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Association of oxidative stress with female infertility - A case control study

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ORIGINAL ARTICLE

Association of oxidative stress with female infertility - A case control study

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Abstract

Objective: To compare stress markers and reproductive hormones in fertile and infertile females, and to relate the markers with age, duration and cause of infertility, and body mass index.

Methods: The case-control study was conducted at Aga Khan University Hospital, Karachi, from March 2017 to February 2018. Females aged 16-50 years regardless of ethnic background were recruited from the Australian Concept Infertility Medical Centre, Karachi, and were equally divided into infertile cases group A, and fertile controls group B. Serum follicular stimulating hormone, luteinizing hormone, estradiol, glutathione reductase and cortisol were measured using enzyme-linked innmunosorbent assay. SPSS 19 was used for statistical analysis.

Results: There were 328 female subjects divided into two equal groups of 164(50%). Serum luteinizing hormone and cortisol was higher in the group A than in group B ($p<0.001$). Serum glutathione reductase was low in group A compared to group B ($p<0.001$). Duration of infertility, serum levels of glutathione reductase and cortisol were also significantly different among infertile females when distributed on the basis of cause of infertility ($p<0.05$). Serum cortisol had negative correlation with glutathione reductase ($p<0.001$). Age and body mass index had a positive correlation with serum cortisol ($p=0.035$; $p=0.63$), while there was a negative correlation with glutathione reductase ($p = -0.732$).

Conclusion: Prolonged duration of infertility, age of females and body mass index enhanced the production of stress hormones and decreased antioxidant activity which augmented the risk of infertility.

Keywords: Oxidative stress, Female infertility, Glutathione reductase, Cortisol. (JPMA 69: 627; 2019)

Introduction

Infertility refers to conception failure by a couple and is perceived as a multifactorial syndrome in all cultures and societies. Approximately 23% couples of reproductive age group are affected in Pakistan, of whom 5% contribute to primary infertility and 18% to secondary causes.[1]

During this process of folliculogenesis, the oocyte undergoes a significant array of genetic, epigenetic and cytoplasmic alterations in order to achieve fertilisation proficiency. This whole course of events depends on a continuous cross-talk between oocytes and granulosa cells that safeguard the coordination of all the events sequenced in the ovary under the influence of paracrine and endocrine factors, hormones and peptides.[2] Maintenance of ovarian reserve is dependent on extrinsic (xenobiotics and anticancer drugs) and intrinsic (endometriosis, diabetes, polycystic ovarian syndrome [PCOS] and ovarian aging) factors.[3]

It has been documented that the quality and quantity of oocytes gradually decline with increase in age due to disturbed balance of the redox activity. Thus aging plays a major role in the decline of the functional capability of oocytes, and affect female fertility.[4] Replacement of aged oocytes by younger oocytes have overcome the decline in female infertility as younger oocytes have a better balance of cellular redox activity compared to the aged oocytes.[5]

Reactive oxygen species (ROS) are continuously produced mainly by the mitochondria and the prompt scavenging of free radicals (FR) is very essential to prevent the occurrence of oxidative damage.[6] Free radical oxidation (FRO) might also result in follicular atresia and oocyte aging in the ovaries.[7] Under normal conditions, ROS production is mitigated by antioxidants which enable the organisms to cope with oxidative environments and help the cell in repairing the damage caused by ROS.[8]

Cellular redox activity is a normal mechanism of male and female reproductive system, but goes unmitigated by antioxidants' effects on female fertility.[9] Studies have related psychological stress and ageing with oxidative imbalance. Chronic stress causes a sustained increased level of cortisol, which leads to conditions like endometriosis by compromising normal cellular

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functions.[10] This oxidative stress (OS), produced by mitochondrial dysfunction, is considered the main cause for chromosomal segregation disorders, maturation and fertilisation failures, or oocyte/embryo fragmentation.[9] Glutathione system plays a key role in protection against the detrimental effects of FR by providing detoxification of organic and inorganic peroxides by utilising reduced glutathione. The regeneration of oxidised glutathione is carried out by glutathione reductase (GR), which uses nicotinamide adenine dinucleotide phosphate (NADPH) as reduced equivalents.[11] GR activity has been found to be low in low-responding females going through in vitro fertilisation (IVF) when compared with high responders and healthy oocyte donors, negatively influencing the ovarian response.[10,12] This suggests that persistent stressful conditions may lead to sustained increase in cortisol levels while at the same time reduced GR activity fails to counter-balance the disturbed micro-environment (Figure). This hypothesis drives us to investigate the stress markers in females who face an unrelenting stress of being infertile instigated on them by their in-laws and society in general right after their marriage. The current study was planned to assess stress markers in fertile and infertile females, and to compare the levels among infertile groups formed on the basis of the causes of infertility.

