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COUNTENANCE OF HEAT SHOCK PROTEIN-70 AND GONADAL AXIS PROFILE IN AGEING MALES: STUDY FROM FAISALABAD-PUNJAB-PAKISTAN

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ABSTRACT

Aging is a complex process of gradual deterioration of physiological functions including fertility. Different explanations have been given, most accepted one is the oxidative stress theory of aging and infertility. Oxidative stress is an imbalance between ROS and anti-oxidant defense system of the humans. During the aging process, there is constant production of oxidative proteins which causes the partial unfolding of proteins leading to aggregates. This protein aggregation leads to a defective cellular proteolytic system which results further production of oxidative products causing damage to macromolecules and apoptotic cell death.Fifty healthy male subjects between the age 20-85 were included in the study. We examined the natural alterations in hypothalamic pituitary gonadal axis in aging male of south East Asia. Normal age-related changes in serum levels of testosterone, LH, FSH and SHBG were noted. HSP 70 relation with these observations was established to check the age-related changes effected on general health status. A questionnaire was used to exclude the systemic diseases, smoking and risk factors. BMI was calculated. RBS and CBC were performed on the same day after centrifugation of sample within two hours. All assays were performed under the standard procedures by manufacturers in the lab by skilled staff. The hormonal assay kit was used for testosterone, LH, FSH and SHBG. The results received and entered in excel sheets. The quantitative sandwich ELISA kit was used to determine the HSP 70 levels in undiluted human serum samples.Maximum range of Hb was observed in the male individual (15.48±11.02) with the age group (21-40) as compared to other

groups. The mean values of MCV, MCH and MCHC were significantly high (5.45±0.31, 82.56 ±5.07 and 29.20±0.00) in Group (81-100), Group (61-80) and Group (1-20)respectively, as compared to other age groups. Nevertheless, the mean values of WBC, neutrophils, lymphocytes, eosinophils and platelets were significantly higher (33.45± 1.77 s/L, 7.47±2.40 %, 63.50±7.72%, 1.50± 0.57 %, and 1.50± 0.57%) in older age group (81-100) correspondingly as compared to other age groups. Contrary to that, the mean values of monocytes and RBC were significantly increased (38.00± 0.00 % and 280.2 ±125.9s/L) in Group (1-20) and Group (41-60) respectively. The mean value of HSP-70 was statistically significantly increased (415.26±11.08 ng/ml) in older age group as compared to other groups with P-value 0.01. Meanwhile, the mean values of LH and testosterone were significantly high (7.16±0.00 mIU/ml and 551.01±0.00 mIU/ml) in age group (I-20) respectively. On the other hand, the mean values of SHBG (38.87±17.57 nmol/l) and FSH (6.70±6.27 mIU/ml) were increased in age Group (21-40) and Group (61-80) respectively, with the contrary to the other age groups.In the present study, it has been concluded that heat shock protein-70 (HSP-70) has a significant role to upregulate the production of free radicals and oxidative stress during ageing. HSP-70 has been used not only as a biomarker for the estimation of endogenous oxidative DNA damage, but also a major risk factor to cause ageing in males.

Keywords: Ageing, LH, FSH, SHBG, HSP-70

INTRODUCTION

Aging is the decline opposition of the body to external environmental stress. Aging is blend of changes in biological, physiological, environmental, psychological, behavioral and social process. In comparing to females who has complete and sudden stoppage of ovarian function in phases of menopause, older males mostly have their normal androgen levels by the testes **[1]**. On the other hand, the cross sectional studies show that in advancing age, there is some lesser levels of testosterone, which still need to be evaluated. There are differences in study design, patient numbers, assay techniques and inclusion criteria. Anticipated mechanisms for an age-related changes in testosterone production include, defects in the hypothalamic-pituitary-testicular axis, an increase in sex hormone binding globulin levels, environmental factors, medication use and chronic illness **[2]**. Throughout ageing, the secretory patterns of the hormones formed by the hypothalamic-pituitary axis change.

