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Original Research Article

POTENTIAL ROLE OF HEAT SHOCK PROTEINS 60, 70, AND 90 IN THE AGING AMONG POPULATION OF PUNJAB-PAKISTAN

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Abstract: *Aging can be characterized as the collision of cellular and organ-destructive processes over the years with actions that maintain homeostasis, vitality, and longevity. The current study was based on an assessment of various indicators of stress, Heat shock proteins and antioxidants in elderly subjects to identifying their significance in ageing. It was aimed to increase the heat shock proteins, decreased antioxidants and vitamins responsible for ageing. A total of 100 individuals were recruited in the current study consisting of 50 young control and 50 older people. The significance of HSPs (60, 70, 90) and oxidative and antioxidant markers, were analyzed through laboratory tests and ELIZA kits using serum samples. The results were evaluated through an independent t-test in SPSS version 16. Elder group showed a significantly higher level of HSP-60, HSP-70, and HSP-90 than the control group. According to study results, the anti-oxidant profile, which includes SOD, CAT, and GSH, exhibited a dramatically declining trend in the aged population. MDA and NO, which are indicators of oxidative stress, were also higher in the elderly population than in the young. The study also showed that the elderly had lower vitamin A, C, D, and E levels than the young. The results reflect the increased level of inflammatory markers specifically heat shock proteins in old people compared to healthy control. It is indication that increased level of heat shock proteins are responsible for aging. It can be cured by limiting the heat shock proteins.*

Keywords: Oxidative stress markers, Antioxidant markers, Vitamins, Heat shock proteins

Introduction

The ageing process involves the decreased capability of the cells to manage environmental challenges and increased oxidative stress resulting from the stress response, called heat shock response. It also results from producing reactive oxygen species from electron transport chain reactions (Hemagirri and Sasidharan, 2022). Oxidative damaged proteins accumulate in some diseases connected to aging, namely, Huntington's disease, Multiple sclerosis, Amyotrophic lateral sclerosis and Parkinson's disease (Tansey et al., 2022). Heat shock proteinin the molecular chaperone classhaperones involve endurance and ageing. They play a cytoprotective role under pathological conditions through refolding and repairing newly synthesized or misfolded polypeptides, preventing cells from stress, processing agents involved in immune response, and degradation of irreparable proteins. Among these HSPs, HSP90 and HSP70 attach with unfolded sequences and cause their folding along with the hydrolysis of ATP and are considered mitochondrial proteins (Cater et al., 2022) whereas HSP60 is the important and foremost heat-inducible protein. Other functions include import, transport, refolding, and avoiding aggregation of mitochondrial proteins. HSPs block extrinsic and intrinsic pathways of apoptosis through communication with key proteins at three stages: pre-mitochondria, mitochondria, and post-mitochondria. Anti-apoptotic HSPs include HSP 27, HSP 70, HSP60, and 90 (Kampinga et al., 2009).

The mechanism for HSPs-based attenuation of apoptosis involves inhibition of apoptosome formation, stress kinase activation, increase in levels of glutathione or reduction in aggregation of protein.

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Inhibition of apoptosis proteasome activating factor (APAF1) by HSP90 and HSP70 ultimately inhibits the association of APAF1 with procaspase-9 to form the apoptosome-activating caspase-3. HSP90 constitutes only 2% of proteins involved in ageing and altered homeostasis (Boopathy et al., 2022). Under the stress of high temperatures, lymphocytes and mesenchymal stem cells attenuate the production of HSP90. Anti-apoptotic HSPs include HSP 27, HSP 70, HSP60, and 90 (Kampinga et al., 2009).

The cellular levels of HSPs are increased in reaction to formation of reactive oxygen species (ROS) in cells to protect them from various other types of stresses (Adly et al., 2008). Because of the ageing process and oxidative stress, cells go through a drop in HSPs resulting in a shorter life span (Haslbeck et al., 2008). Levels of heat shock proteins alter with age is still controversial. These proteins have multiple roles in body in which interaction of both nitric oxide and antioxidant mechanisms (Gurbuxani et al., 2001). Many research studies have proved relation of these proteins with reactive oxygen species, although their cellular and molecular mechanisms are not fully understood. Production of ROS is directly linked with lifestyle, diet and professional habits, and the ROS levels may differ in different populations (Gurbuxani et al., 2001).

The current study was based on an assessment of various indicators of oxidative stress in elderly subjects of different districts of Punjab, Pakistan, and identifying the significance of HSPs in ageing and oxidative stress.

