Original Research Article

EVALUATION OF OXIDATIVE STRESS MARKERS AND HORMONAL PROFILES IN WOMEN DIAGNOSED WITH INFERTILITY IN PORT HARCOURT

ABSTRACT

The study evaluates the contribution of oxidative stress and some fertility hormones to female infertility in Port Harcourt. A total of 140 women aged 15 – 49 years consisting 70 apparently healthy infertile women attending diagnostic fertility clinics in Port Harcourt as test subjects and 70 age-matched healthy fertile women as control were recruited. Subjects were recruited using structured questionnaires after given their informed consent. The levels of some oxidative parameters (MDA, TAC and LPI) and FSH, LH, prolactin, progesterone and estrogen of infertile and the fertile (control) subjects were determined by standard procedures. LPI was determined as the ratio of MDA: TAC. Result showed a statistically significant increased lipid peroxidation index (LPI) in the test subjects than in the fertile group ($p<0.05$). TAC level showed a statistically reduced value in the test subjects than in the control at $p<0.05$. Test subjects exposed to oxidant agents like alcohol, infections and ulcer had significantly increased LPI than their counterparts who were not exposed ($p<0.05$). It was also observed significantly increased LPI value in those test subjects with normal hormone levels than in those with hormone imbalance ($p<0.05$). The outcome of this study suggests that the infertility being experienced by some of the infertile women are due not only to endocrine dysfunction, but some order conditions that induce oxidative stress. Thus investigation of oxidative parameters is highly suggested as an adjunct for effective management of unexplained infertility in women

Keywords: Infertility, fertility, oxidative stress, hormones, peroxidation index, intercourse

INTRODUCTION

Infertility is the incapability to attain gestation within after one year of unprotected, non-contraceptive regular sexual intercourse. Infertility could be primary (when couples have never conceived in their lifetime) or secondary (when another child could not be achieved after a year when one or both partners have previously had a child or children.[1] Over time, infertility has been on steady increase in Nigeria compared with what was obtainable in the past.[2] It has been reported that about 8-12 out of every 100 couples in diverse nationalities are hurt by infertility.[3-5] According to the report of Giwa-Osagie,[6] there are over twelve million infertile persons in Nigeria. In African states, subfertility is projected at 10-25%, the female factors are responsible for the greater percentage of the causes (55%) while the male factors are responsible for 30–40%

of causes. The infertility which causes could not be diagnosed (unexplained) accounts for 5–15% .[6] The burden of infertility in our environs is so high that almost half of women seeking consultation with gynaecologists complain of inability to get pregnant.[7]

Oxidative stress is the term generally used to describe a state of imbalance between pro-oxidant (free radicals) and antioxidants.[8] The free radicals (reactive oxygen species (ROS) and reactive nitrogen species (RNS)) are products of cellular metabolism constantly taking place in the body. They are needed in a certain quantity for normal cell functions.[9] The body usually respond to the excess amount of free radicals produced through an organized system known as antioxidant defense system. This system helps the living organisms to combat the radicals and reduce their toxic effects on cells and tissues. Antioxidants are the many substances that, in their small amount relative to the amount of those substrates that are oxidizable such as DNA, proteins, lipids, and, carbohydrates, will markedly slows down or hinder the substances from been oxidized.[10] The major work antioxidant defense does is to shield the cells and tissues from the damaging effects of reactive species. The reactive species are either produced in living organisms through processes involving inflammation of cell and tissues, disease conditions or normal metabolism (interior sources). Otherwise they are produced from sources like irradiation, food, drugs etc. (exterior sources). In any case, an increased generation of free radicals may instigate oxidative damage.[10]