Furthermore, glucose homoeostasis are also linked with increasing age. In addition, age related effects are tricky to sort out from the control of other factors that are common in aged people such aschronic diseases, inflammation and low nutritional values, all together affect endocrine systems. Usually, the less hormonal activity through the ageing process has been measured to be unfavorable because of the interrelated decline in physical functions **[3]**. All the way through life, the functions of the human body begin to slow but surely decline. Ageing is characterized by changes in practically all organic systems. Nevertheless, other factors such as inflammation and calorie intakealso affect the ageing process and are often associated with age-related chronic diseases. These factors are responsible to influence hormonal activity, which is difficult to distinguish and clarify in clinical practice. During ageing, the secretory patterns of hormones produced by the hypothalamus-pituitary axis change, as it causes sensitivity to negative feedback by end hormones **[4]**. Aging and degeneration of tissues is caused by accumulated damage of macromolecules of adult cells. Oxidative stress and glycation have the capacity of protein, lipid and DNA damage.Free radicals are reactive chemical species, containing unpaired valence electrons in their outer orbit. These unpaired electrons make free radicals, highly reactive towards carbohydrates, lipids, proteins, nucleic acids, and other cellular molecules. Free radicals arise from normal cell metabolism or can be produced by exogenous sources, including radiation, herbicides, cigarette smoking, chronic stress, alcohol abuse, some drugs, and air pollution **[5]**. In mitochondria, oxygen is converted to highly reactive oxygen molecules called reactive oxygen species (ROS). Low and medium concentrations of ROS are involved in cellular resistance against infection mediators, signal transduction, and reaction against mitogens, carboxylation, peroxidation and reduction of ribonucleotides. ROS cause DNA damaging which contains base variations, basic sites, single strand and double strand DNA breakdowns and DNA protein cross-links **[6]**. Reactive oxygen and nitrogen species are formed by numerous endogenous and exogenous progressions, and their negative effects are neutralized by antioxidant defenses. Oxidative stress occurs from the disparity between ROS production and these antioxidant defenses. Aging is a process categorized by the advanced loss of tissue and organ function **[7]**. The oxidative stress theory of aging is grounded on the

proposition that age-associated efficient losses are due to the growth of RONS-induced damages. ROS are substantial mediators in signal transduction mechanisms.Overexpression of ROS leads to amplified oxidative stress. High oxidative stress causes an increase in lipid per oxidation. High levels of ROS are responsible to decline the activity of sperm **[8]**. Various studies have described the relationship between sperm motility and leukocyte contamination in ejaculated semen. The great body of evidence suggested that ROS production, lipid peroxidation, oxidative DNA damage, sperms DNA damage and apoptosis are the major causative factors to decline sperm motility and strength. A number of studies have also observed higher levels of DNA breakage and production of broken higher DNA fragmentation index (DFI) in older men **[9]**.

MATERIALS AND METHODS

PHYSICAL MEASUREMENTS AND SAMPLE COLLECTIONS

A questionnaire was made to collect all personal information, medical history, lifestyle risk factors, smoking history, height, head circumference and blood pressure were measured by using standard methods. BMI was calculated by using the standard formula as weight in Kg divided by height in meter squares. A sample was taken by drawing 6ml blood from the cubital vein by standard methods and protocols. The sample was centrifuged within first 2hours of sample collections. Random blood sugar and CBC was performed the same day.Remaining samples were stored at -80 C until next use. CBC counter Boule Medonic AB manufactured by MERCK SWEDEN was used to perform a complete blood count. Blood sugar was analyzed by Techno 786 serial no E113991, GMI manufactured.