Methodology

2.1 Study design

A total of 100 persons were recruited in the study [via, $n= \sigma 2(Z1-\alpha+ Z1-\beta)2/(\mu_o- \mu a)$] which were divided into two groups 50 healthy and 50 aged persons. Blood sample was collected after informed consent. Patients suffering from chronic medical conditions such as COPD, Asthma Cancer, and Cardiovascular diseases were excluded.

2.2 Biochemical analysis

2.2.1 Estimation of superoxide dismutases (SOD)

SOD was performed according to (Rea et al., 2001). 100µl serum was added in 1.2ml of 0.052 mole sodium phosphate buffer (pH 8.3) in a test tube. Then, 300 µl of nitro blue tetrazolium (300µm), 100µl of phenazinemethosulphate (186µm) and 200 µl of NADH (750 µm) were added. The reaction started after adding NADH. 90-second incubation was done at 30 degrees, the synthesis was interrupted by taking 100 liters of glacial acetic acid. Including 4.0 ml of n-butanol, reaction samples were thoroughly agitated and allowed to sit for ten minutes before ultracentrifugation and separating butanol layer. The chromogen's color intensity in the butanol layer was quantified against n-butanol at 560nm and calculation of SOD concentration was done in units/gram.

2.2.2 Estimation of malanodialdehyde (MDA)

200 ml sample was added in 200 ml of 8.1% Na-dodecyl sulfate (SDS) in test tubes. Then, 1.5 ml of of 0.8percent TBA and 1.5 ml of 20 percent solution of acetic acid solution (pH 3.5) were added. The mixture was filled upto 4 ml with distilled water and heated using water bath at ninety degrees for sixty minutes. Subsequently, it was cooled with ordinary H_2O, distilled H_2O (1 ml) and n-butanol (5 ml), agitated thoroughly, and centrifugation was performed for 10 minutes at 4X1000 rotations per minute. The upper butanol layer was obtained and absorbance was taken at 532 nm.

2.2.3 Estimation of catalase (CAT)

Serum (100µl) was added in 1.9ml of 50mM phosphate buffer (pH 7.0) into a test tube. Reaction was initiated after adding 1 ml of 30 mM H_2O_2. Decomposition of H_2O_2 was measured spectrophotometrically at wavelength of 240 nm.

2.2.4 Estimation of reduced glutathione

Reduced glutathione was performed according to protocol (Kalsoom et al., 2022). 1 ml supernatant was used with 0.2M sodium phosphate buffer (pH 8). GSH was taken as standard, which was made up to 2 and 10nM. After measurement with spectrophotometer (412 nm), yellow color was developed in 10 minutes and values were expressed as nM GSH/g sample.

2.2.5 Estimation of glutathione peroxidase (GSH-Px)

20µl of serum and 980µl of the reaction mixture were added into an eppendorf. Then, it was incubated for five minutes at 25 degree celsius. Reaction was started when 0.5ml of 8.8mmol/L H_2O_2 was added and absorbance value was measured at 340nm.

2.2.6 Estimation of glutathione (GSH)

GSH was measured by using method of Maqbool t et al., 2019 (Maqbool et al., 2019) and absorbance was taken at 412 nm.

2.7 Estimation of vitamin C

50µL sample was taken into a test tube. And then 250µl of 20% of TCA was added and stirred to obtain fine suspension and allowed to settle for five minutes. Centrifugation was done at 10000 rotations per minute for five minutes. Superannuated was taken separately. 300µL of supernatant was taken into falcon tubes. And then 3ml 2% CMC (carboxy methyl cellulase) was added. After that, 600µL of 2% Selenium dioxide was added. Volume was taken up by adding 7.5ml distilled H_2O in test tube and absorbance was read at 380nm.

2.8 Estimation of vitamin E

1ml of serum was added into a test tube and 0.2ml of 0.2 percent Bathophenanthroline in ethanol and 0.2 milliliter of 0.001 M Fe_3Cl was added. Then it was mixed, vortexed and incubated for one minute. Then 0.2milliliter of 0.001 M H_3PO_4 solution was added &

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mixed with chemicals, and absorbance was taken at 534 nm.

2.9 Determination of vitamin A

According to Mansoor et al., 2022 (Mansoor et al., 2022), vitamin A was determined.

2.10 Determination of nitric oxide

Component A (*N*-(1-naphthyl)ethylenediamine) and component B (sulfanilic acid) were mixed in equal amount which formed Griess Reagent which was added (100 µl) in nitrite containing sample (300 µl) and deionized water (2.6 ml) in spectrophotometer cuvette: The mixture was incubated for 30 minutes at 25 degree celsuis. Mixture of Griess Reagent (100 µl) and deionized water (2.9 ml) prepared a photometric reference sample. Absorbance was measured at 548 nm.

