 (1.310 - 4.920) versus 0.780 (0.340 - 0.900) and 0.240 (0.140 - 0.336) ng/ml respectively, p<0.001).
Conclusions: Serum levels of HSP70 are significantly elevated in patients with CSU and are related to disease severity.

Keywords: heat shock proteins, HSP-70, chronic spontaneous urticaria (CSU)

Introduction

Chronic urticaria (CU) whether spontaneous or induced is a common condition affecting about 1 % of the world population and is characterized by appearance of wheals with or without angioedema that lasts for more than 6 weeks. Intense pruritus can cause significant impairment in daily functioning and disrupt sleep ***(****He et al., 2021****).***
The exact pathological mechanisms responsible for CU are not fully understood. Suggested mechanisms encompass immunological, inflammatory and coagulation alterations. However, the pathological hallmark of the condition remains degranulation of cutaneous or submucosal mast cells and to lesser extent basophils resulting in release of histamine and leukotrienes *(Ensina et al., 2019)* [1].
Treatment of CU is based on second-generation antihistamines as first-line option. The recent introduction of omalizumab provided an effective alternative to resistant cases. However, management of CU remains challenging with many patients unfit for available therapies and others express treatment resistance. Pursuit of new therapeutic options targeting other pathological pathways involved in CU is recommended *(He et al., 2021).*
Heat shock proteins (HSPs) are a large group of molecular chaperones widely involved in many aspects of cellular homeostasis. They are named after their molecular weight into many types including HSP27, HSP40, HSP60, HSP70, HSP90, and other larger HSPs. *(Shan et al., 2020; Yang et al., 2021)* [1, 15].
HSP70 has been linked to allergic *(Shevchenko et al., 2021)* [12] and autoimmune *(Tukaj et al., 2020)* [] conditions. One recent study suggested that HSP70 may be involved in the immune response to stressful inflammatory stimuli in patients with chronic spontaneous urticaria *(Kasperska-Zając et al., 2018)* [3, 4].
The present study aimed to assess serum levels of HSP70 in patients with chronic spontaneous urticaria and to correlate these levels with patients' characteristics.

Subjects and Methods

The present case control study was conducted at Helwan and Ain Shams Universities' Hospitals in the time from January, 2020 through June, 2021. The study protocol was approved by the ethical committee of Helwan Faculty of Medicine. All subjects provided informed consent to participate in the study.

The study included 70 patients with chronic spontaneous urticaria (CSU). In addition, there were 70 age and sex-matched healthy control. Patients were excluded from the study if they were smokers or pregnant, if they had advanced liver disease, malignancy, diabetes mellitus, infections (e.g. Hepatitis) or autoimmune diseases (e.g. Systemic lupus erythematosus) or if they under treatment with steroid of anti-inflammatory medications.
All patients were subjected to careful history taking and thorough clinical examination. The severity of their urticaria symptoms were assessed using Urticaria Activity Score (UAS). Symptoms were classified as mild, moderate or intense *(Zuberbier et al., 2018).*
Laboratory investigations included complete blood count (CBC) with differential count, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), urine analysis, stool analysis, thyroid stimulating hormone (TSH) and total serum IgE. Serum levels of HSP70 measured by ELISA method, using commercially available kits after withdrawal of oral antihistamines for 1week.
Data obtained from the present study were presented as mean and standard deviation or median and interquartile range (IQR) for numerical variables and number and percent for categorical variables. Numerical variables were compared using t test, Mann-Whitney U test or Kruskal-Wallis test as appropriate while categorical variables were compared using Fisher exact test or chi-square test. All statistical calculations were processed using SPSS, version 25 (IBM, USA) with p value less than 0.05 was considers statistically significant.

Results

The present study included 70 patients with CSU and 70 healthy controls. No statistically significant differences were noted patients and controls regarding the demographic data. Disease severity was categorized into mild (41.4 %), moderate (41.4 %) and intense (17.2 %) (Table-1).
Comparison between patients and controls regarding serum HSP70 revealed significantly higher levels in the patients' group [median (IQR): 0.615 (0.230 - 0.900) versus 0.260 (0.210 - 0.420) ng/ml, p=0.003] (Fig.1).
Exploring the relation between HSP70 levels and demographic and clinical data revealed that patients with intense disease had significantly higher HSP70 levels when compared with patients with moderate and mild disease [median (IQR): 2.280 (1.310 - 4.920) versus 0.780 (0.340 - 0.900) and 0.240 (0.140 - 0.336) ng/ml respectively, p<0.001) (Table-2, Fig.2).