INCLUSION AND EXCLUSION CRITERIA

The present study includes fifty male subjects aged between 20-85 years. All data were collected from the cross sectional prospective study. In this study, we examined the natural alterations in hypothalamic pituitary gonadal axis in aging male of South East Asia. Normal age related changes in serum levels of testosterone, LH, FSH, SHBG and HSP-70 were established to check the age related changes effected on general health status. The study protocol was approved by Institutional review board and research ethical committee, The university of Lahore. The sample was collected from Faisalabad division Punjab-Pakistan. The written consent was taken from the subjects after explaining the study prospects. The

selected subjects were chosen on the base that they don't have any acute or chronic disease affecting hypothalamic pituitary gonadal axis. It was checked that these subjects didn't use any medications or supplements having effects on body growth and metabolism. The subjects with severe communication skills and acute illness were excluded. The subjects less than 18years old were not included in this study.

BIOCHEMICAL ANALYSIS

All assays were performed under the standard procedures by manufacturers in the lab. Total serum testosterone, FSH, LH and SHBG measurements were done by frozen serum aliquots using competitive chemiluminescence enzyme immunoassay machine Alinity Ci. It is a compact immunoassay system that maximizes CHEMIFLEX chemiluminescence technology, which is Chicago USA made. The hormonal assay kit was used for testosterone Lot no [107850P00] and reference no [07D68-22].FSH Lot no [91274U100] and reference no [07P49-30] was used. LH Lot no [90017U100] and reference no [7P91-20] and for SBHG Lot no [00231L818] and reference no [09P38-20] were used. The results received and entered in excel sheets. Quantitative sandwich ELISA kit APCAM was used to determine the HSP-70 levels in undiluted human serum sample. Sensitivity of the kit was 10pg/ml with a detection range of 125 pg/ml -4000 pg/ml.

STATISTICAL ANALYSIS

Statistical analysis was performed by SPSS statistics 17.0. The results of all variables wereevaluated by using one way ANOVA.

RESULTS

DEMOGRAPHIC FILE OF HEALTH MALE INDIVIDUALS

In **Table 01**, the demographic file of healthy male individuals showed that the mean values of age and BMI were significantly increased (83.50±2.38 and 29.30±2.58) in older age group (81-100) respectively as compared to other age groups. On the other hand, the mean values of weight and height were significantly higher (76.61±16.23kg and 173.21±7.36cm) in the age ranges 41-60 and 61-80 correspondingly.

HEMATOLOGICAL PROFILE OF HEALTHY MALE INDIVIDUALS WITH DIFFERENT AGE GROUPS

In **Table 02**, variables determined in the normal healthy male individuals divided into different age groups, i.e., Group (1-20), Group (21-40), Group (41-60), Group (61-80) and Group (81-100). The levels of haemoglobin (Hb) were significantly

different in all age groups. Maximum range of Hb (15.48±11.02) was observed in the male individual with the age group 21-40 as compared to other groups. The mean values of MCV, MCH and MCHC were significantly high (5.45±0.31, 82.56±5.07 and 29.20±0.00) in Group (81-100), Group (61-80) and Group (1-20) respectively, as compared to other age groups. Nevertheless, the mean values of WBC, neutrophils, lymphocytes, eosinophils and platelets were significantly higher (33.45± 1.77 s/L, 7.47 ±2.40 %, 63.50±7.72%, 1.50± 0.57 %, and 1.50± 0.57%) in older age group (81-100) correspondingly as compared to other age groups. Contrary to that, the mean values of monocytes and RBC were significantly increased (38.00± 0.00 % and 280.2 ±125.9s/L) in Group A (1-20) and Group C (41-60) respectively.

GONADAL AXIS PROFILE OF HEALTHY MALE INDIVIDUALS WITH DIFFERENT AGE GROUPS

In **Table 03, Figure 01** the hormonal profile of healthy male individuals was determined in different age groups. The mean value of HSP-70 was statistically significantly increased (415.26±11.08 ng/ml) in older age group as compared to other groups with P-value 0.01. Meanwhile, the mean values ofLH and testosterone were significantly higher (7.16±0.00 mIU/ml and 551.01±0.00 mIU/ml) in age group (I-20) respectively. On the other hand, the mean values of SHBG (38.87±17.57 nmol/l) and FSH (6.70±6.27 mIU/ml) were increased in age Group (21-40) and Group (61-80) respectively, with the contrary to the other age groups.