2.11 Estimation of HSP60, 70 and 90 by ELISA KIT

ELISA of anti-HSP60, 70 and 90 were performed according to Bio-Vendor as mentioned in the following study (Maqbool et al., 2019; Abid et al., 2020). Briefly, the concentration of these antibodies was measured in human serum sample. Absorbance was taken at 450nm. The antibodies content was calculated using the formula mentioned in kit.

2.12 Statistical analysis

Serum levels will be described as mean ± S.D. Mean levels will be compared in two groups by independent sample T-test. Significance will be set as P< 0.05. SPS version 16 will be used for analysis.

Results

3.1 Hematological profile of aged group vs young control

Table no 1 and figure 1 depict the hematological profile of older patients compared to young control subjects. The low levels of red blood cells (RBCs) and hemoglobin were measured in aged people compared to control individuals. The mean values of white blood cells (WBCs) were increased in the aged population compared to control, and platelets decreased in the ageing population. The decreased levels of Hct% and total bilirubin were observed in elderly subjects compared to control individuals, respectively.

Table 1. Hematological profile of aged group vs young controls

ARIABLES	CONTROL (N= 50)	AGED GROUP (N=50)	*P*-VALUE
RBCs (million/mm^3)	4.92 ± 1.08	4.5 ± 0.957	0.01
WBCs (million/mm^3)	8.23 ± 3.057	10.24 ± 2.06	<0.001
Hbg/dl	14.25 ± 4.19	10.25 ± 3.09	<0.001
PLTs10^9 /L	311.25 ± 24.29	165 ± 15.029	0.01
Hct%	40.23 ± 9.65	35 ± 14.25	0.01
T. Bilirubin (mg/dl)	0.8 ± 0.0056	3.6 ± 0.59	<0.001

3.2 Circulatory stress markers profile of aged group vs young controls

The mean value of MDA,GSH-Px, GSH-PR and NO was increased in an elderly group compared to the control group. This shows that MDA level increases with ageing process compared to young individuals. Levels of antioxidants SOD, GSH and Catalase were decreased in the aged population compared to young ones. Levels of vitamins have shown a distinct result according to their presence and character in the ageing process. We have found the lower values of VIT-A (477.09±52.26mg/dL vs. 588.66±24.25mg/dL), VIT-C (0.325±.015mg/dL vs. 0.526±.0566mg/dL), VIT-E (0.323±.0018mg/dL vs. 0.235±.009mg/dL), and VIT-D (9.8±1.29mg/dL vs. 13.26±2.25mg/dL), in older individuals as compared to healthy young ones. As shown in figure table no 2 figure 3 and 4.

Table 2. Stress markers profile of aged group vs young controls

VARIABLES	CONTROL (N= 50)	AGED GROUP (N=50)	*P*-VALUE
MDA (nmol/ml)	1.03 ± 0.957	4.26 ± 1.09	<0.001
SOD (U/gHb)	0.237 ± 0.058	0.016 ± 0.00157	0.02
GSH (µmol/L)	9.24 ± 3.28	2.37 ± 0.569	0.03
CAT (U/gHb)	3.77 ± 0.957	1.34 ± 0.659	0.01
VIT A (mg/dL)	588.67 ± 24.26	477.08 ± 52.27	0.01
VIT C (mg/dL)	0.527 ± 0.057	0.326 ± 0.016	0.02
VIT E (mg/dL)	0.335 ± 0.008	0.324 ± 0.0019	0.01
VIT D (mg/dL)	13.36 ± 2.35	9.7 ± 1.19	<0.001
NO (µmol/L)	20.66 ± 3.165	67.48 ± 8.79	0.03
GSH-Px (µmol/ml)	9.45 ± 1.69	6.5 ± 1.18	0.02
GSH-Pr (µmol/ml)	2.43 ± 0.69	2.64 ± 0.856	0.03