Moreover, alteration in rate at which reactive species are generated as well as the effectiveness of the antioxidant defence mechanisms in living cells may result to oxidative stress (OS), giving rise to development of some pathological conditions. When there is increase in production of ROS/RNS or there is a reduced antioxidant status (or both), the natural antioxidant defence mechanisms of the body may be overpowered, thereby creating an unfavourable

environment for the normal functioning of the various systems of the body including reproductive system in the females. This could lead to development of some reproductive disease conditions including endometrioses, polycystic ovary syndromes (PCOS) and unexplained infertility. Also associated with this state of oxidative disturbance are pregnancy complications including preeclampsia, abortion, intrauterine growth restriction (IUGR) and repeated loss of pregnancy loss.[8,11-13] The degrading effect of oxidative stress (OS) on quality of ova has been previously described in mouse. Hence, fertilization as well as gestation rates in humans are adversely affected by OS.[14] Sterility could suffer as a result of reduced antioxidant status in the human body. Thus antioxidant therapy or consumption of antioxidant-containing food can be of great help in management or even prevention of sterility.[15]

Several current studies have linked excessive free radical productions with some controllable lifestyle factors like alcohol consumption, smoking of cigarette, use of some recreational drugs and exposure to irradiations.[16] The substances have ability to generate high volume of reactive species. Exposure to some occupational and environmental factors such as heavy metals like lead can also promote ROS/RNS generation. Hence women exposed to these factors may possibly experience disturbed reproductive system, resulting in infertility.

The peroxidative action of oxidants on polyunsaturated fatty acids (PUFAs) leads to the production of malondialdehyde (MDA) alongside many other secondary products. Because MDA is relatively stable it is often used as a marker of OS. The gamete as well as the genital tracts are rich in enzymatic antioxidants (superoxide dismutase (SOD), glutathione peroxidase, glutathione reductase, catalase) as well as non-enzymatic antioxidants (glutathione, vitamins E, and C and uric acid).[17]

It will be an almost impossible task to measure one by one all the antioxidants present in a living organism. Hence the more convenient way of accessing the antioxidant status of an individual is to determine the total antioxidant capacity (TAC). The amount of the overall activities of non-enzymatic antioxidants taking place in an organism is referred to as total antioxidant capacity.[18]

Although subfertility is a major challenge confronting couples in Nigeria, there is dearth of reports on the role and implication of oxidative stress in the etiology of infertility in Nigeria. This study is the first recorded report involving the use of oxidative stress markers in the investigation of infertility in infertile women in Port Harcourt. This study was, therefore, aimed at evaluating the impact of oxidative stress markers and hormonal profiles in women diagnosed with infertility in Port Harcourt, Nigeria.

MATERIALS AND METHODS

Study Area

This work was done in Port Harcourt, Rivers State of Nigeria

Subjects' selection: A total of 70 infertile female subjects, under reproductive ages (15 – 49 years), who willingly consented to participate in the study were randomly selected among patients attending diagnostic centers and fertility clinics in Port Harcourt including Rivers State University Teaching Hospital (RSUTH) and Image Diagnostic Center, Port Harcourt. Ethical approval for the study was obtained from the Rivers State Ministry of Health, Port Harcourt. A

forced-choice (closed ended) questionnaire was used to collect relevant information required for inclusion or exclusion of subjects. The well-structured questionnaires were given to each participant and they were guided by a trained laboratory staff to fill the forms. Also a total of seventy (70) healthy and fertile female subjects, who were within the reproductive ages of 15 – 49 years were recruited as controls using the questionnaire.

Study Design: This research is designed as a case controlled, and the sampling technique used was random and convenience sampling techniques.[19] The sample size was obtained by using the formula for calculation of sample size in a case-control design as described by Jaykaran & Tamoghna,[20]

Inclusion Criteria:

a) **Case group:** Women included in this group were those :

i. Married for at least 12 months, and have been having regular, unprotected sexual intercourse for at least 12 months.

ii. within the ages 15-49 years.[21]

iii. not under any contraceptive use for at least one year.

iv. Whose male partners has been investigated for fertility and found fertile with normal seminal fluid parameters.

b) **Control group**: those included in this group were:

i. Fertile women having at least a child in the past one year and are not under any contraceptive drug.

ii. Those within the fertility ages of 15-49 years.

Criteria for Exclusion as Controls:

Women under any of the following conditions were excluded from the study:

i. Those who have suffered from serious illness or hospitalized in the past 3months.
ii. Chronic illnesses like cancer, hypertension, asthma and diabetes mellitus which could interfere with result obtained.
iii. Those with history of recurrent/untreated genital tract infections within 1 year
iv. Those with history of ulcer for the past one year
v. Persons under drugs for infertility
vi. All regular alcohol consumers and cigarette smokers were excluded.