Discussion

The present study found significantly higher serum HSP70 levels in Egyptian CSU patients as compared to controls. In addition, we found significantly higher levels of HSP70 in patients with intense disease in comparison to those with mild and moderate disease. Similar findings were reported by the study of *Kasperska-Zając et al. (2018)* [3, 4] on 58 patients with active CSU.
The contribution of HSP70 to CSU pathogenesis may be explained by the recognized relation between HSP70 and mast cell degranulation. Mast cells play pivotal roles in immediate-type and inflammatory allergic and nonallergic reactions. Cross-linking of the high-affinity receptor for IgE (FcepsilonRI) on mast cells activates a signaling pathway leading to Ca2+ mobilization and is followed by degranulation and the release of histamine and other preformed mediators, as well as de novo synthesis of arachidonic acid metabolites *(Mortaz et al., 2005).* In fact, active role of mast cells in stress adaptation is related to presence of Hsp70 in secretory granules *(Shabel'nikov et al., 2012).*
One study investigated induction of HSP70 expression during antigen stimulation in mouse bone marrow-derived mast cells (BMMC). The study found that extracellular HSP70 induced phosphorylation of linker for activation of T cells and a series of downstream signaling molecules in BMMC *(Li et al., 2018).*
In addition, *Mortaz et al., (2007)* experimental study investigated the effects of heat shock and acetylsalicylic acid on the activation of mast cells and the release of cysteinyl leukotrienes. They found an association between increased HSP70 levels and leukotrienes release. In another work, they showed stimulatory effect of HSP70 on toll like receptors *(Mortaz et al., 2006).*
Increasing HSP 70 with higher severity score can by explained by *Molvarec et al (2009)* who reported that HSP 70 expression is generally correlated with the grade of inflammation, elevated circulating HSP 70 possibly reflects the tissue damage or active release from cells in response to stressful insults. It seems that circulating HSP 70 in CSU possibly reflects its over expression in the skin and might be released from cells involved in the urticarial inflammatory response.
In conclusion, the present study found that serum levels of HSP70 are significantly elevated in patients with CSU and are related to disease severity.

Table 1: Demographic and clinical characteristics of the studied groups

	Patients N=70	**Controls N=70**	**p value**
Age (years) mean ± SD	33.3 ± 10.2	35.4 ± 9.0	0.26
Male/female n	32/38	36/34	0.35
Residence n (%)			
Urban	41 (58.6)	50 (71.4)	0.11
Rural	29 (41.4)	20 (28.6)	
Occupation n (%)			

Employee	13 (18.6)	18 (25.7)	0.83
Farmer	1 (1.4)	-	
Housewife	16 (22.9)	20 (28.6)	
Manual worker	17 (24.3)	20 (28.6)	
Professional	14 (20.0)	15 (21.4)	
Student	9 (12.9)	7 (10.0)	
Disease severity n (%)			
Mild	29 (41.4)	-	-
Moderate	29 (41.4)	-	
Intense	12 (17.2)	-	

Table 2: Relation between HSP70 levels and demographic and clinical data in the studied patients

	HSP70 Median (IQR)	**p value**
Sex		
Male	0.380 (0.220 - 0.800)	0.14
Female	0.683 (0.240 - 1.240)	
Residence		
Urban	0.640 (0.241 - 920)	0.23
Rural	0.334 (0.200 – 0.840)	
Occupation		
Employee	0.820 (0.620 - 0.900)	0.49
Farmer	0.241	
Housewife	0.302 (0.225 - 0.735)	
Manual worker	0.334 (0.140 - 1.379)	
Professional	0.651 (0.230 - 0.780)	
Student	0.820 (0.420 - 1.400)	
Disease severity n (%)		
Mild	0.240 (0.140 - 0.336)	<0.001
Moderate	0.780 (0.340 - 0.900)	
Intense	2.280 (1.310 - 4.920)	

