



## Short Communication

# Heat Shock Protein-70 is expressed in Higher Quantities under Thermal Stress Conditions in Longevity Individuals

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### ABSTRACT

The cellular response to stress is mediated by intracellular proteins, called heat shock proteins (HSPs). Among various known stressors, heat is a major factor that induces the production of HSP70. Keeping in view the very hot conditions of Dera Ismail Khan (D.I. Khan) division where the temperature remains at 45-50°C during the months of June to September, it was hypothesized that heat stress conditions do induce the overexpression of HSPs, especially Hsp70. It was thus attempted to find out the possible role of Hsp70 in those human being having the age of 90 years or above, called longevity people, against heat stress conditions. Whole blood samples of 45 longevity individuals and 20 samples of control people were collected in D. I. Khan during September 2018 to October, 2019 after proper approval from Gomal University ethical review board and written consent of each individual was taken prior to collection of blood sample. For serum collection, blood samples were centrifuged at 1000xg for 15 minutes. Quantitative measurement of Hsp70 protein was done using sandwich ELISA technique. The maximum serum Hsp70 level observed was 42ng/ml and minimum serum Hsp70 value was 13ng/ml, with median value of 28ng/ml. In control group, maximum concentration observed was 38ng/ml while the minimum serum level was 18ng/ml with median value of 13ng/ml. In longevity males, serum Hsp70 levels increased in individuals between 89 to 91 years of age, peaked between 92 to 97 years but comparatively lower having the age of above 98 years. On the other hand, serum concentration of Hsp70 in longevity females were highest in those having 89 to 92 years, lower in 93 to 97 years and, like males, lowest having age above 98 years. The study hence, showed that longevity individuals had higher quantities of serum Hsp70 compared to control group, and again, males contained higher concentration than longevity females, showing that thermal stress was the agent leading to over-expression of Hsp70 especially in longevity individuals but in longevity individuals. Whether this increased expression had any impact on longevity is still not clear that needs to be deciphered.

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### Authors' Contributions

JK designed the project, collected samples and wrote the manuscript. TF and MR did the experimental work and sample collection. HS edited the manuscript. DM helped in sample collection and financial support.

### Key words

Heat shock protein, Longevity, Serum, Heat stress, ELISA.

Stress is a sudden natural change that can lead to injury either at molecular, cellular or organism level. Cells respond to such changes through intracellular proteins known as Heat Shock Proteins (Hsp). Hsps are synthesized at minimal level in normal conditions, but under stressful conditions, they are produced in excessive quantities (Lindquist *et al.*, 1988; Villar *et al.*, 1993). Of these, the Hsp70 is a highly inducible and most actively synthesized protein in the cell upon heat shock (Binder *et al.*, 2000). It

preserves the healthy state of the cell by preventing denaturation of proteins and making the correct assembly (Georgopoulos *et al.*, 1993; Hartl, 1996). Moreover, it is also capable of binding to plasma membrane, promotes the influx of Ca<sup>2+</sup>, causes activation of certain the transcription factors, and promotes the augment the synthesis and release of pro-inflammatory cytokines (Basu *et al.*, 1998; Asea *et al.*, 2000; Binder *et al.*, 2000). Such activities of Hsp70 and certain more against any abnormal change lead to protect cellular life but the excess is bad. Importantly, Hsp70 is minimally prone to heat-induced apoptosis in old age individuals compared to non-longevity individuals (Ambra *et al.*, 2004). Heat shock genes are regulated by transcription factors called heat shock factors (HSFs). Of the HSFs, HSF1 is the most important transcription factor because of its prominent role in regulating the process of transcription immediately after stressful conditions

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(Richter *et al.*, 2010). HSF1 is transcribed regularly at a constant level. It is present in the cytosol where heat shock proteins, notably the Hsp70 or Hsp90, combine with it in order to keep it in an inactive form (Lindquist *et al.*, 1988). In various human studies, regarding aging, Hsp70 seems to be a propitious biomarker for the indication of aging as gene variants and blood levels of heat shock 70 protein are found to increase the lifespan. It is capable of being formed immensely in the presence of heat shock. Among various known stressors, heat is a major factor that induces the production of Hsp70. Hence, keeping in view the contrasting findings regarding the expression of Hsp70 in different geographical regions and in different age groups, and the hot environmental conditions of D.I. Khan division and its surrounding area as well where the temperature reaches 50°C during the months of June, July and August, and is severe cold during the months of December and January, it was attempted to find out any possible relation between Hsp70 and longevity. It is thus hypothesized that the adverse environmental condition of D.I. Khan does induce over expression of heat shock protein70 in longevity individuals. Blood samples were collected from longevity individuals and quantitative measurements of the protein were made in comparison with non-longevity individuals.