Blood sample collection: The blood samples were collected on the day 21 of menstrual cycle of the subjects by venepuncture, dispensed into plain bottles and centrifuged after clotting using bench centrifuge. The serum separated and frozen at -20 ^{0}C till assay

Determination of Serum Fertility Hormone Concentrations

Human FSH, LH and prolactin (PRL) levels were determined using Solid Phase enzyme-linked immunosorbent assay (ELISA) method of Engvall & Perlmann.[22] Estrogen as well as progesterone was determined using competitive binding Enzyme immunoassay (EIA) method of Van-Weemen and Schuurs.[23]. No special pretreatment was necessary for this assay as all grossly hemolyzed, lipaemic, or turbid samples were excluded in the assay. It was also ensured that no sample containing sodium azide was used.

Determination of MDA Concentration

Thiobabituric acid reactive substance **(**TBARS) colorimetric assay technique of Bernheim *et al*.[24] was used. This assay is based on the reaction of a chromogenic reagent, 2-thiobarbituric acid, with MDA at 25^{0}C. One molecule of MDA reacts with two molecule of 2-thiobarbituric acid via

a knoevenagel-type condensation to yield a chromophore with absorbance maximum at 532nm. The intensity of the stable pink color formed is proportional to the amount of MDA present in the sample.

Determination of TAC Concentration

Serum total antioxidant capacity (TAC) levels were determined spectrophotometrically using CUPRAC-BCS assay method of Campos *et al.*[25] This assay evaluates the capacity of the antioxidants of a sample to reduce the Cu^{2+} to Cu^{+} in the presence of a chelating agent. These chelators form colored stable complexes with Cu^{+} that have a maximum absorption at 450 – 490 nm. The CUPRAC assay measures the thiol-group antioxidants and other plasma antioxidants such as ascorbic acid, α-tocopherol, β-carotene, uric acid, albumin, and bilirubin. The reduction potential of antioxidants in the sample/standard effectively reduces Cu^{+2} to Cu^{+}, thus changing the ion's absorption characteristics. This reduced form of copper will selectively form a stable 2:1 complex with the chromogenic agent (the Chelator- bathocuproinedisulfonic-acid disodium salt (BCS)) with absorption maximum at 450 nm. A known concentration of trolox is used to create a calibration curve, from which the TAC concentration in samples is extrapolated. The concentrations are expressed as mM/L Trolox equivalent.

Lipid peroxidation index (LPI) was calculated as the ratio of MDA to TAC.

RESULTS

MDA, TAC and LPI were measured in a total of 70 infertile women (case) and 70 fertile women (control). The frequency and percentage distribution of the observed clinical characteristic of the studied population (case group) is shown in table 1. Out of the seventy (70) infertile women recruited, 13 (18.6%) were between 20-29 years, 41 (58.6%) were between 30 -39 years, while

16 (22.8%) were within 40-49 years. 16 (22.9%) of the subjects were affected by primary infertility, while 54 (77.1%) were affected by secondary infertility. Also, 53 (75.7%) of the women have suffered childlessness for less than five years, while 17 (24.3%) of the women have stayed childless for at least 5 years but not more than ten (10) years. Similarly, a total of 16 (22.9%) subjects had pelvic inflammatory diseases as a result of urinary/genital tract infections, 5 (7.1) were alcohol drinkers, 10 (14.3) had ulcer, 3 (4.3) had infections and also drink alcohol, while 36 (51.4) were not exposed to any of the aforementioned oxidant agents.