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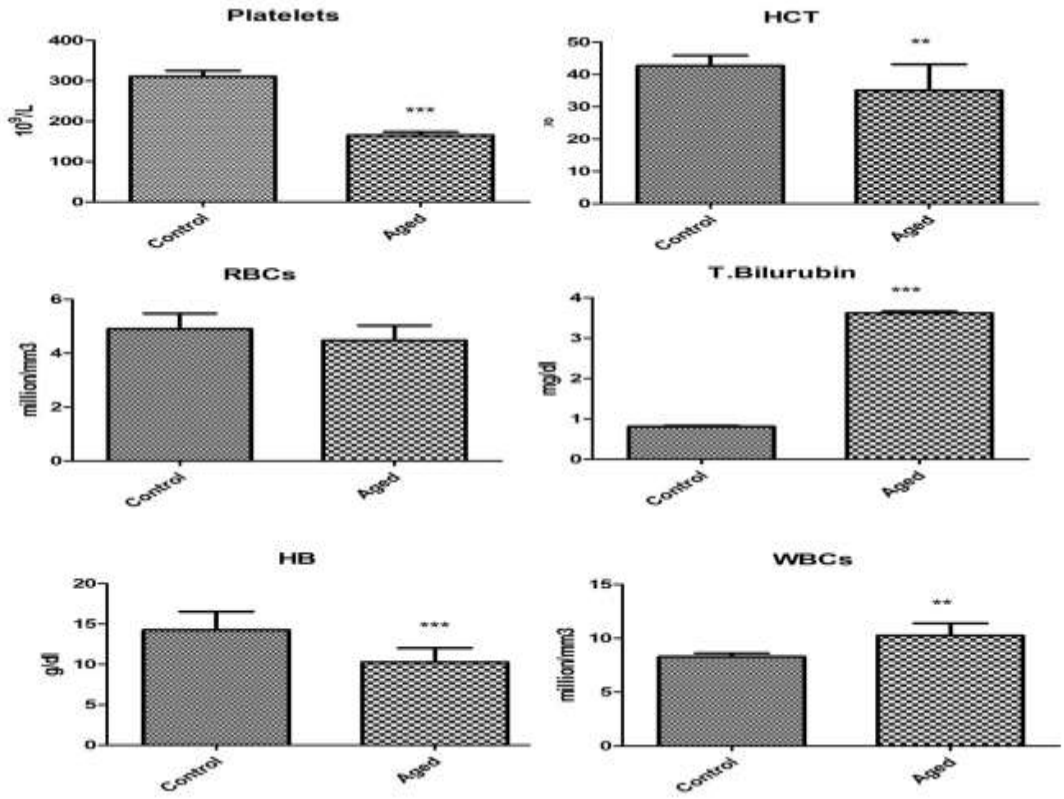

Fig 1 is showing hematological profile of aged group vs young controls. Where increased level of WBCs, T.Bilurubin and decrease level of RBCs, HCT, HB, Platelets can be observed in aged group compared to control. ** and *** showing significant difference between control and aged group, P≤0.05.

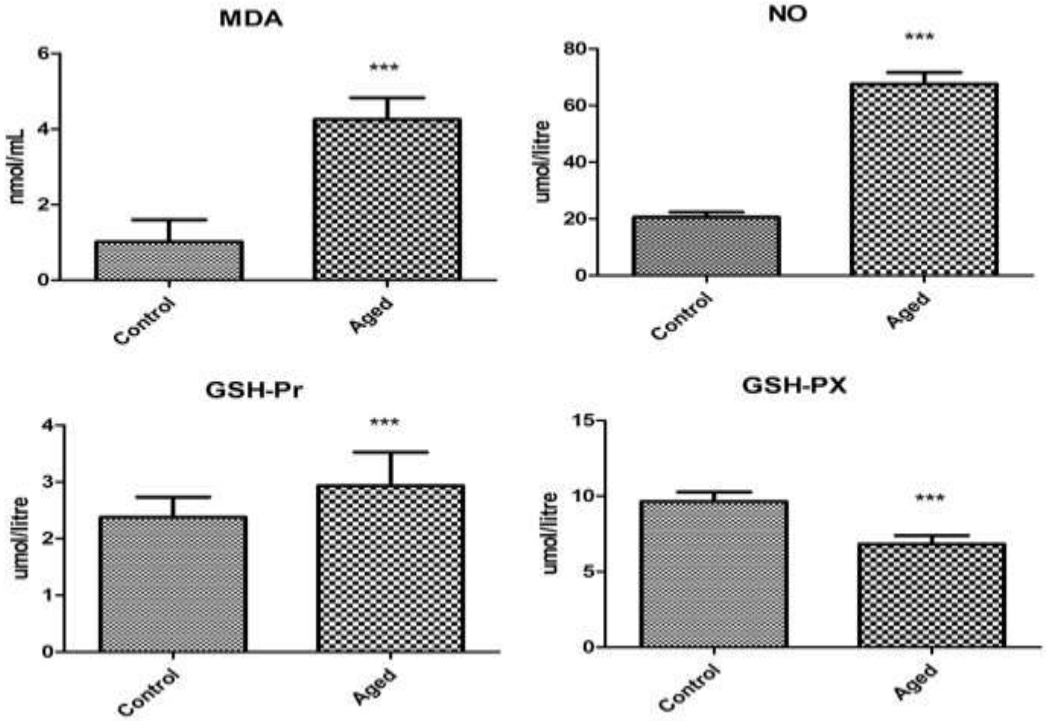

Fig 2 is showing increased stress marker in aging group compared to control where *** showing the significance difference between control and aging group. P≤0.05.

