Heat Shock Protein Response to Chronic Cold Exposure in Antarctic Expedition Members

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Abstract- The heat shock response is seen when cells are exposed to extremes of thermal environment, which may be acute or chronic. The heat shock response is featured by increased expression of heat shock proteins (HSPs). One such key stresses is extreme cold environment as seen in Antarctica. The Antarctic continent on the planet Earth is full of environmental challenges. It is considered as natural stress model. The objective of this study was to study the effect of chronic cold environment on HSP levels. Seventeen healthy men of XXVI Indian Antarctic expedition with mean age of 39.7±1.95 years and age ranged from 29 to 56 years participated in this study. Antibodies of IgG, IgA and IgM classes against HSP65 were investigated by indirect ELISA method. Samples were collected in 2 phases. In phase-1, pre-expedition samples were collected before leaving to Antarctica at National Center for Antarctic and ocean research (NCAOR), Goa. In phase-2, end expedition samples were collected after 11 months of stay in Antarctica in an Indian permanent station (Maitri) during polar days. The raw data on analysis using statistical tool revealed that the anti-HSP65 IgM antibody were significantly elevated (p= <0.001). It was observed that the anti-HSP65 antibodies were increased in expedition members compared with the control group who stayed in India. The present study concluded that HSP expression increased in Indian Antarctic expedition members who were exposed to chronic cold stress in Antarctica.

Index Terms- HSP65, Chronic cold stress, Antarctica, Immunological response.

INTRODUCTION

Stress are of various types. They are physical stress, chemical stress, biological stress and psychological stress. Physical stresses are due to heat, cold, ultraviolet radiation, noise and vibration. One of the key stresses in Antarctica is cold stress. With its long winters of unremitting darkness, cold and isolation, Antarctica is perhaps the harshest sustained human environment on earth. The environment is very challenging and Antarctica is a natural Laboratory in human response to sustained physiological and emotional stresses. Changes in cardiovascular system, endocrine function, immunology and psychological adaptation have been studied in Antarctica. Stress factors are also postulated to induce the expression of certain proteins called heat shock proteins.

Heat Shock Proteins (HSPs) are the most phylogenetically conserved, ubiquitous, intracellular molecules (1). HSPs were first discovered in 1962 by Ritossa and co-workers in overheated Drosophilia melanogaster larvae (2). However the term heat shock proteins is a misnomer, as they are induced not only by heat shock but also by cold environment. These proteins are also expressed under various stressful conditions including pathological, environmental and physiological insults (3, 4). They are induced by wide range of cellular insults like oxidative stress, nutritional deficiencies, ultraviolet irradiation, exposure to chemicals, bacterial infection, viral infection, necrosis etc. (5). HSPs are found in all eukaryotic cells, which protect the cell against stress factors such as thermal stress, hypoxia, hypoglycemia and hormonal changes by initiating its synthesis. Based on their molecular weight these proteins are classified into six families viz., Hsp10, Hsp40, Hsp60, Hsp70, Hsp90 and Hsp100. HSPs function as molecular chaperones in numerous processes such as folding and unfolding of proteins, assembly and disassembly of protein complexes and antigen processing under physiologic and stress conditions (6). Recent research have shown that HSPs in extracellular compartment elicit different functions like antigen presentation, intercellular signaling, and induction of production of cytokines which mediate immunity. HSPs represent as prominent antigens in several infectious diseases and autoimmune diseases and thereby mediate humoral and cellular immune response. HSPs are increased in autoimmune arthritis (7), Guillian-Barré syndrome (8), atherosclerosis (9) and multiple sclerosis (10).

Thermal stresses trigger a complex program of gene expression and biochemical adaptive responses (11). The ability to survive and adapt to thermal stress appears to be a fundamental requirement of cellular life. Changes in gene expression are an integral part of the cellular response to thermal stress. HSPs are perhaps the best-studied examples of genes whose expression is affected by heat shock. Induction of HSP synthesis is transcriptionally regulated both in prokaryotic and eukaryotic cells. In prokaryotic cells, sigma 32 acts as a positive transcription factor for heat shock protein expression (12). In eukaryotic cell Heat Shock factor I (HSF-I) acts as a transcription factor.