Table 1: Demographic Characteristics of the Case Subjects

Characteristics	Group	Percentage (%)	Total
Ages (years)	20 – 29 (13)	18.6	100
	30 - 39 (41)	58.6	
	40 – 49 (16)	22.8	
Types of Infertility	Primary (16)	22.9	100
	Secondary (54)	77.1	100
Duration of infertility (Years)	1 – 5 (53)	75.7	100
	6 – 10 (17)	24.3	
Hormonal factor	Normal (23)	32.9	100
	Ovarian insufficiency (8)	11.4	
	Hyperprolactinaemia (35)	50.0	
	Hypogonadotrophic hypogonadism (4)	5.7	
Exposure to oxidants	Not exposed (36)	51.4	

agents	Exposed to infection (16)	22.9	100
	Alcohol (5)	7.1	
	Ulcer (H. Pylori) (10)	14.3	
	Infection and alcohol (3)	4.3	

Hormonal Characteristics of Case and Control

Table 2 presents the mean ± SEM of fertility hormones (LH, FSH, prolactin, progesterone and estradiol in the studied population. The mean ± SEM of FSH, LH, and Prolactin were found to be higher in the infertile women with values of 10.72 ±2.32 mIU/mL, 12.62 ± 2.09 mIU/mL and 30.3 ± 3.04 ng/ml respectively than in the control group who are fertile women with values: 6.30 ± 0.28 mIU/ml, 9.32 ± 1.53 mIU/mL and 21.87 ± 4.13 ng/mL respectively. However, the increased values were not statistically significant ($p>0.05$). Estradiol and progesterone levels were lower in the case group of 38.02 ± 3.87pg/mL and 3.50 ± 0.39ng/ml respectively than in the control group with values of 75.59 ± 2.73pg/mL and 7.37 ± 0.70 ng/mL respectively. These differences were statistically significant ($p<0.05$).

Table 2: **Hormonal Characteristics of Case and Control Groups ((Mean ± SEM)**

Parameters	**Controls N= 70**	**Tests N= 70**	**t-value**	**P-value**	**Remarks**
Age (years)	34.01 ± 0.72	35.79 ± 0.66	0	>0.9999	NS
FSH (mIU/ml)	6.30 ± 0.28	10.72 ± 2.32	1.892	0.0606	NS

LH (mIU/ml)	9.32 ± 1.53	12.62 ± 2.09	1.272	0.2057	NS
Estradiol (pg/ml)	75.59 ± 2.73	38.02 ± 3.87	7.905	<0.0001***	S
Progesterone (ng/ml)	7.37 ± 0.70	3.50 ± 0.39	4.847	<0.0001***	S
Prolactin (ng/ml)	21.87 ± 4.13	30.3 ± 3.04	1.642	0.0116*	S

Key: FSH-follicle stimulating hormone, LH-leutinizing hormone, NS – not significant, S – statistically significant, * $p<0.05$, *** $p<0.0001$

Levels of Fertility Hormones and Oxidative Parameters in the Test and Control Subjects According to Age Group.

Table 3 presents the mean concentrations (mean ± SEM) of hormonal and oxidative parameters according to age groups. The infertile subjects were classified into three age groups (20 – 29 years, 30 -39 years and 40 -49 years) respectively. The mean values of LH and FSH were highest in the 40 – 49 years category. The mean ±SEM of LH and FSH for the 40 – 49 years age group were 22.3 ± 6.31mIU/ml and 26.09 ± 8.42mIU/ml respectively while for the 30 -39 years age group the LH and FSH value were 8.46 ± 1.77mIU/ml for LH and 6.66± 1.53mIU/ml for FSH respectively. The mean values of LH and FSH for the 20 – 29 years were 13.83 ± 5.21mIU/ml for LH and 4.61± 1.00mIU/ml for FSH. There were statistically significant difference between the means of the LH and FSH among the three age categories ($p<0.05$). Prolactin level was highest among the 20 -29 years age group (43.15± 12.66ng/ml) and lowest among the 40 -49 years age group (25.29 ± 3.94ng/ml) but the difference was not significant $p=0.1211$. Progesterone and eostrogen levels were lowest among the 40 -49 years group (2.41 ± 0.49ng/ml (progesterone) and 29.36 ± 5.88pg/ml for estrogen and the differences in means were not statistically significant ($p>0.05$) among the three age groups. The level of oxidative peroxidation was highest among the 30 – 39 years age group (LPI_{30-39} = 49.10 ± 13.96, LPI_{20-29} =32.39 ± 8.90,

LPI_{40-49}= 26.61 ± 6.98) respectively. However, no significant difference (p>0.05) was found in the level of oxidative peroxidation index among the three groups.