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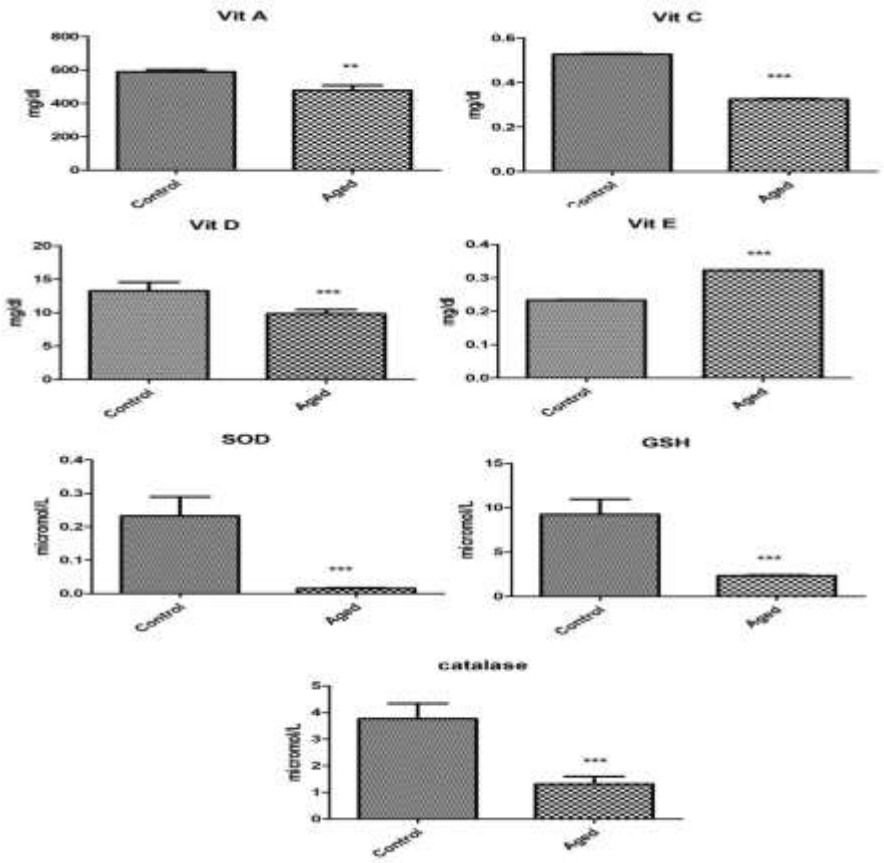

Fig 3 is showing decreased antioxidant and vitamins level in aging group compared to control where *** showing the significance difference between control and aging group. P≤0.05.

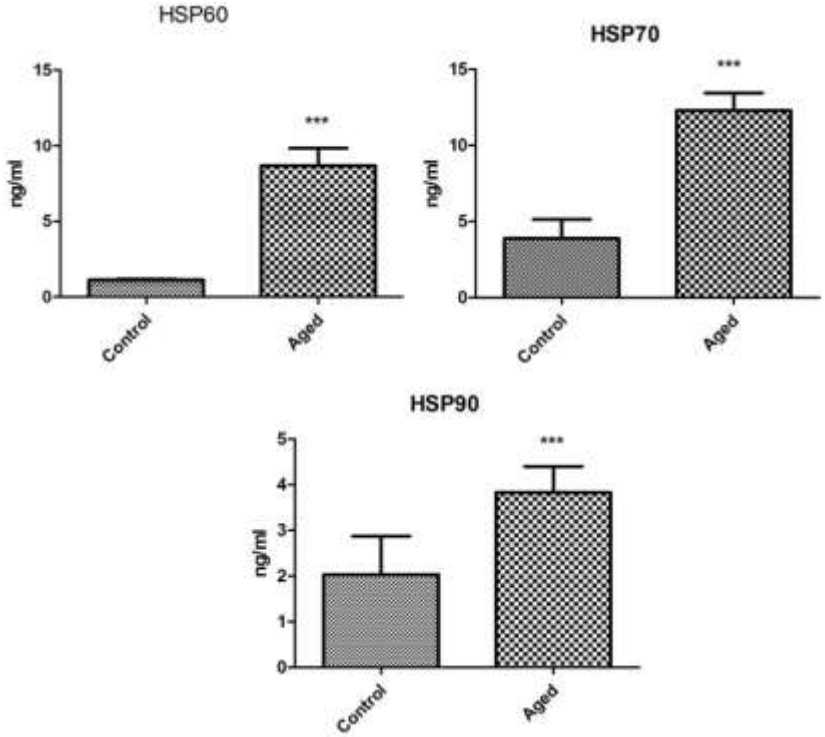

Fig 4 showing increased level of HSP60,70 and 90 in aging group compared to control where *** showing the significance difference between control and aging group. P≤0.05.