McClung, Yamada and coworkers in their study of HSP gene expression in peripheral blood mononuclear cells from human subjects participating in a 10-day heat acclimatization protocol

showed that levels of HSP72 and HSP90 were increased by 17.6 ± 6.1% and 21.1 ± 6.5% respectively (13, 14). In another study when subjected to whole-body heat stress (sitting in a heat stress chamber for 30 minutes at 73°C) there was increase in Extracellular HSP72 (15). In industrial workers working in an environment with extreme heat, antibodies to HSP27 and HSP70 was significantly higher (16). Similarly even at 32°C a cold-stress response is elicited and several genes are activated in mammalian cells. The induction of HSPs in brown adipose tissue in mice exposed to cold ambient temperature was shown to be mediated by norepinephrine released in response to cold (17). In human skin biopsies exposed to 4^0, 15^0, 20^0, and 37°C for 1 h it was found that at 15^0C and below there was increased synthesis of HSP72 and HSP90 in human keratinocytes (18). Previous studies done in Antarctica mud clam, zebra fish, and insects have shown an up regulation of HSP. As per our knowledge there are no studies done in humans to study the effect of chronic cold environment on HSPs in Antarctica. Therefore we hypothesize that cold stress in Antarctica will cause an increase in HSP levels in humans. Therefore the aim of this study was to assess the correlation of cold stress on HSP expression by assessing IgG, IgA and IgM antibodies towards HSP-65 in the serum of Antarctica expedition team members.

MATERIALS AND METHODS

The study group consisted of 17 members of XXVI Indian scientific expedition to Antarctica who were screened for medical and psychological abnormalities prior to selection. The study was conducted in two phases. In phase-1(Jan 2007), the baseline data (pre-expedition samples) were collected before leaving to Antarctica at National Center for Antarctic and ocean research, Goa. In phase-2 (Dec 2008), end expedition samples were collected after 11 months of stay in Maitri, Antarctica during polar days. Fasting state venous blood samples from antecubital vein were collected and centrifuged. Further the serum was stored in vacutainer and preserved at a temperature -24^0C. On completion of expedition the phase 2 samples were brought back to NIMHANS microbiology lab for analysis. In Antarctica (Schirmacher Oasis) the average temperature during summer and winter range was +5^0c to -15^0c and -10^0c to -30^0c respectively. Seventeen non-diseased healthy serum samples (Blood Bank, NIMHANS) served as control group.

Enzyme-Linked Immunosorbent Assay (ELISA) procedure:
Antibodies of IgG, IgA and IgM classes against HSP-65 were investigated by indirect ELISA method in serum of pre-expedition, end-expedition, and control samples. ELISA plates (Maxisorp; Nunc, Roskilde, Denmark) were coated with 50µl of HSP-65 (20 ng/ml) in phosphate buffered saline (PBS) by incubating overnight at 4 °C. (HSP-65 courtesy of van Embden). After washing three times with PBS containing1% Tween20 (PBST), the plates were blocked with 1% fat free milk (Anik spray, India) in PBST at 37 °C for 2 h in a moist chamber. Diluted serum samples (1:500) in 1% milk-PBST were added in duplicates, 50µl/well and incubated at 37 °C for 2 h in a moist chamber. After washing with PBST (×7), peroxidase-conjugated specific anti-human IgG, IgM or IgA (Dako, Denmark; 1:5000 for G and 1:1000 for A and M) in 1% milk–-PBST was added (50µl/well) and the plates were incubated at room temperature for 90 min. The plates were then washed with PBST (×7) and added with peroxidase substrate and ortho-phenylenediamine (OPD) chromogen (Sigma, USA) in citrate phosphate buffer and incubated for 15 min. at room temperature. The reaction was stopped using 50µl of $2NH_2SO_4$ and absorbance was read at 492 nm as optical density (OD).