#### *Material and methods*

Venous blood samples were collected from 54 longevity individuals, with their ages in the range of 90 to 100 years. Among the longevity individuals 35 were males and 20 were females (Table I). The only centenarian individual found was male. Blood samples of non-longevity individuals having the ages in the range of 20-45 years (Table I) were also collected as control. Approximately 3 ml blood was taken from each individual in corning tubes. For serum collection, blood samples were centrifuged at 3000xg for 15 min. Serum in the form of supernatant was collected in a separate tube and stored at -20°C to carry out the assay. Serum Hsp70 levels was determined using sandwich ELISA technique (Elascience; Human HSP-70/HSPA9). The sera were accordingly analyzed using the classical sandwich enzyme-linked immunosorbent assay (ELISA) method as described earlier (Rea *et al.*, 2001; Pockley *et al.*, 2000) with slight modifications. Shortly, stored sera were thawed and the total protein content was quantitatively determined through Bradford method using bovine serum albumin as a standard. The serum was then treated with 0.9% Triton X-100 for 15 min at 15°C with gentle shaking for releasing trapped Hsp70 (Gastpar *et al.*, 2005; Lancaster *et al.*, 2005). The samples were then put onto 96-well microplate (Nunc Immunoplate Maxisorp; Life Technologies) that had been coated with

murine monoclonal antihuman Hsp70 (clone C92F3A-5; StressGen) in carbonate buffer, pH 9.2 (2.5 µg/mL) for 24 h at 15°C on gentle agitation, and then washed with PBS containing 1.5% Tween 20 (PBS-T). Blocking was then done by incubating with 1.5% bovine serum albumin in PBS-T. Detection of Hsp 70 was done by adding rabbit polyclonal anti-Hsp70 antibody (SPA-812; StressGen) and this bound polyclonal antibodies were detected with alkaline phosphatase-conjugated murine monoclonal antibody to rabbit immunoglobulins, followed by p-nitro phenyl-phosphate substrate (Sigma Aldrich). The resultant absorbance was measured at 405 nm with a BioRad Benchmark Plus plate reader, and the concentrations (ng/ml) of Hsp70 were determined by reference to standard curves (BIOSOFT).

#### *Results*

It was hypothesized that hot environmental condition of District D.I. Khan, KP, Pakistan can induce over expression of Hsp70 in longevity individuals. To investigate the hypothesis, serum Hsp70 concentration levels were determined in longevity individuals with their ages in the range of 90-100 years. The same protein was quantitatively measured in non-longevity individuals having ages in the range of 20-30 years as control. The mean concentration among the individuals of 90 year, 91, 92 and 93 years of age were 38.37, 38.35, 37.74 and 38.23 ng/ml respectively, while those of 94 years, 95 years, 96 years, 97 years and 98 years were 40.01, 38.83, 37.42 and 35.99 ng/ml. Interestingly, the only centenarian had comparatively smallest concentration of 28.3 ng/ml among all the longevity individuals (Table I). Maximum concentration of 42.1 ng/ml was found in a male and a female with 95 years and 94 years of age, respectively while the minimum concentration of 28.3 ng/ml was recorded in centenarian male (Table I). Among the non-longevity, the BS zoology and MPhil/PhD students, the highest serum concentration of 38.23 ng/ml of Hsp70 was found in a 26 years old student while the lower quantity of 11.49 ng/ml was recorded in a 19 years old male BS student (Table I). Comparing age-wise mean concentration among the longevity individuals, no significant difference was found. Similarly, there was no significant difference when the mean concentration of the protein was compared between male and female longevity individuals (Table I). In case of non-longevity individuals, the smaller mean concentrations of Hsp 70 were recorded in individuals with age 19. Interestingly, gradual increase in the mean concentration was observed from 20 years of age to 30 years (Table I).