Table 01: Demographic Profile of Normal Healthy Male Individuals

VARIABLES	GROUP	MEAN±STD	P-VALUE
Age(year)	1-20	20.00±0.00	0.00
	21-40	31.31±6.06	
	41-60	52.31±5.99	
	61-80	72.21±6.56	
	81-100	83.50±2.38	
Weight (kg)	1-20	65.00±0.00	0.296
	21-40	70.23±11.99	
	41-60	76.61±16.23	
	61-80	71.21±12.69	
	81-100	73.28±13.42	
Height (cm)	1-20	170.00±0.00	0.361
	21-40	169.84±5.63	
	41-60	168.15±7.41	
	61-80	173.21±7.36	
	81-100	170.00±7.25	

BMI	**1-20**	**22.50±0.00**	**0.80**
	21-40	**24.33±3.76**	
	41-60	**26.99±5.17**	
	61-80	**23.68±4.35**	
	81-100	**29.30±2.58**	

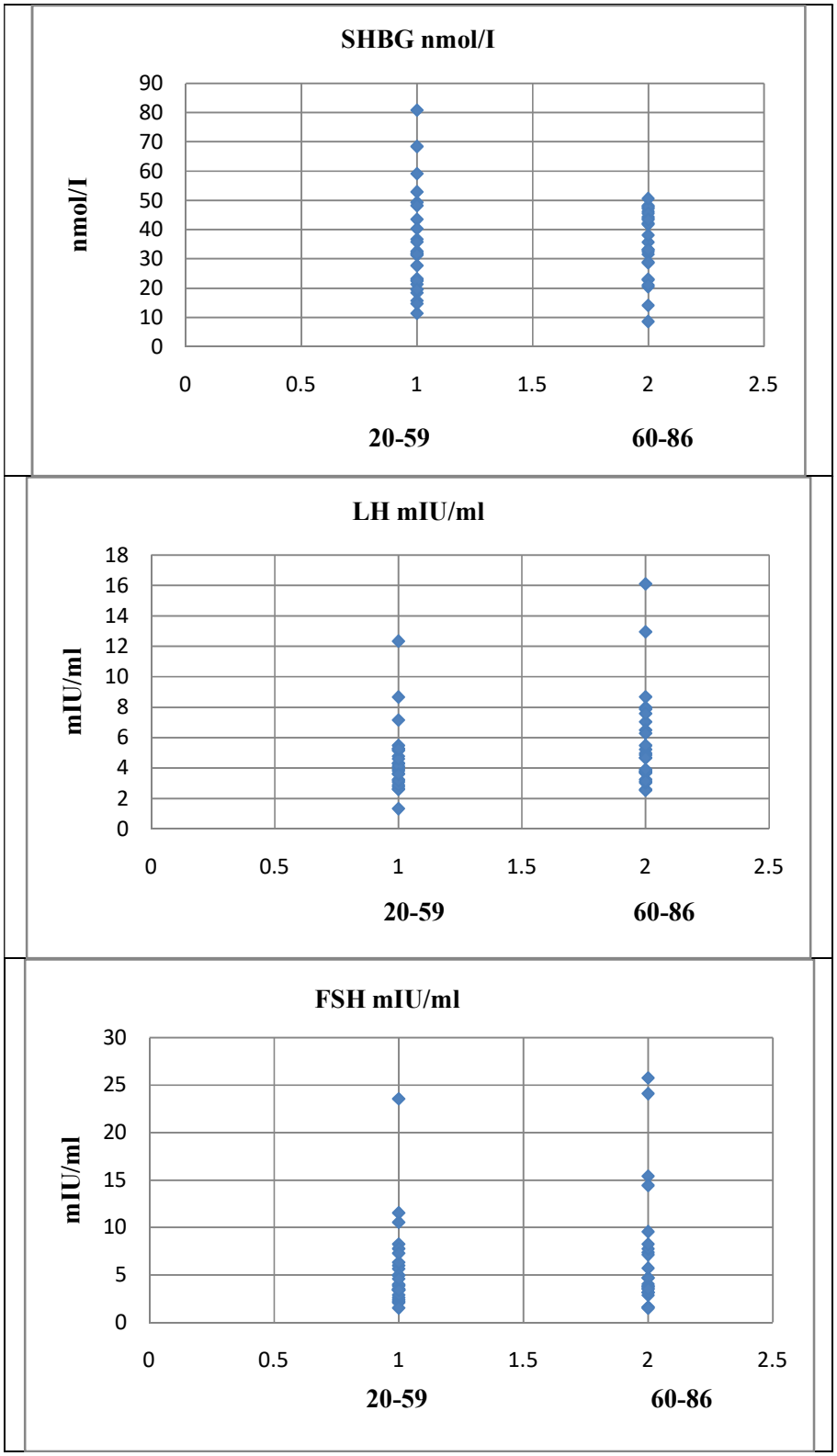
Table 02: Hematological Profile of Normal Healthy Male Individuals