Statistical analysis
Relevant descriptive statistical analysis was done using Microsoft ® Office Excel® 2013 Professional. Data were expressed as mean ± SD. Paired sample correlation test was used to compare anti HSP 65 antibodies levels in pre-expedition and end-expedition samples. Independent samples T-test was used to compare anti HSP65 antibodies levels between the serum of expedition members and the control group. A 'P' value <0.05 was considered as statistically significant. Statistical analysis was done using SPSS Version software (version 16.0, SPSS Inc., Chicago, Illinois).

RESULTS

The mean age of 17 subjects was 39.7±1.95 years. The age ranged from 29 to 56 years. The Mean BMI was 24.81 kg/m^2 and BMI ranged from 21.1 to 27.7 kg/m^2. Anti-HSP65 kDa antibodies in the serum of pre-expedition samples (n=17), end-expedition samples (n=17) and control samples (n=17) were detected by ELISA method using antibodies of IgG, IgA and IgM classes. The Mean ± SD of IgG, IgA and IgM classes during pre-expedition and end- expedition are tabulated in Table 1. The significant rise at end- expedition values were observed in IgG and IgM vales as shown in Table 1.

Table 1. Paired Samples Statistics

		N	Mean	Std. Deviation	Std. Error Mean
Pair 1	Pre expedition IgG	17	0.81	0.21	0.05
	End expedition IgG	17	0.91	0.34	0.08
Pair 2	Pre expedition IgM	17	0.79	0.36	0.09
	End expedition IgM	17	0.83	0.38	0.09
Pair 3	Pre expedition IgA	17	0.24	0.06	0.02
	End expedition IgA	17	0.27	0.14	0.03

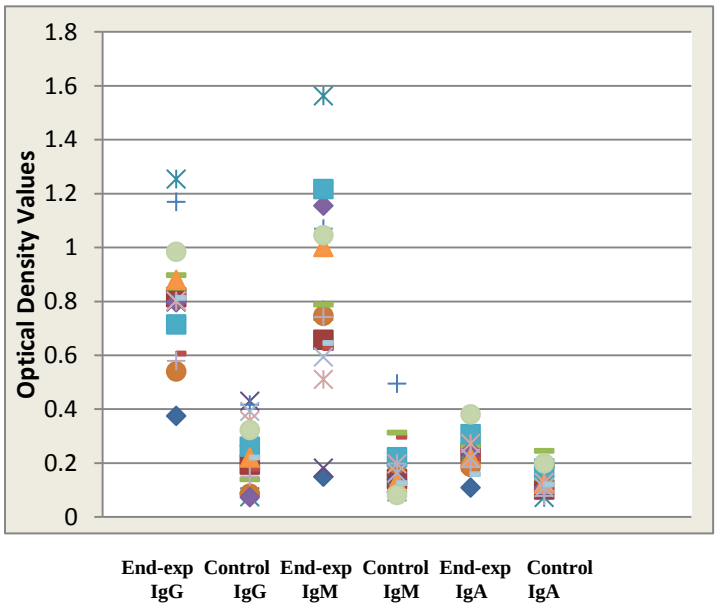
The paired samples correlations between the pre-expedition and end expedition values of IgG, IgA and IgM classes are tabulated in Table 2. The IgM values was found to be positively correlated and was statistically significant (p <0.001) as shown in Table 2.

Table 2. Paired Samples Correlations

		N	Correlation	p-value
Pair 1	Pre expedition IgG & End expedition IgG	17	0.088	0.737
Pair 2	Pre expedition IgM & End expedition IgM	17	0.899	<0.001

Pair 3	Pre expedition IgA & End expedition IgA	17	0.719	0.001

The individual optical densities of the IgG, IgA and IgM classes of expedition members and the control group at the end-expedition were compared and are depicted in scatter graph in Figure 1. IgM showing significant rise.