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3.3 Inlfammatory markers profile of aged group vs young controls

The results depicted in Figure 4 reflect that inflammatory markers, specifically heat shock protein behaviour in old people, is statistically highly significant ($p \leq 0.05$). The data interpretation of HSP-60, HSP-70, as well as HSP-90, shows significantly increased levels in the old age group (8.68±2.085ng/ml, 17.28±3.295ng/ml, 3.82±0.956ng/ml) in comparison with young subjects (1.18±0.095ng/ml, 4.99±1.085ng/ml, 2.01±1.056 ng/ml).

Table 3. Inlfammatory markers profile of aged group vs young controls

VARIABLES	CONTROL (N= 50)	PATIENTS (N=50)	*P*-VALUE
HSP-60 (ng/ml)	1.18 ± 0.095	8.68 ± 2.085	0.01
HSP-70 (ng/ml)	4.99 ± 1.085	17.28 ± 3.295	0.02
HSP-90 (ng/ml)	2.01 ± 1.056	3.82 ± 0.956	0.03

Discussion

The current study was based on the calculation of various stress markers in older adults accompanied by identifying the role of HSPs in ageing, relevant to the work of Balkan and co-workers (Balkan et al., 2002). In several studies on laboratory animals, other scientists have discovered the function of oxidative damage. Humans, on the other hand, have little evidence. According to ageing theory of free radical and oxidative stress published in 1956 by Denham Harman, oxidative damaged DNA and proteins increase with age. In our study, we aimed to check and testify to how the response of heat shock proteins is activated. The large range of depilatory disorders in which oxidative stress plays a significant role would greatly benefit from an effective Hsp-based therapy (Kalmar and Greensmith, 2009). The two ageing theories that survived were free radical and mitochondrial theories. According to such beliefs, oxidative stress in mitochondria can result in cyclic event in which damaged mitochondria produce more reactive oxygen species than normal, which in turn causes damage to gradually worsen (Romano et al., 2010). Malondialdehyde (MDA) was an essential marker identified in our research. According to our study and data from previous studies, levels were increased in elderly subjects. Furthermore, there is no difference in MDA concentrations between males aged 20-25 years and men aged 39-49 years when comparing the results of this study with the results of a prior publication (control group at 19). In our study, SOD was decreased in the elderly subjects, but some observational studies suggested that high SOD levels are associated with decreased mortality (Kalmar and Greensmith, 2009). Results of some scientists concluded that women had greater SOD activity than men, which is perhaps one of the striking findings Semenova et al., 2022). However, the mechanism of differences in levels of SOD between males and females remains unclear, but possible reasons include differences in metabolic rates, leading to different levels of oxidative damage. The other reason could be endocrinological differences, resulting in different sensitivity and responses to oxidative stress. Glutathione (GSH) is a non-protein thiol. Our research found its levels in elderlyolder people to be lower. In general, any disease associated with elevated points of ROS lowers GSH levels or the GSH / GSSG ratio. In a study conducted by Liu et al. (2015) (Liu et al., 2015), the role of HSP was investigated in oxidative stress conditions induced by selenium deficiency. They concluded that MDA level was increased, and glutathione peroxidase (GSH-Px) and GSH activities decreased in the Selenium-deficient group. Additionally, levels of mRNA and expression of HSPs raised in Selenium-deficiency group in comparison with control group. GS-protein synthesis results from the oxidation of GSH after the cells are exposed to induction of HSP response. A study concluded the hypothesis that the activity of GPx decreases with age in moderately disabled women. In an investigation of moderately aged members of 55–69, no connection was found between GPx levels and age (Viña et al., 2005). An examination showed that GPx action is generally steady until age 65, and afterwards starts to decrease. This result is supported by in vitro research of adult neutrophils, which show lesser amounts of GPx. Results displayed validity of the evidence that GPx declines beyond 65 is substantial, predictable, and age-related (Kudva et al., 2015). In our study, the levels of Catalase (CAT) were observed to be decreased in the aged subjects compared to the young subjects, whereas, in some studies, its levels were found to be increased in women over 30 years. In the peroxisome of Arabidopsis thaliana, tiny heat shock protein Hsp17.6CII (AT5G12020) is interacted with and activate catalases (Liu et al., 2015). Any medication that is effective in decreasing levels of oxidative stress will likely have a significant impact on the treatment of a wide range of disorders because oxidative stress plays a significant part in a variety of diseases and their mechanisms (Forman and Zhang, 2021). Several studies have shown that activating HSF1 and increasing formation of HSPs and co-chaperones, both protect cells against the harmful effects of protein misfolding, apoptosis, and inflammation (Kalmar and Greensmith, 2009). HSPs protect cells in

