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RESEARCH ARTICLE

Status of α-tocopherol concentration and oxidative stress in infertile females of Vijayapur District, northern Karnataka

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ABSTRACT

Background: Female fertility declined in Karnataka. Oxidative stress leads to anovulation, dysfunctional oocytes, fertilization failure, implantation failure, or miscarriage. Vitamin E (α-tocopherol) has the ability of fertilized egg to implant into uterine wall properly. **Aims and Objective:** The aims and objectives of the study were to establish the serum concentration of α-tocopherol and lipid peroxide (LPO) level in infertile females of northern Karnataka compared to age-matched control. **Materials and Methods:** Infertile females 20-35 years of age were selected prospectively, and age-matched fertile females were included in the study and divided into two groups: 45 normal fertile females (Group I) and equal number of infertile females (Group II). Body mass index (BMI), blood glucose level, and hemoglobin (Hb) levels were estimated with standard methods. α-tocopherol concentration was determined by non-antibody coated microplate method by using enzyme-linked immunosorbent assay reader. LPO level was assessed by malondialdehyde quantification using spectrophotometry. **Results:** Infertile females showed any statistical difference in BMI and random blood glucose, but Hb levels reduced significantly as compared to fertile females. The mean duration of infertility was 5.69 years. In infertile females, serum α-tocopherol was decreased significantly from 16.88 to 8.87 μg/L which is 45.78% and serum LPO level was increased from 1.25 to 2.13 μmol/L, which is 70.40% as compared to fertile females. **Conclusion:** Infertile females of northern Karnataka showed lower α-tocopherol levels and greater potential for oxidative stress as compared to fertile females. These findings demonstrate imbalance in ROS production and antioxidant.

KEY WORDS: α-Tocopherol; Female Infertility; Oxidative Stress; Karnataka

INTRODUCTION

There has been an alarming decline in the fertility rates of women in Karnataka in the past decade; a study of the available 2011 census data has revealed.[1] The study conducted by the Population Research Centre at Institute for Social and Economic Change shows the fertility rate has declined to two children per women in the northern and southern region of Karnataka state in the past 5 years, resulting in a faster negative growth in the child and young population in future. Recent evidence suggests that while oxidative processes play an essential role in human reproduction; a state of oxidative stress may contribute significantly to the inability to conceive.[2] Oxidative stress has been implicated in endometriosis, recurrent pregnancy loss, and poor embryo quality.[3] Human studies into the effect of antioxidants on reproductive outcomes have shown some promising results.[3-6] One study of 45 women that had experienced recurrent pregnancy loss found significantly decreased total antioxidant capacity (TAC) and increased total oxidative

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status among these women compared to healthy pregnant controls.[7] α-tocopherol is the chemical name for the most active form of vitamin E. Vitamin E (α-tocopherol) is an exogenous, lipid soluble potent antioxidant molecule.[8] It is thought to be a direct free radical scavenger by activating the intracellular antioxidant enzymes and saving the cell membranes from lipid peroxidation.[9] A study performed on rats whose diet was devoid of vitamin E, showed those rats to become infertile. This study showed female rats had a higher rate of miscarriages than rats who were not vitamin E deficient. The rats were then given wheat germ oil which is high in vitamin E, and their fertility was completely restored.[10] Interventions of antioxidants may quench oxidative stress and offer novel therapeutic options in the management of female infertility.[11] Hence, this study was conducted to establish the concentration of α-tocopherol and oxidative stress in infertile females compared to age-matched controls to exclude the possibility of cause of α-tocopherol deficiency and oxidative stress in infertile females of Vijayapur District, northern Karnataka.

MATERIALS AND METHODS

A prospective study was conducted in the Department of Physiology, Al-Ameen Medical College between January 2016 and December 2016 on 45 infertile patients and 45 fertile females who consecutively attended the obstetrics and Gynecology Outpatient Department of the Al-Ameen Medical College Hospital, Urban Health Centre and Government Civil Hospital of Vijayapur District, northern Karnataka, India. The study was approved by the Research Ethics Committee of the Institution, and all patients who were evaluated gave written informed consent to participate.