**Table I.- Age-wise distribution of longevity and non-longevity individuals and Hsp70 level in their serum.**

Age group (years)	Gender	n	Hsp70 level (ng/ml)
<b>Longevity</b>			
90	Male	8	35.2-40.2
	Female	2	38.5-39.4
91	Male	6	34.67-39.65
	Female	4	36.7-41.0
92	Male	5	28.73-40.52
	Female	5	34.5-40.22
93	Male	3	37.37-40.34
	Female	4	36.33-38.23
94	Male	3	38.63-39.72
	Female	2	40.27-42.1
95	Male	3	39.72-42.13
	Female	3	34.74-37.72
96	Male	2	33.44-41.41
	Female	0	Nil
97	Male	2	33.43-38.55
	Female	0	Nil
98	Male	1	35.57
	Female	0	Nil
99	Male	0	Nil
	Female	0	Nil
100	Male	1	28.12
	Female	0	Nil
Total		54 (Male, 34; Female, 20)	
<b>Non-longevity</b>			
19	Male	3	11.49-12
	Female	0	Nil
20	Male	3	13.36-25.35
	Female	1	12.23
21	Male	2	32.22-33.28
	Female	2	17.8-18.85
22	Male	0	Nil
	Female	0	Nil
23	Male	3	30.35-35.36
	Female	0	Nil
24-25	Male	0	Nil
	Female	0	Nil
26	Male	2	35.6-38.32
	Female	0	Nil
27	Male	1	32.2
	Female	0	Nil
28	Male	0	Nil
	Female	0	Nil
29	Male	0	Nil
	Female	1	36.9
30	Male	2	34.7-35.3
	Female	0	Nil
Total		20 (Male, 16; Female, 4)	

*Discussion*

The idea behind quantitative measurements of Hsp70 protein in longevity individuals, in comparison with non-longevity individuals in adverse and continuous heat-stress environmental conditions of D.I. Khan division of southern belt of KP for more than nine months in a year, was to see any possible influence of the protein on longevity. Previous findings regarding the relationship of extracellular Hsp70 with aging and longevity were heterogeneous. In an earlier report (Rea *et al.*, 2001) conducted on individuals having their ages in the range of 20 to 96 years, inverse relationship between serum Hsp70 and age was observed as the protein had progressive decrease with age. Similarly, in another finding conducted on Chinese males of 15 to 50 years of age, the same progressive decline of serum Hsp70 with age was reported but a positive correlation of the protein with age was also observed in younger individuals (Jin *et al.*, 2004). These findings hence report decline in serum Hsp70 levels as human ages but this decline within an individual over time has not been indicated, although in vitro experiments on cell lines of centenarians showed increased expression of Hsp70 against heat stress conditions (Ambra *et al.*, 2004), showing the protein as a marker of health, indicator of stress (Jin *et al.*, 2004; Ambra *et al.*, 2004). Another possibility of low concentration of serum Hsp70 shown in earlier report (Njemini *et al.*, 2006) was the use of modified form of commercially available kit that was detecting comparatively lower concentration of the protein because of washing the samples with Triton X-100. Here, the findings of the present study are totally in contrast and in disagreement with what has previously been described. The mean concentrations of Hsp70 in longevity individuals, both males and females were comparatively significantly higher than non-longevity individuals. No significant difference was found in the mean concentrations between male and female longevity individuals. Interestingly, the mean values had increase with increase in age in non-longevity individuals. This significant difference between the present study and previous studies can be due to the reasons: (i) continued heat stress conditions in D.I. Khan division throughout the year, (ii) life style of people especially longevity individuals, living in the remote areas in the villages, working hard in the field throughout the year, lack even the basic health facilities and thus never rely on medicines, (iii) possibly, inheritance factor is also contributing to some extent as 70% the non-longevity individuals possessing comparatively higher concentration of Hsp70 belong to the family of longevity individuals, (iv) increased synthesis of Hsp70 protein in longevity individuals seems to have compensating influence against diseases, a signal molecule and a marker of good health. The protein

probably acts more than a chaperon in these individuals, stimulate the adaptive immune response and regulate other biochemical processes vital for healthy long life, and (v) it has been shown that low concentration of Hsp70 is directly related to decreased exposure to inflammation and inflammation has influence on cardiovascular diseases (Pockley and Frostegard, 2005). But interestingly during this study, the longevity individuals had comparatively higher concentrations of Hsp70 and nobody was found to have cardiovascular diseases that needs to be deciphered.

#### Conclusion

Increased synthesis of Hsp70 protein in longevity individuals seems to have compensating influence against diseases, a signal molecule and a marker of good health. The protein probably acts more than a chaperon in these individuals.

#### Statement of conflict of interest

The authors have declared no conflict of interests.

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