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conditions of severe shock, such as ischemia (Manikandan et al., 2022). Many stimuli can induce HSP expression in various cells, making them important for therapeutic use. Though the factors affecting age related effects on HSP-70 are unspecified, in vitro, research indicates that age related differences in intracellular HSP70 formation are due to a lack of transcription of heat shock genes, which is due to lower activation and binding of the HSF1 to HSE (Rea et al., 2021). In the current research, it was found that serum HSP70 levels decreased with age. When only participants with detectable HSP70 levels were included in the study and the few removed, no noteworthy difference was found amongst the HSP70 levels of young control subjects compared to older healthy subjects. Accordingly, this could contradict our results for intracellular HSP70 (Njemini et al., 2007). One studies had similar results to the current study that the serum HSP70 is elevated with ageing (Rea et al., 2001). Levels of vitamins have shown a different results according to their presence and character in the ageing process. Different vitamins have demonstrated their levels considerably different in AGEs group as compared to young individuals. We have found lower values of vitamin-A in older individuals as compared to healthy young ones. Hematological profile was also studied between older people and the young subjects. The low levels of red blood cells (RBCs) and hemoglobin were recorded in aged people compared to control individuals.

Conclusion

Our research focused on several indicators of oxidative stress in the elderly, and the involvement of heat shock proteins in the aging process. It was based on comparing three factors, namely hematological profile parameters, circulatory stress indicators and inflammatory marker profile parameters, comparing older and young participants. According to current study, elevated HSP70, HSP60 and HSP90 levels are linked with inflammation and weakness in old patients. Moreover, a specific response was observed in each of the oxidative stress markers, where MDA and NO were elevated in aged subjects, whereas levels of anti-oxidants and vitamins were shown to be decreased in elderly subjects. Most of the hematological parameters were found to be reduced. By limiting the level of Heat shock proteins, the process of ageing can be prevented.

Limitations

Some important limitations should be considered when interpreting the results of this study. Individuals with a chronic conditions such as COPD, Asthma, Cardiovascular illness, Alzheimer's were excluded. This can be a limitation because levels of oxidative stress and heat shock proteins could vary due to such conditions.

Conflict of interest

The authors declared absence of conflict of interest.

References

Abid, F., Saleem, M., Muller, C. D., Asim, M. H., Arshad, S., Maqbool, T., & Hadi, F. (2020). Anti-proliferative and apoptosis-inducing activity of Acacia Modesta and Opuntia Monocantha extracts on HeLa cells. *Asian Pacific Journal of Cancer Prevention: APJCP*, **21**(10), 3125.

Adly, M. A., Assaf, H. A., & Hussein, M. R. A. (2008). Heat shock protein 27 expression in the human testis showing normal and abnormal spermatogenesis. *Cell Biology International*, **32**(10), 1247-1255.

Balkan, J., Kanbagli, Ö., Mehmetçik, G., Mutlu-Türkoglu, Ü., Aykaç-Toker, G., & Uysal, M. (2002). Increased lipid peroxidation in serum and low-density lipoproteins associated with aging in humans. *International Journal For Vitamin and Nutrition Research*, **72**(5), 315-320.

Boopathy, L. R. A., Jacob-Tomas, S., Alecki, C., & Vera, M. (2022). Mechanisms tailoring the expression of heat shock proteins to proteostasis challenges. *Journal of Biological Chemistry*, 101796.

Cater, J. H., Mañucat-Tan, N. B., Georgiou, D. K., Zhao, G., Buhimschi, I. A., Wyatt, A. R., & Ranson, M. (2022). A novel role for plasminogen activator inhibitor type-2 as a hypochlorite-resistant serine protease inhibitor and holdase chaperone. *Cells*, **11**(7), 1152.

Forman, H. J., & Zhang, H. (2021). Targeting oxidative stress in disease: Promise and limitations of antioxidant therapy. *Nature Reviews Drug Discovery*, **20**(9), 689-709.

Gurbuxani, S., Bruey, J. M., Fromentin, A., Larmonier, N., Parcellier, A., JaÈaÈttelaÈ, M., ... & Garrido, C. (2001). Selective depletion of inducible HSP70 enhances immunogenicity of rat colon cancer cells. *Oncogene*, **20**(51), 7478-7485.