Subject Selection

All the subjects were matched according to age (20-35 years), socioeconomical status and dietary habits. About 45 normal fertile females were included for the study and served as Group I. 45 infertile female patients were taken for the study as Group II. The study patients were being followed with the diagnosis of infertility, defined according to the World Health Organization as the failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse.[12]

The inclusion criteria for infertile females were confirmation of primary and secondary infertility in reproductive age (20-35 years) in study area (northern Karnataka) in 1-11 years of disease duration. The patients in the control group had no pelvic disease associated with infertility when submitted to diagnostic laparoscopy that was used as part of the procedures for the investigation of infertility.

The exclusion criteria for both groups included the presence of diseases such as diabetes mellitus, cardiovascular disease, dyslipidemia, systemic lupus erythematosus and other rheumatologic diseases, HIV infection, or the use of hormonal medications and hormonal or nonhormonal anti-inflammatory medications.

Routine Investigations

All study subjects were examined and notated down their age, the duration of infertility, measured height and weight using scales on barefoot then calculated body mass index (BMI) by using Quetelet's index.[13] Hemoglobin (Hb) was estimated by the auto analyzer method as stated in user's manual.[14] Random blood sugar glucose (RBS) was determined using Accu-Chek® Active Glucometer, Roche Diagnostic Corporation, Germany. Blood gluco-strips (Roche Diagnostic Pvt. Ltd., Mumbai, India).[15]

Sample Collection and Processing

Fasting 3 mL of venous blood was collected in plain test tube, processed in Laboratory Centrifuge Machine (R-4C), Remi, India, at 2000 rpm for 10 min to separate serum and stored at −20°C for later analysis. The stored serum samples were analyzed in the Environmental Health Research Unit of the Department of Physiology at our institute for determination of lipid peroxide (LPO) and vitamin E (α-tocopherol) levels as described in the next subsection.

The serum LPO was measured by the method of Satoh.[16] Serum samples were thawed until they reached a temperature of 37°C and immediately were used for the estimation of LPO. About 1 mL of sample was mixed with 2 mL of 15% trichloroacetic acid, 0.375% thiobarbituric acid, and 0.25 N hydrochloric acid and then was heated in a boiling-water bath for 15 min. After cooling, the precipitate was processed by centrifuge at 1,000 g for 10 min. The absorbance of the supernatant was measured at a 535 nm wavelength using an ultraviolet visible double beam spectrophotometer (SL 159), Elico, India. The concentration of thiobarbituric acid-reactive substances was determined by considering the coefficient of molar absorptivity of the product, and the results are reported as μmol MDA per liter (μmol/L). A standard curve was constructed using a stock solution of 10 mM MDA prepared from tetramethoxypropane (Sigma Chemical Co., St. Louis, MO), and the concentrations detected in the samples were within this curve, showing good linearity with the standard. The assays were performed in duplicate, and the results are reported as the mean.

Vitamin E (α-tocopherol) concentration in serum was determined by the method of Jargar et al.[17] Two centrifuge tubes labeled as T and B (i.e., sample and blank), in the sample tube added 750 μL absolute ethanol and serum, respectively. Serum added slowly with shaking to obtain a finely divided protein precipitate. To the blank tube added 750 μL distilled water and 750 μL absolute ethanol. Both tubes were covered

tightly by wrap paper and shaken vigorously for at least 30 s. To these tubes 750 μL xylene was added. These tubes were covered again by wrap paper and shaken vigorously for at least 30 s and centrifuged all tubes for 10 min at 3000 rpm. Further 500 μL of the xylene layer (supernatant) transferred into properly labeled clean small size test tubes. Then, in each tube added 500 μL of α, α-bipyridyl and 100 μL ferric chloride. 2 min later absorbance was measured by adding 200 μL of above solutions into a plain enzyme-linked immunosorbent assay (ELISA) microplate (non-antibody coated) wells, respectively, and read in an ELISA reader (ERBA-Lisa Scan II) at 492 nm. The serum α-tocopherol concentration of the sample was obtained using the standard curve prepared earlier with standard procedure. The assays were performed in duplicate, and the results are reported as the mean.

