ORIGINAL ARTICLE

IMPACT OF ANTIOXIDANTS AND PRO-OXIDANTS ON FEMALE FERTILITY

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ABSTRACT

Background: Oxidative stress, an imbalance of pro-oxidants and antioxidants, is reported to be associated with female infertility. This study was aimed to compare cortisol (oxidative stress marker) and vitamin E (antioxidant) levels in the fertile and infertile female population of Karachi, Pakistan.

Materials and Methods: A total of 88 females, recruited from Australian Concept Infertility Medical Centre, were divided equally into infertile cases and fertile controls. Serum cortisol and vitamin E (VE) levels were measured using commercially available enzyme-linked immunosorbent assay (ELISA) kits. Statistical comparison was done by Chi-square and Mann-Whitney U-test. Correlation between two continuous variables was determined by Spearman's Correlation. The P value < 0.05 was considered significant in all cases.

Results: The median age for the fertile group was 31.0 (IQR= 27.0-37.0) and for the infertile group, 32.0 (IQR= 28.0-38.0). Of the infertile females, 70.5% (n= 31) had primary infertility while 29.5% (n= 13) had secondary infertility. A significant difference in the cortisol levels was seen between the fertile and the infertile groups. (p value= 0.001). VE levels were significantly decreased in the infertile females (p value= 0.026). The levels of cortisol and VE were found to be in a weak negative correlation in the infertile women (-0.163).

Conclusion: A balance between oxidants and antioxidants is required to maintain the reproductive potential in females. Decrease in the antioxidant vitamin E, and an increase in pro-oxidant cortisol, may be associated with a risk of infertility in females.

KEYWORDS: vitamin E, fertility, pro-oxidants, antioxidants

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INTRODUCTION

Reactive oxygen species (ROS) produced normally, are required for gene expression, cell signaling, and redox homeostasis[1]. Normally, ROS are balanced by the scavenging antioxidant system. Hence oxidative balance is required for all the normal metabolic functions in mammals including cell proliferation, differentiation, apoptosis, and death[2]. This balance between pro-oxidative agents and antioxidant defense mechanisms is also required for the reproductive activities in female, such as folliculogenesis, ovulation, endometrial changes in different luteal phases, fertilization, placental growth, embryogenesis, and implantation[3]. Oxidative stress (OS), an imbalance, influences physiological functions and leads to pathological effects including damage to the cell membrane and DNA, cell degradation and injury[1,4]. The mechanisms responsible for pathological effects are lipid damage, inhibition of protein synthesis, and ATP depletion[5].

Infertility affects 15% of couples of reproductive age[6]. OS is reported to be associated with infertility[3,7]. Studies have shown that OS causes various pathological conditions in the female reproductive

tract, including endometriosis, tubal and peritoneal pathologies, which ultimately lead to infertility[3]. Elevated ROS, produced from erythrocytes and apoptotic endometrioma cells, as well as activated macrophages cause OS[8]. OS results in lack of oocyte maturation, oocyte degeneration, and apoptosis[9]. Cellular mechanisms of OS include lipid peroxidation, increased membrane permeability, degraded membrane integrity, inactivated enzymes and DNA damage, which ultimately leads to rapid cell death. Elevated ROS can also affect in vitro fertilization (IVF) as they induce embryonic fragmentation and hinder the development of blastocysts in vitro and hence pregnancy success rate[7,9].

Vitamin E (tocopherol) (VE) acts as an essential lipid soluble anti-oxidant, scavenging hydroperoxyl radicals in a lipid milieu. ROS react with it 1000 times faster than when binding to polyunsaturated fatty acids in the membranes[10]. It can contribute to the protection of cells and tissues against the deleterious effects of free radicals[11]. VE deficiency has been linked to infertility. Numerous studies have been conducted to see the anti-oxidant effects of VE in nullifying the oxidative stress contributing to infertility[10,12]. Fatemeh et al has evaluated the role of VE and/or folic acid (FA) in protection against oxidative damage. There was decreased apoptosis in the cells but the effect was more due to the VE than the synergy of VE and FA[12]. Jennifer et al concluded that high antioxidant diet including VE leads to decrease in peripheral oxidative stress markers, and increased antioxidant markers in women with endometriosis[13].