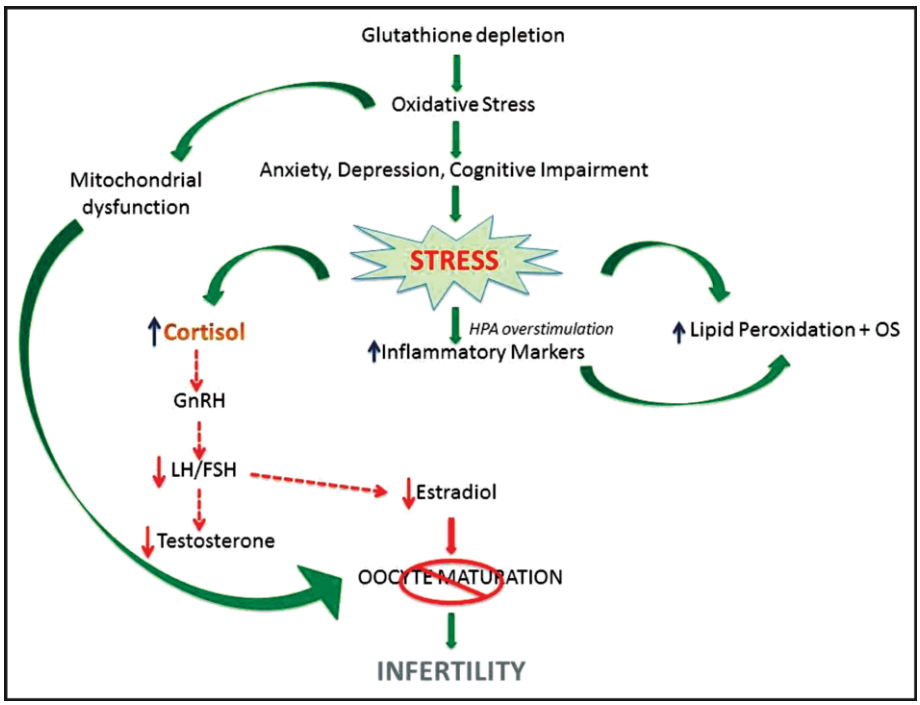

Figure: In presence of Glutathione reductase (GR) (antioxidant) depletion, there is an imbalance of redox activity generating oxidative stress leading to anxiety and depression and also causing mitochondrial dysfunction. Hypothalamus pituitary axis (HPA) is over-stimulated by stress, triggering excess secretion of cortisol from the adrenals, releasing the inflammatory markers along with increased lipid peroxidation and oxidative stress (OS). Over-secretion of cortisol suppresses the secretion of gonadotropin releasing hormone (GnRH_, which in turn decreases the release of luteinizing hormone (LH) and follicular stimulating hormone (FSH). Failure to stimulate the ovaries to secrete estradiol and in the coexistence of mitochondrial dysfunction, oocyte maturation failure occurs as a resultant Infertility ensues.

Subjects and Methods

The case-control study was conducted at Aga Khan University Hospital, Karachi, from March 2017 to February 2018. The subjects were recruited from the Australian Concept Infertility Medical Centre (ACIMC), Karachi, and they were divided into infertile cases group A and fertile cases group B.

The sample size was calculated using online software at Open Source Epidemiologic Statistics for Public Health to achieve 80% power to detect an odds ratio (OR) of at least 2. The hypothetical proportion of control with exposure was 22%, with expected prevalence of infertility 23% and two-sided confidence (1-alpha) 5%.[13]

Those included as cases had duration of infertility over two years, aged 16-50 years, and from all ethnic backgrounds. Healthy females of matching age and backgrounds but with a child below 5 years of age were included as controls.

Women with assisted conception in previous pregnancies, history of recurrent miscarriages, thyroid problems, gynaecological tumours, hypertension, diabetes and women with serious general health status were excluded. Females on oral contraceptive pills and hormonal treatments or practising any contraceptive measures were also excluded.

The study was approved by the institutional ethics review board and all subjects furnished written informed consent.

Clinical data, including age, height, weight, blood pressure, menstrual/ obstetric and gynaecological history, dietary pattern and family history of each subject was noted. Weight was assessed by digital weighing scale in kilogrammes and standing body height in inches (converted into meters) by the height scale (floor type ZT-120 EVERICH, China). South Asian criteria of body mass index (BMI)[14] was used and it was calculated using the

standard formula.[15]

Using aseptic techniques, venous blood from ante-cubital vein was collected from the subjects by observing pain-free procedures at the time of recruitment. Around 6ml of venous blood was withdrawn and the serum was isolated after centrifugation before being immediately stored at -80°C until further analysis.