Variables	Group	Mean±Std	P-value
HB g/L	**1-20**	**14.50±0.00**	**0.824**
	21-40	**15.48±11.02**	
	41-60	**13.50±2.26**	
	61-80	**13.93±1.64**	
	81-100	**14.95±1.89**	
MCV	**1-20**	**4.97±0.00**	**0.553**
	21-40	**5.23±0.71**	
	41-60	**5.06±0.38**	
	61-80	**5.09±0.55**	
	81-100	**5.45±0.31**	
MCH	**1-20**	**86.10±0.00**	**0.657**
	21-40	**80.46±7.84**	
	41-60	**82.24±5.78**	
	61-80	**82.56±5.07**	
	81-100	**81.00±4.84**	
MCHC	**1-20**	**29.20±0.00**	**0.974**
	21-40	**27.14±4.21**	
	41-60	**27.37±2.62**	
	61-80	**27.45±2.41**	
	81-100	**27.07±2.90**	
WBCs/L	**21-40**	**33.27±2.25**	**0.462**
	41-60	**33.15±1.42**	
	61-80	**33.24±1.17**	
	81-100	**33.45± 1.77**	
Neutrophils %	**1-20**	**6.00±0.00**	**0.421**
	21-40	**7.77± 2.00**	
	41-60	**6.91± 2.18**	
	61-80	**7.23±1.46**	
	81-100	**7.47±2.40**	
Lymphocytes %	**1-20**	**60.00±0.00**	**0.962**
	21-40	**63.07 ±6.72**	
	41-60	**63.05± 7.68**	
	61-80	**63.15±8.24**	
	81-100	**63.50±7.72**	
Monocytes %	**1-20**	**38.00± 0.00**	**0.931**
	21-40	**33.97± 6.42**	
	41-60	**34.94±7.56**	
	61-80	**33.80 ±7.81**	
	81-100	**33.00 ±7.02**	

Eosinophils %	**1-20**	**1.00±0.00**	**0.925**
	21-40	**1.43±3 0.50**	
	41-60	**1.44±0.50**	
	61-80	**1.40± 0.50**	
	81-100	**1.50± 0.57**	
Platelets	**1-20**	**1.00± 0.00**	**0.924**
	21-40	**1.41±0.49**	
	41-60	**1.44±0.50**	
	61-80	**1.40±0.50**	
	81-100	**1.50± 0.57**	
RBCs/L	**1-20**	**240.0±0.00**	**0.393**
	21-40	**269.3±69.72**	
	41-60	**280.2 ±125.9**	
	61-80	**233.9± 9.15**	
	81-100	**225.5±37.25**	

Table 03: Gonadal Axis Profile of Healthy Male Individuals With Different Age Groups