Although widespread research has been conducted on VE and its antioxidant effects in order to cure infertility, most of these studies have used either rats or human males as test subjects. No such study has been conducted upon female infertility, especially in Pakistan, although the impact of stress on fertility is well documented.[14] Moreover, the literature also lacks association between the anti-oxidant capabilities of VE and cortisol. Thus, the aim of this research is to bridge this gap and to investigate the correlation between VE and cortisol in female infertility.

METHODS

In this study 44 infertile and 44 fertile female patients were recruited from the Australian Concept Infertility Medical Center (ACIMC) between the ages of 20-55 years. The study was approved by the Ethics Committee of the Aga Khan University (4426-BBS-ERC-16) and all patients gave written informed consent to participate in the study. Blood samples were collected from all these patients in sterile tubes with no anticoagulant. Serum was stored in sterile aliquot tubes at -20ºC until assayed. Cortisol was measured by Cortisol ELISA Kit, (Catalog # Co103S by CAL BIOTECH) and VE concentrations were measured by Vitamin E and cortisol in female fertility.

Statistical analysis of the data was performed using IBM Statistical Package for the Social Sciences (IBM SPSS Statistics for Windows, Version 20.0. Armonk, New York). Categorical data was presented as frequency and percentages, while continuous data was presented in terms of median and interquartile ranges. Statistical comparison between the categorical variables was done by Chi-square. Mann-Whitney U was applied on non-parametric data of continuous and categorical variables. Correlation between two continuous variables was determined by Spearman's Correlation. The P value < 0.05 was considered significant in all cases.

RESULTS

A total of 88 females were a part of this study and 50% were infertile. The median age for the fertile group was 31.0 (IQR= 27.0-37.0) and for the infertile group, 32.0 (IQR= 28.0-38.0). Of the infertile, 70.5% (n= 31) had primary infertility while 29.5% (n= 13) had secondary infertility. Table 1 compares the hormone cortisol and the vitamin E levels between the fertile and the infertile women. A significant difference in the cortisol levels was seen between the fertile and the infertile group. (p value= 0.001). VE levels were significantly decreased in the infertile females (p value= 0.026). No significant age difference was seen between fertile and infertile females (p value= 0.474). Duration of marriage also had no significant relation with fertility in this study (p value= 0.139). Regarding regularity of menstrual cycle in the two groups, it was seen that menstrual cycles were more regular in fertile group and irregular in infertile group (p value= 0.001). The family system, joint or independent, was seen to have a significant impact on the fertility status (p value= 0.016). 59.1% of the fertile females were living independently, while only 34.1% of infertile were living independently.

Table 1: Comparison of study characteristics in Fertile and Infertile female

	Fertile (n=44)	**Infertile (n=44)**	**P-value**
	Median (interquartile range)	**Median (interquartile range)**	
Cortisol (ng/dl)	26.6 (22.9-28.9)	278.8 (235.2-333.6)	**0.001**
VitE (nmol/L)	0.585 (0.513-13.6)	0.475 (0.350-25.0)	**0.026**
Age of female (years)	31.0 (27.0-37.0)	32.0 (28.0-38.0)	0.474
Duration of marriage (years)	7.0 (5.0-10.0)	8.5 (4.8-16.0)	0.139
	N (%)	**N (%)**	
Menstrual Cycle			**0.001**
Regular	44 (100)	32 (72.7)	
Irregular	0	12 (27.3)	
Family System			**0.016**
Joint	18 (40.9)	29 (65.9)	
Independent	26 (59.1)	15 (34.1)	

The levels of cortisol and vitamin E were found to be in a weak negative correlation in the infertile women with a spearman's rho correlation coefficient of -0.163. Figure 1 illustrates the difference in the cortisol levels between fertile and the infertile women. Cortisol levels are seen to be very high among the infertile women in comparison to the fertile women.

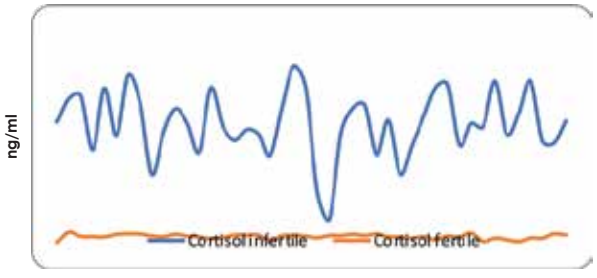

Figure 1: Comparison of cortisol levels in fertile and infertile females

Figure 2 illustrates the difference in the Vitamin E levels between fertile and the infertile women. The levels are variable with higher levels of vitamin E seen in fertile than infertile women.

