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Evaluation of Selected Trace Elements and Glutathione Peroxidase Levels in Female Infertility

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ABSTRACT

Infertility is an important world-wide reproductive disorder and some couples are being affected everyday as the population increases. The study aimed to evaluate the levels of selected trace elements (zinc, magnesium, selenium) and glutathione peroxidase in female subjects diagnosed with infertility. The study investigated a total of 90 subjects; 25 subjects diagnosed of primary infertility, 25 subjects diagnosed with secondary infertility and 40 apparently healthy individuals which served as the control group within the age range of 20-45years. Samples were obtained from the patients who attended the obstetrics and gynecology department, Federal Medical Centre, Ido-Ekiti, Ekiti State, Nigeria. Zinc (Zn) and selenium (Se) were estimated using atomic absorption spectrophotometer (AAS), magnesium (Mg) was estimated spectrophotometrically, while glutathione peroxidase (GPx) was evaluated using enzyme linked immunosorbent assay (ELISA) technique. Zinc (Zn), magnesium (Mg) and glutathione peroxidase (GPx), were significantly lower (P>0.05) in subjects with infertility in relation to the control subjects, while selenium (Se) was found to be significantly higher (P<0.05) in subjects with infertility compared with control subjects. Selenium (Se) and magnesium (Mg) were significantly lower (P<0.05) in subjects diagnosed with primary infertility compared with subjects diagnosed with secondary infertility. No significant difference (P<0.05) was found in serum zinc (Zn) levels and glutathione peroxidase (GPx) between subjects with primary and secondary infertility. The study concluded that the parameters are useful and should be included in the routine assessment, diagnosis and monitoring of cases of infertility.

Keywords: Glutathione peroxidase, Infertility, Selenium, Zinc

Introduction

Infertility is an important worldwide reproductive disorder, and some couples are being affected every day as the population increases. Infertility problems affect various aspects of human beings. The prevalence of infertility varies from country to country ranging from less than 5% to over 30% **(1, 2)**. In Nigeria, it has been extrapolated that about 3–4 million couples have infertility problems, affecting 10%–30% of couples **(3, 4)**. Infertility is defined as a disorder of the reproductive system and the inability to become clinically pregnant after 1 year or more of regular unprotected sexual intercourse **(5)**. Primary infertility is when a woman is unable to give birth to a child, either due to the failure to become pregnant or due to the failure to carry a pregnancy to term **(6)**. Secondary infertility, on the other hand, is the inability to conceive for 1 year or more (2 years in some epidemiological studies) after having conceived at least once before **(7)**. Causes of female infertility are many: including acquired or genetic causes, inability to ovulate, and malformation of the eggs (Figure 1). These causes could be due to deficiencies of 1 or more micronutrients or nutritional disorders that play significant roles in reproduction and may complicate conception **(8)**. Smoking, alcoholism, and obesity are some of the risk factors capable of affecting fertility (Figure 2). Moreover, exposure to pollutants in the environment and some toxic substances directly can become toxic to human gametes (eggs and sperm), resulting in both decreased numbers and poor quality of gametes, which may eventually lead to infertility **(9, 10)**.

Trace elements, such as zinc (Zn) and selenium (Se), are important to health. Zn, for instance, is an essential trace element in human nutrition needed in every cell **(11)**. Zn plays a key role in human growth and development, especially in reproduction **(12, 13)**. Zn is essential in the synthesis of DNA and RNA, needed at every step of the cell cycle **(12)**. Zn is involved in the building up and breakdown of many organic compounds. It is said to be involved in the regulation and expression of many genes **(14, 15)**. Zn has anti-apoptotic and antioxidant properties **(16, 17)**.