DISCUSSION

In this study we have investigated the immune responses of IgG, IgA and IgM classes towards HSP65 in the serum of pre-expedition and end-expedition samples of expedition members. It has been observed from the results that the anti-HSP-65 antibody especially IgM was highly significant (p= <0.001) in the end-expedition samples compared with pre-expedition samples. This shows that HSP expression increases in conditions of stress like extreme cold environment like Antarctica. However, in healthy control samples the anti-HSP antibody expression was limited.

HSPs are induced when a cell undergoes various types of environmental stresses like heat, cold and oxygen deprivation. One such key stress factor is extreme cold environment like that seen in Antarctica. In Antarctica (Schirmacher Oasis) the average temperature during summer and winter range was $+5^0$c to -15^0c and -10^0c to -30^0c respectively. Currently, the physiological mechanism(s) underlying increased HSP synthesis in cold environment is not known. The possible reason for increase in HSPs could be due to following reasons. It is seen that when cells are exposed to cold stress there is denaturation of proteins (19, 20), and increased flux of non-native proteins which if left unprotected results in misfolding and aggregation of proteins. The presence of abnormally folded proteins in a cell may be a key signal for the transcriptional activation of HSP genes. Thermally denatured or otherwise misfolded proteins induce the synthesis of Hsp70 via a biochemical mechanism involving HSF1, which is a transcription factor (21, 22). HSF1 is normally maintained in an inactive conformation in the cytosol because it is bound to Hsp90, Hsp70, or other molecular chaperones (23). In the presence of denatured proteins, these chaperones release HSF1 to bind to the hydrophobic regions of the unfolded proteins. This frees HSF1 to trimerize, enter the nucleus, and binds to specific DNA sequence of the promoter region called heat shock element (HSE) and activate the transcription of HSP genes (22). Expression of HSPs has been correlated with cold stress in plants, insects, and rodents (24) which is in accordance with the present study.

In a study carried out by Liu and co-workers, they have found that transient cold shock at 4^0C did not induce the HSP response in human fibroblasts and HeLa cells but upon recovery at 37^0C, the HSF-1 mediated induction of HSP70 and HSP90 was observed which is in contrast to our findings (18). It was proposed that the severe cold exposure activates signals for a cell stress response and also interferes in the process such as transcription and translation as to preclude stress protein expression until rewarming occurs. Cold exposure leads to generation of free radicals and other toxic metabolites that are capable of inducing stress response.

As molecular chaperones, HSPs participate in the folding of misfolded proteins. HSPs also confers thermo-tolerance to cells and organisms by preventing denaturation and aggregation of cellular proteins (25). Members of the HSP 70 family of proteins for example have been shown to interact with nascent polypeptide chains and prevents them from aggregation (26). This functional role of HSPs is consistent with the observation that many of the stress conditions which produce heat shock response has the ability of either damaging proteins directly or causing cells to synthesize or accumulate aberrant proteins. Perhaps the increased accumulation of abnormal proteins signals the need and thus the induction of HSPs. Given that cold can denature protein and denatured protein can induce the heat shock response.

In our study the results revealed that anti HSP65 antibodies were highly significant in expedition members compared with the control group. This means that there was increase in HSP levels in expedition members due to various stresses in Antarctica. This shows that expedition members were more stressed than the control group.

LIMITATIONS

The subjects were not exposed to cold stress throughout the day. On an average they were exposed to higher degree of cold stress for about 3-4 hours per day as they were inside the controlled environment in the station. They were also subjected to other stressors like isolation, alteration of circadian rhythm in polar night and polar days, preserved & desiccated food, separation from family etc. Post expedition analysis was not carried out, which would have given the degree of changes in HSP levels after returning to tropical temperature. Correlation with other biochemical stress markers were not done.

CONCLUSION

In this study we found that there was significant increase in the HSP65 (IgM) in the expedition members of XXVI Indian Scientific expedition to Antarctica in response to 11 months exposure to chronic cold stress. However, further studies in Antarctica on larger sample size, and post expedition immunoglobulin levels would play a vital role in understanding the mechanism of alterations of molecular chaperones levels and to rule out the role of other possible factors involved in elevated level of HSP.

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