Commercially available enzyme-linked immunosorbent assay (ELISA) kits were used for biochemical estimation of serum follicle stimulating hormone (FSH) (Cat# DKO010; Diametra), luteinizing hormone (LH) (Cat# DKO009; Diametra), estradiol (BC1111; BioCheck, Inc), glutathione reductase (GR) (Cat# SG-00523; Sino Gene Clone Biotech Co., Ltd), and cortisol (Cat# DKO001; Diametra).

Statistical analysis was done using SPSS 19. Descriptive analysis of continuous variables like biophysical and biochemical parameters were expressed as mean ± standard deviation (SD). Comparisons were performed using student-t test for parametric variables and Mann-Whitney U test for non-parametric variables. Associations between circulating hormone levels and infertility were determined using Spearman's rank correlation. The p-value of >0.05 will be considered significant

Results

There were 328 subjects who were divided into two groups 164(50%) each. The overall mean age was 30.6±5.75 years. There was a significant difference observed in weight and BMI of the fertile and infertile groups ($p<0.001$). Non-significant changes were seen in age at marriage ($p>0.050$) while duration of marriage was significantly different ($p<0.001$) in the two groups. Serum FSH level was not significantly different ($p>0.05$) whereas LH was significantly higher in group A compared to group B ($p<0.001$). Serum GR levels were significantly low and serum cortisol levels were significantly high in group A compared group B ($p<0.001$) (Table-1).

Additionally, the duration of infertility, serum levels of GR and cortisol were also found to be significantly different

Table-1: Clinical and biochemical data of study groups.

Variables	Group A Infertile females n = 164 (Mean ± SE)	Group B Fertile females n = 164 (Mean ± SE)	P value
Age (years)	30.8 ± 5.5	30.4 ± 6.01	0.202
Weight (kg)	69.76 ± 15.72	67.07 ± 13.43	<0.001
Height (cm)	165.53 ± 3.73	165.9 ± 2.38	0.033
BMI (kg/m^2)	25.41 ± 5.40	24.33 ± 4.70	<0.001
Age at marriage (years)	21.9 ±5.8	23.36 ± 4.8	0.314
Duration of Marriage (years)	8.04 ± 5.7	5.7 ± 4.2	<0.001
Duration of infertility (years)	8.242 ± 1.6	3.67 ± 0.4	<0.001
FSH (IU/mL)	8.2 ± 1.1	8.89 ± 0.34	0.235
LH (IU/mL)	10.05 ± 0.34	5.34 ± 0.32	<0.001
Estradiol (pg/ml)	192.48 ± 18.9	211.18 ± 19.3	<0.001
Glutathione reductase (pg/ml)	115.22 ± 80.67	872± 322.99	<0.001
Cortisol (ug/ml)	27.12 ± 6.41	10.84 ± 2.41	<0.001

LH: Luteinizing hormone, FSH: Follicular stimulating hormone, BMI: Body mass index.

Table-2: Comparison of variables among causes of infertility (Infertile Group).

Variables	Causes of Infertility					P value
	Endometriosis n = 15	PCOS n =76	Tubal n =18	Male factor n =13	Unexplained n =42	
Age (years)	30.15 ± 5.7	30.96 ±5.01	30.26 ± 7.1	28.8 ±4.5	31.65±6.2	0.753
BMI (kg/m^2)	25.4 ± 4.8	26.241 ± 6	25.908 ± 4	24.63 ± 6	25.391 ± 6	0.133
Duration of infertility (years)	6.8 ± 4.06	7.934 ± 5	9.01 ± 7	7.69 ± 6	9.897 ± 6	<0.001
Estradiol (pg/ml)	259.9 ± 79.9	172.9 ± 29.9	139.1 ± 38.6	221.9 ± 60.7	240.7 ± 46.8	<0.625
Glutathione reductase (pg/ml)	110.73 ± 46.65	106.0 ± 86	114.21 ± 59	133.41 ± 77	111.27 ± 30	<0.001
Cortisol (µg/ml)	27.12 ± 6	111.26 ± 30	15.83 ± 7	10.04 ± 6	21.924±5	<0.001

PCOS: Polycystic ovarian syndrome
BMI: Body mass index.

among group A infertile females when distributed on the basis of cause of infertility ($p<0.05$). Cause of infertility was endometriosis in 15(9%), PCOS 76(46%), tubal factor 18(11%), unexplained cause 42(25%) and male factor 13(8%) (Table-2). Age, BMI and estradiol were not significantly different ($p>0.05$).