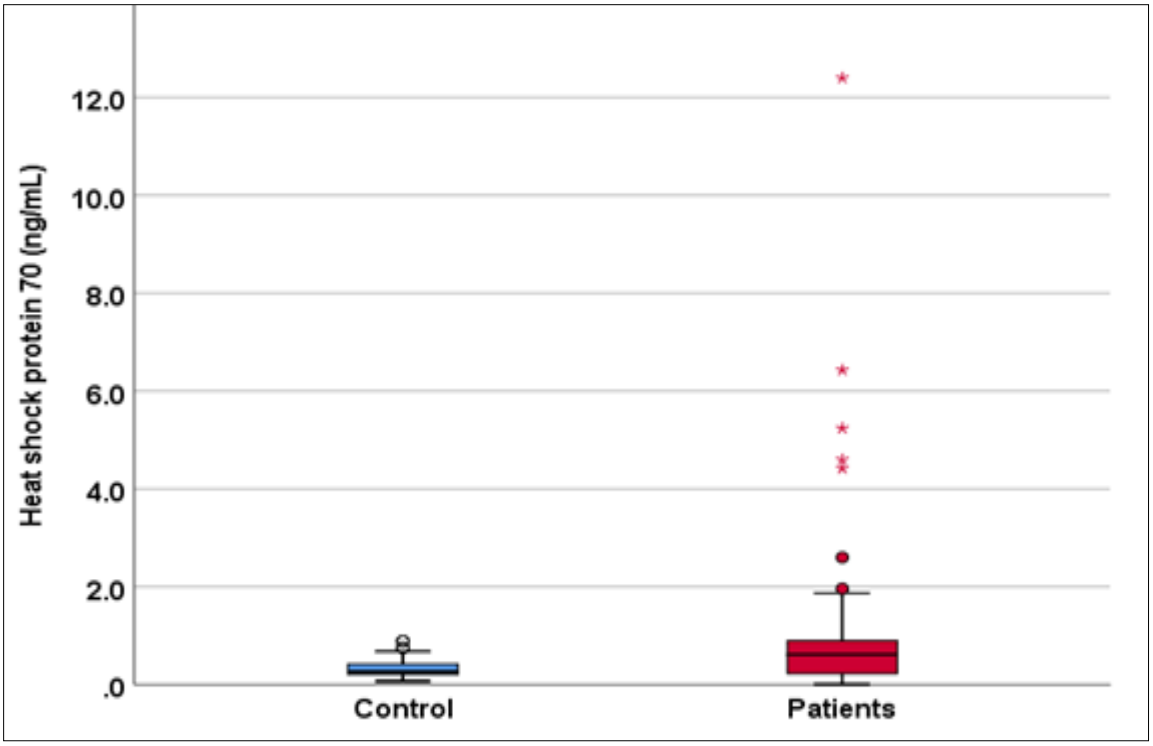

Fig 1

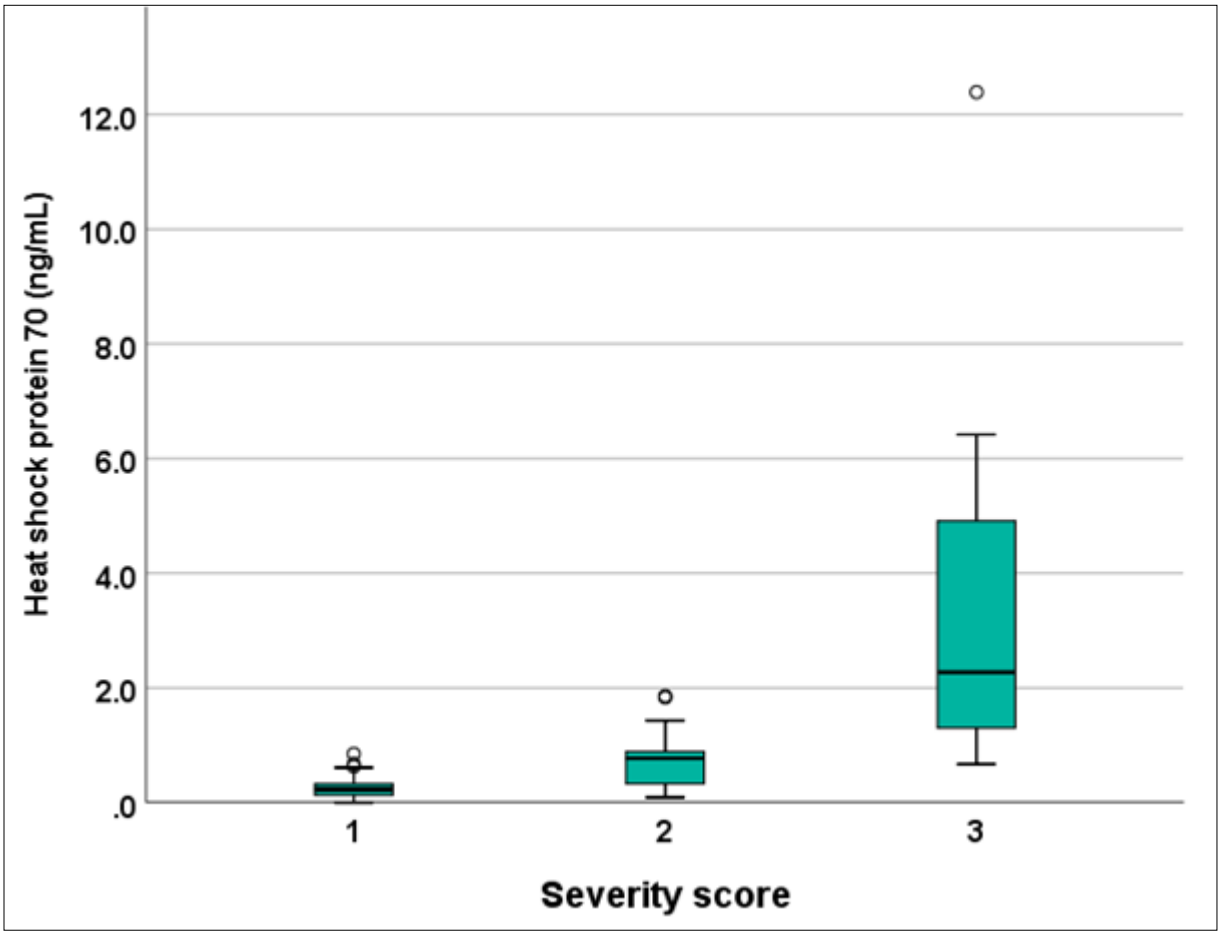

Fig 2

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