Parameters	Group	Mean±Std	P-value
SHBG (nmol/l)	**1-20**	**22.70±00.0**	**0.498**
	21-40	**38.87±17.57**	
	41-60	**32.22±15.27**	
	61-80	**35.35±10.00**	
	81-100	**25.60±20.04**	
LH (mIU/ml)	**1-20**	**7.16±0.00**	**0.757**
	21-40	**4.8±2.55**	
	41-60	**5.36±3.48**	
	61-80	**5.40±2.66**	
	81-100	**3.51±1.05**	
FSH (mIU/ml)	**1-20**	**1.52±0.00**	**0.850**
	21-40	**6.6-±5.50**	
	41-60	**6.35±5.74**	
	61-80	**6.70±6.27**	
	81-100	**3.86±0.92**	
Testosterone (mIU/ml)	**1-20**	**551.01±0.00**	**0.229**
	21-40	**496.96±238.83**	
	41-60	**347.66±163.47**	
	61-80	**459.23±128.40**	
	81-100	**503.67±292.29**	
HSP-70	**1-20**	**162.66±8.88**	**0.019**
	21-40	**283.25±12.56**	
	41-60	**299.65±4.58**	
	61-80	**306.35±5.26**	
	81-100	**415.26±11.08**	


SHBG nmol/I
nmol/I
90
80
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50
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20
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0
0.5
1
1.5
2
2.5
20-59
60-86
LH mIU/ml
mIU/ml
18
16
14
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20-59
60-86
FSH mIU/ml
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60-86

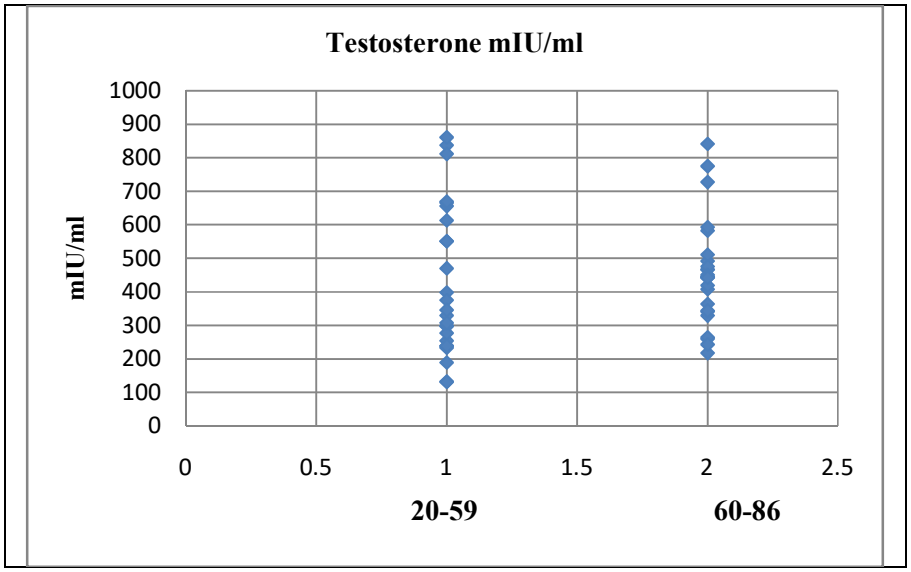

Figure 1: Levels of Gonadal Axis Profile In Healthy Male Individuals With Different Age Groups

DISCUSSION

The aim of the present study is to determine the role of heat shock protein-70 (HSP-70) and gondal axis profile such as testosterone, LH, FSH and SHBG in young healthy males that significantly involved in the progression of aging.Variations in the events of numerous endocrine systems occur during ageing, counting different hormonal secretory outlines and variation of feedback sensitivity **[10]**. These physiological variations can be measured byevaluating hormone concentrations in older persons with and without endocrine disease. Although, the extent of these changes significantly differs between different age groups. In order to determine these variation are due to the ageing, or whether they are linked to other complications, such as interrelated chronic diseases, inflammation and nutritional status **[11]**. Age-related modifications cause ongoing changes in hormone levels and spermatogenesis in men. Accordingly, these advanced alterations result in a decrease in both quality and quantity of spermatozoa. Additionally, some studies established that obesity, lack of exercise, and age-related comorbidities are more effective than chronological aging in deteriorating testosterone levels in aged men **[12]**. The effects of aging on male reproductive system are relatively complex by physiological process and environmental factors. Human aging is described by an advanced, intrinsic, and generalized disproportion of control of many regulatory systems **[13]**. Progressive