Statistical Analysis

Analysis of data was performed using Microsoft Excel and EPI INFO 2002. Standard statistical methods were used to determine the mean, standard deviation (SD), and median and range (minimum-maximum). Unpaired *t*-test was used to compare the results of various study parameters in the two groups. All the values were quoted as the mean ± SD. The $P < 0.05$ was considered statistically significant difference and represented by asterisk "*" between two groups.

RESULTS

The routine investigations in Group I (fertile females) and Group II (infertile females) are summarized in Table 1.

Fertile females and infertile females showed any statistical significant difference between mean age in years (27.64 vs. 29.07, $P = 0.0609$), BMI in kg/m^2 (23.04 vs. 25.52, $P = 0.1087$), and RBS in mg/dL (77.64 vs. 77.80, $P = 0.4421$). However, there was statistically significant difference showed in mean Hb concentration (12.73 vs. 9.69 mg/dL, $P = 0.0001$) between Groups I and II. The mean duration of infertility was 5.69 years.

Infertile females in Group II showed highly statistically significant decrease ($P = 0.0001$) in mean serum α-tocopherol concentration (8.87 μg/L) which is 45.78% reduction and increase ($P = 0.0001$) in mean LPO level (2.13 μmol/L) which is 70.40% elevation as compared to fertile females in Group I (16.88 μg/L, 1.25 μmol/L, respectively) as depicted in Figures 1 and 2.

DISCUSSION

This study has been the first in the literature to establish the concentration of α-tocopherol in infertile females of northern Karnataka.

Study results on routine investigations: Both fertile females (Group I) and infertile females (Group II) found any statistically significant difference in their age. BMI was noticed more (statistically non-significant), and Hb level was obtained less (statistically significant) in infertile females when compared with fertile females. The mean duration of infertility was 5.69 years. Study results on special investigations: Infertile females revealed two-fold decline in serum α-tocopherol concentration (most potent antioxidant) and two-fold elevation in LPO level (oxidative stress marker) as compared to fertile females.

Obtained results in our study demonstrated that the group of infertile females had lower Hb levels compared with the fertile females group. Németh et al.[18] demonstrated alleviation of oxidative stress by vitamin E leading to a significant increase in Hb levels. The mechanism by which vitamin E enhances the serum levels of erythropoietin and Hb is not clear. However, on the basis of some of the past studies, a direct effect of vitamin E on the erythroid cells for induction of Hb appears plausible. For example, in mouse model, vitamin E

Table 1: Routine investigations in both the study groups (*N*=90)

Routine investigations	Group I (fertile female) (*N*=45)	Group II (infertile female) (*N*=45)	*P* value
Age (in years)	27.64±4.46 28 (20-35)	29.07±4.36 29 (20-35)	0.0609 [NS]
BMI (kg/m^2)	23.04±1.44 24 (20-35)	25.52±2.31 25.35 (20-35)	0.1087 [NS]
Hb (mg/dL)	12.73±0.88 12.67 (11-14.34)	9.69±0.81* 9.44 (8.45-12)	0.0001
RBS (mg/dL)	77.64±4.49 77 (70-88)	77.80±5.21 77 (68-88)	0.4421 [NS]
Infertility duration (in years)		5.69±2.71 6 (1-11)	

All the values quoted as the mean±SD and median (min-max). Unpaired *t*-test was used to compare the results between two groups. *The $P<0.05$ was considered statistically significant difference and represented by asterisk. NS: Statistically not significant. BMI: Body mass index, Hb: Hemoglobin concentration, RBS: Random blood sugar, SD: Standard deviation

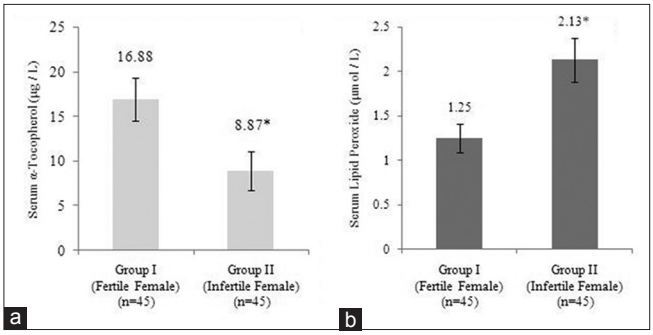

Figure 1: Status of serum α-Tocopherol concentration (a) and Serum lipid peroxide level (b) in both the study groups (*N*=90). All the values quoted as the mean±standard deviation. Unpaired *t*-test was used to compare the results between two groups. *The P<0.05 was considered statistically significant difference and represented by asterisk