Table 3: The Mean ±SEM of Fertility Hormones and Oxidative Stress Markers in the Infertile Population by Age Group.

Ages (years)	LH (mIU/ml)	FSH (mIU/ml)	PRL. (ng/ml)	Prog. (ng/ml)	E2 (pg/ml)	MDA (µM/L)	TAC (mM/L)	LPI
20 – 29	13.83± 5.21[a]	4.61± 1.00[a]	43.15± 12.66	3.29± 0.98	53.05 ± 12.05	8.45± 1.57	0.69± 0.24	32.39± 8.90
30 – 39	8.46± 1.77 [a]	6.66± 1.53[a]	28.19± 2.84	3.99± 0.55	36.63 ± 4.77	15.83± 3.10	0.64± 0.11	49.10± 13.96
40 – 49	22.3± 6.31[b]	26.09± 8.42[b]	25.29 ± 3.94	2.41± 0.49	29.36± 5.88	9.64± 1.50	0.80± 0.17	26.61± 6.98
P-value	0.0241	0.0008	0.1211	0.2533	0.1343	0.2181	0.2797	0.6794
F-value	3.942	7.892	2.179	1.402	2.069	1.558	0.7569	0.5104
Remarks	S	S	NS	NS	NS	NS	NS	NS

Mean with different superscripts (on each column) are statistically different from each other. LH-leutinizing hormone, FSH-follicle stimulating hormone, PRL-prolactin, Prog.-progesterone, E2-Estradiol, MDA-malondialdehyde, TAC- total antioxidant capacity and LPI-lipid peroxidation index, NS – not significant, S – significant, * – statistically significant and ** – very significant.

Oxidative Characteristics of Case and Control Groups.

Table 4 provides the mean concentrations (Mean ± SEM) of oxidative parameters (MDA, TAC and LPI) of infertile and fertile (control) groups in the studied population. The mean concentrations (Mean ± SEM) of MDA and LPI were higher in the infertile group (13.05 ± 1.90 µM/L and 40.85 ± 8.52 respectively) than in the fertile group (9.34 ± 0.92 µM/L and 16.21 ±2.50). Whereas the difference was not statistically significant (p>0.05) for MDA, it was LPI

($p<0.05$). The total antioxidant capacity (TAC) was lower in the infertile group (0.69 ± 0.09 mM/L) when compared with the fertile control group (1.33 ± 0.14 mM/L) and the difference was statistically significant ($p<0.05$).

Table 4: Oxidative Characteristics of Case and Control Groups (Mean ± SEM)

Parameters	Control group N= 70	Infertile group N= 70	T-value	P-value	Remarks
Age (years)	34.01 ± 0.72	35.79 ± 0.66	0	0.9999	NS
MDA (µM/L)	9.34 ± 0.92	13.05 ± 1.90	1.754	0.0816	NS
TAC (mM/L)	1.33 ± 0.14	0.69 ± 0.09	3.897	0.0002***	S
LPI	16.21 ± 2.5	40.85 ± 8.52	2.774	0.0063**	S

KEY: S – Significant, NS – not significant, ** – very Significant, *** – highly significant

Mean Levels of MDA, TAC and LPI in the Infertile Group According to Normal Hormone Levels and Abnormal Hormone Levels Compared

The mean concentrations of MDA, TAC and LPI according to normal hormone levels and abnormal hormone levels in the infertile subjects are represented in table 5. The oxidative parameters (MDA, TAC and LPI) were determined for the infertile women with abnormal hormone levels and the infertile women with normal hormone levels. The values were compared with control group of normal fertile women with normal hormone levels. The mean concentration of MDA in the infertile women with abnormal hormone levels was 14.04 ± 2.48µM/L compared to its lower value of 11.88 ± 2.85 µM/L in the infertile women with normal hormone level and both values were higher than that for the control group and the variation did