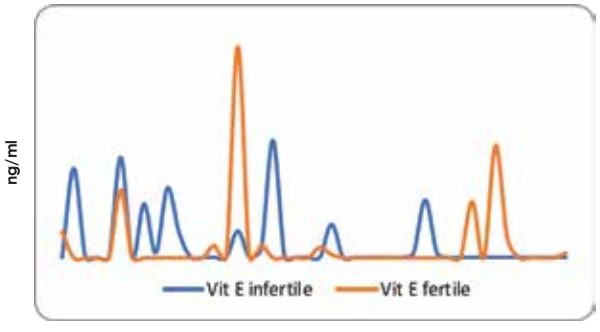

Figure 2: Comparison of Vitamin E levels in fertile and infertile females

DISCUSSION

Oxidative stress occurs either due to abnormally high concentrations of free radicals or decreased antioxidant levels[3]. Protection against ROS damage is provided by the antioxidants, which include both enzymatic and non-enzymatic antioxidants. VE is the most effective lipid-soluble, non-enzymatic antioxidant and protects against lipid peroxidation. In our study, significant decrease in VE in infertile females was seen. This is consistent with previously reported association of decrease in VE with infertility[10,12]. Thus the protective effect of VE against ROS damage is decreased in infertile females. This oxidant status causes precipitous pathologies affecting female reproduction and causing female infertility. OS affects early embryo development by changing the key transcription factors and gene expression[15]. In addition to this both implantation and fertilization of eggs is also affected.[16]

This data has reported a significant increase in cortisol levels in the infertile group of females as compared to the fertile group. Cortisol has an anti-inflammatory effect which inhibits natural killer (NK) cell activity and causes suppression of cell immunity[17]. This anti inflammatory effect of cortisol is normally required, to limit the inflammatory cascade that occurs during ovulation and causes proteolytic breakdown of the follicle wall and ultimately follicular rupture.[18] However increases in cortisol levels produce extreme situations, which affect the normal ovulatory mechanism, the estrous cycle and block ovulation[19]. This increase in cortisol in the infertile group may be due to stress, as high corticotrophin-releasing hormone is secreted by the activation of hypothalamic neurons in stressful conditions[17]. It has also been reported that high cortisol levels interfere with reproduction because at the pituitary level they inhibit the secretion of gonadotropins, especially LH, and in the ovaries, ovarian function is affected[20].

In this study, it was observed that menstrual cycles were more regular in the fertile group and irregular in the infertile group. It has been established that healthy women go into OS for 2/3rd of the menstrual cycle, this is called oxidative cyclicity[21]. This OS is compensated for by the antioxidant systems so that lipid peroxidation remains constant and equilibrium is maintained for the entire menstrual cycle[22]. But unchecked OS produces irregularities in the menstrual cycle.

It was reported that there was a significant negative correlation between VE and serum cortisol levels in the infertile group. This shows the imbalance between the antioxidant VE and the pro-oxidant cortisol. Physiologically, the body can defend itself against ROS by using its reserves of antioxidants including VE[11]. An imbalance, either due to the reduction of antioxidants or the increased generation of oxidizing species, generates oxidative stress. Thus female reproductive activities influenced by OS include oocyte maturation, ovarian steroidogenesis, ovulation, implantation, formation of blastocyst, luteolysis and luteal maintenance in pregnancy[3]. The negative effects of OS on female reproduction can also occur by affecting the follicular fluid, peritoneal fluid, hydrosalpingeal fluid as well as oocyte quality[23].

Additionally, this study has also evaluated that there was a significant difference in the family system of the infertile group as compared to the fertile group. It was found that in the infertile group, a joint family system was more common compared to the fertile group. Joint family system has been known to cause difficulties in marital adjustments. Both marital adjustment and social support affect the psychological status of the female. It is reported that psychological distress in females causes female sexual dysfunction and oxidative imbalance, which

can affect fertility[24,25]. Thus infertility is conditioned by social structural realities.

CONCLUSION

Balance of oxidants and antioxidants is required to maintain the reproductive potential in females. Decrease in the antioxidant vitamin E and increase in the pro-oxidant cortisol may be associated with a risk of infertility in females. However, further research should be done to evaluate the effect of antioxidants on infertility and its use in its treatment.

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