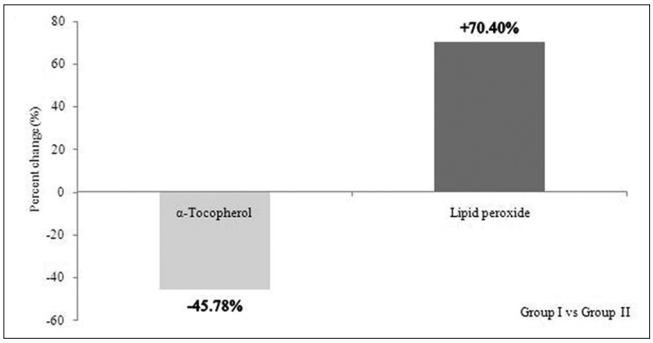

Figure 2: Percent change of α-tocopherol and lipid peroxide in infertile females (Group II) with respect to fertile females (Group I)

was shown to increase erythroid colony-forming unit-derived colonies in a dose-dependent manner.[19,20] The results obtained demonstrated that the group of infertile females had lower α-tocopherol concentration compared with the fertile females group. These data suggest that infertile women may have a greater consumption of α-tocopherol, a potent antioxidant, reflecting a possible increase in LPO level as a marker of oxidative stress in the serum of these women. In agreement with our findings, Murphy et al.[21] demonstrated that vitamin E values were significantly lower in infertile women, a fact that may explain the more rapid oxidation of lipoproteins detected in women with infertility. Szczepanska et al.[22] evaluated the activity of the antioxidant enzymes glutathione peroxidase and superoxide dismutase, as well as the TAC of the infertile women of no apparent cause and also detected a reduction of both TAC and the activity of the enzymes. Normal levels of oxidative stress in ovaries, endometrium, fallopian tube, embryos, and peritoneal fluid play a role in tissue remodeling, hormone signaling, ovarian steroidogenesis, folliculogenesis, maturation of oocyte, tubal function, and cyclical and endometrial changes.[23,24] However, pathologic levels of oxidative stress have resulted in considerable damage to cell structure.[25] Moreover, some evidence reported that oxidative stress is involved in developing female infertility.[5,18,26] Substantial increase in oxidative stress levels has been linked with damage to the DNA of the oocytes and spermatozoa, resulting in defective fertilization.[24] Even if fertilization is achieved, reactive oxygen species induce apoptosis leading to implantation failure, abortion, and embryo fragmentation.[26] Under normal condition, antioxidants are capable to inhibit reactive oxygen species production, scavenge presenting free radicals, and trigger the repair of cell structure damage, which is induced by reactive oxygen species.[26] The available evidence suggests gynecologic oxidative stress is an important mediator of conception. Altogether, our results suggest greater production of oxidative stress by infertile women, supporting the view that greater consumption of vitamin E would result in lower serum levels of this vitamin.

As vitamin E is an antioxidant, it helps in stabilizing the free radicals, increases the thickness of endometrial lining and improves Hb level in infertile females. Thus, decreased level of α-tocopherol concentration in our study suggests that infertile females need to take supplements of vitamin E to prevent infertility. The sample size was small to obtain basic serum α-tocopherol level in the females of the study area. Further study needs to evaluate the association between vitamin E levels and female infertility.

CONCLUSION

The low level of α-tocopherol concentration and high level of LPO in infertile females suggests that α-tocopherol may be consumed for scavenging of free radicals and elucidating the mechanism of disease pathogenesis. The study suggests that vitamin E intake may play a substantial role in preventing or facilitating female infertility in Vijayapur District of northern Karnataka.

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