Age and BMI had a positive correlation with serum cortisol ($r=0.117$, $p= 0.035$; $r=0.026$, $p=0.63$), while there was negative correlation with GR ($r=-0.019$, $p=-0.732$). Moreover, serum cortisol had significant negative correlation with GR ($r=-0.380$, $p<0.001$).

Discussion

Female infertility is a major social problem and impaired oocyte quality is an important factor underling infertility in females.[16] The current study evaluated that infertile females had high body weight and BMI. This association of high BMI and female infertility is consistent with an earlier study.[4] This effect of high BMI may be due to overproduction of ROS that causes hyperglycaemia and increasing level of visceral fat.[17]

The current study also demonstrated that infertile females were significantly younger at the time of marriage and the duration of marriage was significantly higher compared to fertile females. Younger women tend to be more apprehensive to be recognised as a parent and are easily pressurised by society and, thus, are more prone to developing depression and stress. With a longstanding time of infertility, this depression causes oxidative dysfunctions.[18] Stress is not only a risk factor for female sexual dysfunction, but also produces oxidative imbalance which further affects fertility.[19] Further psychosocial issues might also occur due to treatment failure. All these factors affect quality of life, marital adjustment and sexual impact and, as a result, almost 40% couples undergoing assisted reproduction technology (ART) still cannot conceive.[20]

The current study has shown that serum FSH was not significantly different between the two groups, but LH was different. Another study has also demonstrated the same finding, and reported that it may be due to the fact that hypersecretion of LH causes premature oocyte maturation, reduced fertilisation, decreased cleavage rate, and miscarriage.[21] LH has significant role in the follicular development and oocyte maturation. Hence, hyper-secretion of LH has adverse effects during the follicular phase which causes problems in fertilisation and conception. Raised LH levels may have direct or indirect effects on the developing oocyte and endometrium by elevating levels of testosterone and oestrogen levels.[21] The interplay of LH and FSH causes increase in estradiol production which is required for growth and development of follicles.[22]

According to the study's results, infertile females had low serum levels of GR and high serum cortisol levels. Further it was reported that there was a significant negative correlation between GR and serum cortisol levels. GR plays a key role in protection against the detrimental effects of free radicals by providing detoxification of organic and inorganic peroxides.[11] Hence, the decreased endogenous antioxidant enzyme GR causes disruption of cell defence mechanism against mitochondrial ROS and affects the oocyte maturation, fertilisation, embryo development and pregnancy.[23] A study also reported low GR activity in low-responding females going through IVF, hence low GR levels show decreased ovarian response.[12] These findings provide further evidence that the decrease in GR is associated with infertility. It has also been reported that persistent stressful conditions may lead to sustained increase in cortisol levels and if at the same time reduced GR activity is also present, then this fails to counter-balance the disturbed micro-environment. Both physical and emotional types of stress cause activation of the neurons that secrete corticotropin-releasing hormone and ultimately result in higher plasma cortisol levels.[24]

Additionally, the current study also evaluated that significant increase in the duration of infertility, decreased serum levels of GR and increase in cortisol were found in different types of infertile females. The results are similar to those of other authors.[10,25,26] Chronic stress causes a sustained increased level of cortisol, which causes immunosuppression by inhibiting both natural killer (NK) cell activity and T lymphocytes which ultimately leads to the development of endometriosis.[10] In addition to immunological alterations, hormonal changes are also produced which are strongly associated with endometriosis.[10] Both activated macrophages and their immune mediators are increased in endometriosis, which are responsible for causing interference in the function of gamete, fertilisation, both development and implantation of embryo.[10]

The findings of this study in PCOS have shown both decrease in GR and increase in cortisol. It has also been reported by other studies that OS in female reproduction has association with PCOS. Increased levels of ROS leads to decreased fertilisation rates and embryo quality in women suffering from PCOS. Fertilisation potential of oocytes is affected through disruption of meiotic spindle formation.[25] Our study illustrated similar results as PCOS in unexplained infertile females; reduced GR and elevated cortisol.

Several studies have reported oxidative stress as the cause of unexplained infertility. Factors like N-acetyl cysteine (NAC) and folate-metabolising pathways polymorphisms hindering conversion of homocysteine to methionine come into account of unexplained infertility in females, but supplementation of NAC was unable to induce ovulation in such patients.[26]

More studies needs to be done with larger numbers of patients and controls to confirm the results of the current study.

Conclusion

Different concentrations of OS markers and antioxidant enzymes may negatively impact ovarian response. Increased years of being infertile, age of females and BMI enhanced the production of stress hormones and decreased antioxidant activity which impaired ovarian steroidogenic activity and, hence, augmented the risk of infertility.

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Conflict of Interest: None.

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