paternal age is extensively recognized as 40 years or older at the time of the conception **[14]**. Paternal age does not disturb fertility directly as an independent feature; it could be noteworthy in arrangement with the parental age **[15]**. Current studies have designated that progressive paternal age has a major impact on the hazard of certain diseases and associates with a number of complications as compared to young person. The children of aged fathers display high occurrence of certain genetic anomalies, childhood cancers, and numerous neuropsychiatric disorders **[16]**. In addition, the latest advances in assisted reproductive techniques give rise to have a child to older men, even with poor semen parameters **[17]**. Although the studies provide a link between advanced paternal age and certain disorders, there is yet not any diagnostic or screening test panels to detect these disorders using patient gametes. The genetic abnormalities and diseases risk of offspring should be assessed by parent-offspring trios' studies for base substitutions. Additionally, the epigenetic statute of spermatozoa may change with aging and it is not completely clear whether these changes are transmitted and affect the health of the offspring. In epigenetics, whole genome sequencing and new technologies may be useful to understand the effects of aging on male reproductive system and offspring **[18]**.

Reactive oxygen species (ROS) are formed by numerous endogenous and exogenous procedures. Oxidative stress is caused by the inequity between free radicals and antioxidants defense which is mainly involved in "aging theory" **[19]**. Biomarkers of oxidative stress may be beneficial as a diagnostic tool or therapeutic target. Antioxidant therapy such as resveratrol and other nutritional complexes, collected with reasonable aerobic exercise, may absolutely affect the clinical impairment encouraged by oxidative stress **[20]**. Aging is accompanied by a decline of self-defensive mechanisms and by increase damages at the molecular, cellular, and organismal level as a result of a constant experience to opposing environmental strains **[21]**.The reactive species generate the stress reaction, resulting in the production of heat shock proteins (HSPs) that play an important role in cytoprotection. HSP is most significant in physiological and pathological developments and also work as biomarkers to assess the amount of disease. The study of Kim*et al* (2008) suggested that HSPs plays an important part in aging **[22]**. The biotic significance of these age-related variations in HSP-70 levels in normal individuals

arecurrently unidentified and needs additional research. Nevertheless, it is thought that HSP-70 may be a biomarker for aging because some of the genes that are considered to affect aging and longevity are the ones that control the courses of somatic conservation and repair, such as stress-response system **[23]**. HSP-70 also shows an important role in defense against numerous stressesand functions as a molecular chaperone, enabling the folding and restoration of misfolded and impaired proteins resulting from aging **[24]**. Several studies recommended that HSP-70 has significantly involved influencing immune response, which is strongly associated to oxidative stress and aging processes **[25, 26]**. In the present study, high level of HSP-70 was observed in older age groups as compared to other groups, which suggested the upregulation of reactive oxygen species and oxidative stress. It has been established that older patients with acute heat-induced illness showed lower Hsp70 levels than younger ones **[27-32]**.

CONCLUSION

In the present study, ageing is the complex process which involves lifestyle, hereditary and environmental factors. The free radical theory of ageing suggested that,lifespan is measured by the capability of organisms to mange damages induced by natural by-products or reactive oxygen species (ROS). Hydroxyl radicals and superoxide anions are highly reactive and to be responsible for damages to proteins, lipid and DNA. Therefore, it has been concluded that heat shock protein-70 (HSP-70) has a significant role to upregulate the production of free radicals and oxidative stress during aging. HSP-70 has been used not only as a biomarker for the estimation of endogenous oxidative DNA damage, but also a major risk factor to cause ageing in males.

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