not show any significance (p=0.1375). TAC mean concentrations were 0.84 ±0.12 mM/L in infertile women with abnormal hormone levels group and 0.33 ± 0.06mM/L (lower) in infertile women with normal hormone level group; both values were lower than the value for the fertile women with normal hormone group of 1.33 ± 0.14mM/L and the difference in the mean concentrations was statistically significant (p <0.0001). The LPI mean concentrations were higher in the infertile women with normal hormone group (59.36 ± 23.34) than in the infertile women with abnormal hormone group (32.71 ± 5.36). Both values were higher than the value for the fertile women with normal hormone (16.21 ± 2.50) but no significant difference (p>0.05) between the means of LPI of the infertile women with abnormal hormone group and LPI of the fertile women with normal hormone group, however, significant (p<0.05) variation between means of LPI of infertile women with normal hormone group, infertile women with abnormal hormone levels and the fertile women with normal hormone group was seen.

Table 5: Mean Levels of Oxidative Markers (MDA, TAC & LPI) in the Infertile Group According to Normal Hormonal Levels and Abnormal Hormonal Levels Compared.

Group	**MDA (**μM/L**)**	**TAC (mM/L)**	**LPI**
Normal fertile women with normal hormone levels (control)	9.34 ± 0.92	1.33 ± 0.14[a]	16.21 ± 2.50[a]
Infertile women with normal hormone levels	11.88 ± 2.85	0.33± 0.06****[b]	59.36 ±23.34**[b]
Infertile women with abnormal hormone levels	14.04 ± 2.48	0.84 ± 0.12*[c]	32.71 ± 5.36[c]
P-value	0.1375	<0.0001	0.0027
F-value	2.013	10.29	6.188

Remark	NS	S	S

Mean with different superscript (on each columns) are statistically different from each other. NS – not significant, S – statistically significant, * –significant, ** – very significant, *** – highly significant.

DISCUSSION

The issue of infertility is now a global problem facing every population of all societies, both developed and developing countries are been increasingly affected.[3,5] Effective treatment and management of this menace requires a holistic approach born out of a comprehensive understanding of factors affecting the disease. Infertility has been often related to endocrine disorder affecting the hypothalamo-pituitary-ovarian axis, eliciting imbalance in the female hormonal profile. Researchers are currently linking infertility with oxidative stress.[8,12]

The result of this study showed that there was a significantly higher induction of oxidative stress in the infertile women when compared with the fertile control subjects. The LPI and TAC were significantly ($p=0.0063$ and $p<0.0002$) higher in the infertile women when compared with the fertile control. This result is in agreement with studies of Agawal *et al.*[8], Attaran *et al.*[26] and Oyewole *et al.*[17].

The mean concentration of MDA in this study was insignificantly ($p>0.05$) higher while the LPI was significantly ($p<0.05$) higher in the infertile group than in the fertile group. A strong positive correlation of MDA with the lipid peroxidation index (LPI) ($r = 0.661$) was also observed. The study also showed a significantly ($p<0.05$) lower level of total antioxidant capacity (TAC) in the infertile women than the fertile women and the LPI was negatively correlated with TAC in the infertile women ($r= -0.30$, $p= 0.014$). Since LPI was used as index of

oxidative stress, a rise in MDA and fall in TAC elicited an increase in oxidative stress.[27] This study showed that there was significant oxidative stress in the infertile compared to the fertile women and that the overall activity of antioxidant system in the infertile women was weaker than in the fertile women. The weaker antioxidant system may have being responsible for the observed oxidative stress expressed in the infertile group as shown by the raised value of the lipid peroxidation index. This result is in agreement with Oyewoye *et al.*[17] who estimated the total antioxidants capacity (TAC) levels in the follicular fluid of women undergoing IVF and found that the TAC level in the follicular fluid that produced oocytes which become fertilized where significantly higher than in those whose oocytes did not get fertilized, meaning that fertile gametes contain strong antioxidants. In the present study the diminished TAC may have occurred as a result of increased oxidant activities since an elevated oxidant level infers fatigued antioxidant defense, thereby eliciting the incapability of the scavenger to defuse the oxidants' toxic effects.[13] Therefore, the diminished TAC may be responsible for the oxidative stress experienced by the infertile women in the studied population. Hence, antioxidant supplement therapy may be of help in management of infertility in this area. These findings are also supported by the earlier work of Tripathi *et al.*[15] who proved that antioxidants could be helpful in treatment of infertility.