Haslbeck, M., Kastenmüller, A., Buchner, J., Weinkauf, S., & Braun, N. (2008). Structural dynamics of archaeal small heat shock proteins. *Journal of molecular biology*, **378**(2), 362-374.

Hemagirri, M., & Sasidharan, S. (2022). Biology of aging: Oxidative stress and RNA oxidation. *Molecular Biology Reports*, **49**(6), 5089-5105.

Kalmar, B., & Greensmith, L. (2009). Induction of heat shock proteins for protection against oxidative stress. *Advanced drug delivery reviews*, *61*(4), 310-318.

Kalsoom, A., Altaf, A., Ashraf, M., Ali, M. M., Aftab, S., Sattar, H., ... & Maqbool, T. (2022). In Vitro Evaluation of Cytotoxic Potential of

Caladium lindenii Extracts on Human Hepatocarcinoma HepG2 and Normal HEK293T Cell Lines. *BioMed Research International*, *2022*.

Kampinga, H., Hageman, J., Vos, M., Kubota, H., Tanguay, R., & Bruford, E (2009). Guidelines for the nomenclature of the human heat shock proteins. Cell Stress and Chaperones. **14**(1):105-11.

Kudva, A. K., Shay, A. E., & Prabhu, K. S. (2015). Selenium and inflammatory bowel disease. *American Journal of Physiology-Gastrointestinal and Liver Physiology*, **309**(2), G71-G77.

Li, G., Li, J., Hao, R., & Guo, Y. (2017). Activation of catalase activity by a peroxisome-localized small heat shock protein Hsp17. 6CII. *Journal of Genetics and Genomics*, **44**(8), 395-404.

Liu, C. P., Fu, J., Xu, F. P., Wang, X. S., & Li, S. (2015). The role of heat shock proteins in oxidative stress damage induced by Se deficiency in chicken livers. *Biometals*, **28**, 163-173.

Lu, C., Yang, D., Klement, J. D., Colson, Y. L., Oberlies, N. H., Pearce, C. J., ... & Liu, K. (2022). H3K9me3 represses G6PD expression to suppress the pentose phosphate pathway and ROS production to promote human mesothelioma growth. *Oncogene*, **41**(18), 2651-2662.

Manikandan, P., Vijayakumar, R., Alshehri, B., Senthilkumar, S., Al-Aboody, M. S., Veluchamy, A., & Haribaskar, R. (2022). Exploring the biological behavior of Heat shock protein (HSPs) for understanding the Anti-ischemic stroke in humans. *Journal of Infection and Public Health*, **15**(4), 379-388.

Mansoor, G., Tahir, M., Maqbool, T., Abbasi, S. Q., Hadi, F., Shakoori, T. A., ... & Ullah, I. (2022). Increased Expression of Circulating Stress Markers, Inflammatory Cytokines and Decreased Antioxidant Level in Diabetic Nephropathy. *Medicina*, **58**(11), 1604.

Maqbool, T., Awan, S. J., Malik, S., Hadi, F., Shehzadi, S., & Tariq, K. (2019). In-vitro anti-proliferative, apoptotic and antioxidative activities of medicinal herb Kalonji (Nigella sativa). *Current pharmaceutical biotechnology*, **20**(15), 1288-1308.

Njemini, R., Lambert, M., Demanet, C., Kooijman, R., & Mets, T. (2007). Basal and infection-induced levels of heat shock proteins in human aging. *Biogerontology*, **8**, 353-364.

Rea, I. M., McNerlan, S., & Pockley, A. G. (2001). Serum heat shock protein and anti-heat shock protein antibody levels in aging. *Experimental gerontology*, **36**(2), 341-352.

Romano, A. D., Serviddio, G., De Matthaeis, A., Bellanti, F., & Vendemiale, G. (2010). Oxidative stress and aging. *Journal of nephrology*, **23**, S29-36.

Semenova, N. V., Rychkova, L. V., Darenskaya, M. A., Kolesnikov, S. I., Nikitina, O. A., Petrova, A. G., ... & Kolesnikova, L. I. (2022). Superoxide dismutase activity in male and female patients of different age with moderate COVID-19. *Bulletin of Experimental Biology and Medicine*, **173**(1), 51-53.

Tansey, M. G., Wallings, R. L., Houser, M. C., Herrick, M. K., Keating, C. E., & Joers, V. (2022). Inflammation and immune dysfunction in Parkinson disease. *Nature Reviews Immunology*, 1-17.

Viña, J., Borrás, C., Gambini, J., Sastre, J., & Pallardó, F. V. (2005). Why females live longer than males? Importance of the upregulation of longevity-associated genes by oestrogenic compounds. *FEBS letters*, **579**(12), 2541-2545.

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