The comparison of the level of oxidative stress in the infertile subjects based on hormone classification showed significant ($p<0.05$) increase in the mean LPI value among infertile women with normal hormone levels above those with abnormal hormone levels (imbalance) when compared with the fertile women (control group). Mean TAC level was significantly ($p<0.0001$) lower in the infertile subjects with normal hormone levels than those with abnormal hormone levels compared with control fertile women with normal hormone levels. This suggests that the

infertility being experienced by some of infertile subjects may not be due to endocrine dysfunction, rather some other conditions that induce oxidative stress may be responsible, a position that is in agreement with the reports of Tarin *et al.*[14] and Huang *et al.*[16].

The present study further compared the oxidative parameters in the infertile women with normal hormone profile based on exposure to oxidant agents with the fertile control group. The result showed a significant decrease in TAC level between those (infertile women with normal hormone profile) exposed to oxidants agents (infections, alcohol, and ulcer) and those who were not exposed to any of the aforementioned agents (but are infertile with normal fertility hormone levels) when compared with control subjects ($p<0.05$). The LPI was also significantly ($p<0.05$) higher in the exposed subgroup than the non-exposed when compared with control. This result suggests that there may be a significant state of oxidative stress in the exposed subgroup than the non-exposed, which resulted in the experience infertility. This observation is in agreement with reports of several researchers who have demonstrated the roles of the aforementioned oxidant agents in induction of oxidative damage.[16,28-29]

Alcohol is primarily eliminated from the body through an oxidative mechanism occurring in the liver. Alcohol hepatic metabolism produces acetaldehyde which upon further dehydrogenation yields acetic acids with acetyl and methyl radicals. These metabolites generate a high amount of oxidants.[30] The overproduced ROS promotes lipid peroxidation, decrease antioxidant enzyme activities (SOD), and deplete GSH concentration, thereby establishing oxidative stress.[30] Alcohol induced OS can initiate the oxidation steps of the Maillard reaction which promotes AGE (advanced glycation end products) formation. Accumulation of the toxic product, AGE, is linked with the upregulation of antioxidant activities. The binding of AGE to its receptor (RAGE) induces a state of inflammation through activation of NF-Kappa B (a

transcription factor) and then cytokine expression.[28] Thus, alcohol use can speed up oxidative stress through some mechanisms that involved enhancement of apoptosis, alteration of cell structures and damaging of tissues. A study showed that when mouse embryo was exposed to ethanol, it experienced an increased oxidants generation, lipid peroxidation, apoptosis and in vitro deformation, and that when SOD and/or vitamins were administered simultaneously, the effect of oxidative stress was reduced.[29]

Tubal infertility has been largely related to infections of the genital tract and consequently oxidative stress.[28] Tubal damage has been reported as the most common cause of secondary infertility in our environment.[31] Augusta *et al*.[32] observed an association between reproductive hormones and oxidative markers in infertile women infected with chlamydia and reported a moderate increase in LH with a significant low TAC level in chlamydia positive infertile women compared with chlamydia negative fertile control subjects. Macrophages and polymorphonuclear leukocytes are inducted through the inflammatory response to infections of the genital tract. The activities of macrophages and cytokines result in greater ROS generation and consequently oxidative-induced cell destruction.[33] Similarly, a strong positive correlation of MDA with LPI (r= 0.964) in the oxidant-exposed subgroup was also observed in this study. Since LPI is used as the index of oxidative stress in this study, it then implies that the increased oxidative stress in this subgroup could be due to increased MDA production that is linked with increased free radical generation occurring through the metabolic processes of the oxidant molecules that subdued the antioxidant defense system as previously reported.[34] Therefore interventions that eliminate exposure to oxidant sources including infections, alcohol, irradiations, cigarettes and ulcer (H-pylori) may be of help in infertility managements.

CONCLUSION

There is a significant increase in oxidative stress markers in women diagnosed with infertility in the population studied which have been caused by exposure to antioxidant agents to which most of the women might have been exposed. Thus, evaluation of oxidative stress parameters should form part of the panel of analysis used in the investigation of infertility in women in the studied population.

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