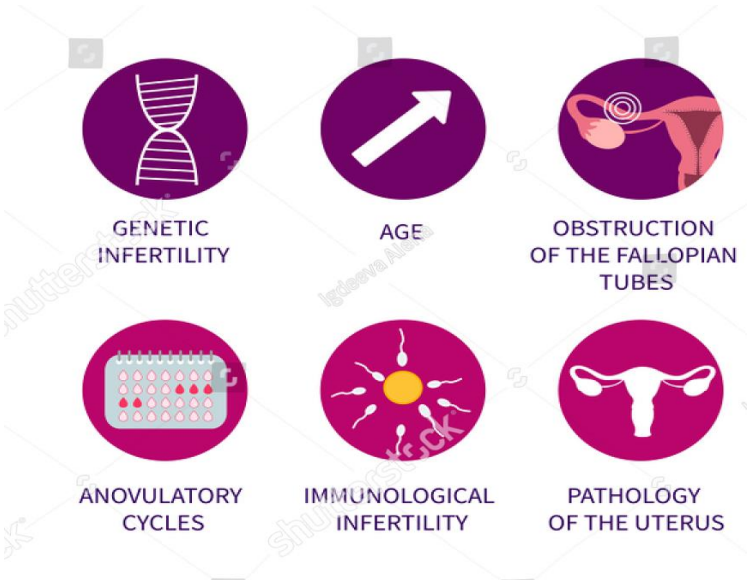

Figure 1. **Some causes of infertility (18)**

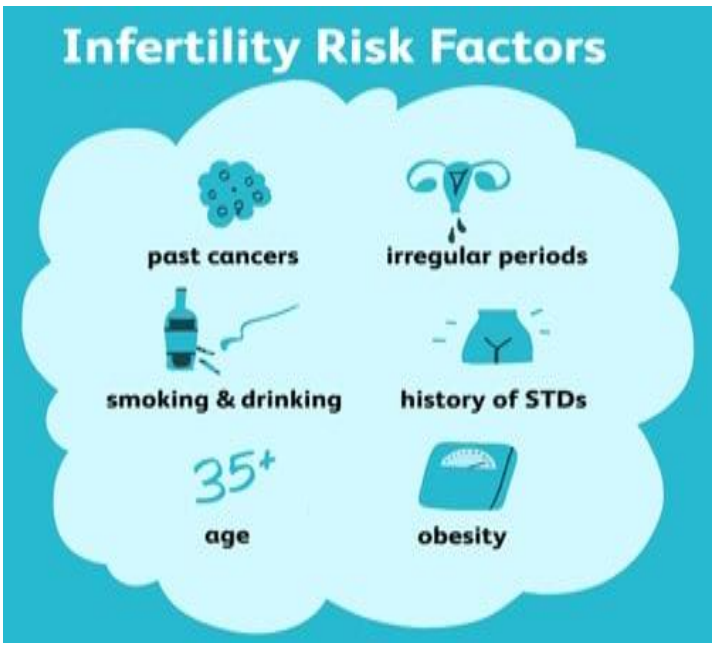

Figure 2. **Some risk factors of female infertility (19)**

Inadequate consumption of trace elements could result in deficiency of the immune system, multiple infections, more severe infections, growth retardation, late sexual maturation, and infertility **(20)**. Magnesium (Mg) is a very much abundant mineral in the body, especially as an intracellular divalent cation, as well as in a number of metabolic reactions in the body **(21, 22)**. On the average about, 50%, 50%, and 1% of Mg are found in the bone, tissues/organs, and blood, respectively **(21, 22)**. Some of the processes in which Mg is a cofactor include the synthesis of protein, production of cellular energy, storage, reproduction, synthesis of DNA and RNA, and stability of mitochondrial membranes **(23–26)**. The nutritional role of Se is realized by its inclusion in selenoproteins that have selenocysteine at their active center **(27)**. Selenoproteins are involved in a number of biological processes, including but not limited to fertility and reproduction **(28)**. As an aid in reproduction and pregnancy, glutathione peroxidase (GPx) is involved in the detoxification of peroxides (hydrogen peroxide and lipid peroxides), forming harmless products and thereby preventing damage to cell by reactive oxygen species **(29)**. Both Se and GPx are associated with follicular fluid **(30)** and fertility disorders. Accordingly, this study assessed the levels of Zn, Mg, Se, and GPx in female infertility.

Methods

This is a cross-sectional design using a stratified random sampling method. Stratification was by age and diagnosis. The study was conducted at the Federal Medical Centre (FMC), Ido-Ekiti, Ekiti State (Nigeria), among women aged 21–45 and diagnosed with infertility (primary or secondary). The study investigated 50 subjects diagnosed with primary (25 subjects) and secondary infertility (25 subjects); also, 40 apparently healthy subjects were enrolled as control subjects. The subjects were informed about the research, and they voluntarily gave their consent before they participated in the study. All sample analysis was carried out in the laboratory of the Medical Laboratory Science Department of Afe Babalola University, Ado-Ekiti. Ethical approval was obtained from the Health Research and Ethics Committee of the Federal Medical Centre, Ido-Ekiti, Ekiti State. The purpose of the research was explained to each participant using an informed consent for literate participants and a verbal explanation for illiterate participants. Participants were not forced to answer questions, but at their free will. The participants were assured of confidentiality and voluntary participation and that there were no financial benefits whatsoever.

Sample Collection and Preparation

A venous blood sample of about 6 mL was collected from the cubital fossa using a needle and syringe and dispensed into a universal plain (non-anticoagulant) bottle. The blood was allowed to clot and spun down at 12,000 rpm for 5 minutes to separate the serum from the cells. The serum samples for estimation of Zn, Mg, Se, and GPx were stored at temperatures of -20^{o}C until analysis. Glutathione peroxidase ELISA kit was purchased from Melsin Medical Co., Limited, China and analyzed using Readwell ELISA plate analyzer from Robonik India Private Ltd., India. Magnesium reagent kit was from Teco Diagnostics, USA and analyzed spectrophotometrically using a spectrophotometer from Pioway Medical Lab Equipment Co. Ltd., China.

Analysis of Biochemical Parameters

Estimation of Zn and Se

Zn and Se were determined using an atomic absorption spectrophotometer (AAS; AAS Buck Scientific 210VGP, from BUCK Scientific Inc., USA). Zn was measured at a wavelength of 213.9 nm, while Se was measured at 313.7 nm.

Principle: Zn and Se absorb light that is emitted by the metallic cathode lamp at the corresponding wavelength. The amount of the light absorbed is proportional to the concentration of the metal in the solution, and the results were displayed on the instrument in ppm. These were converted to mg/dL for Zn and ug/L for Se, respectively **(31)**.

Estimation of Mg

Principle: Mg reacts with calmagite in an alkaline medium to form a red-colored compound that is measured spectrophotometrically at 530 nm. The color produced is proportional to the concentration of the Mg present in the sample **(32)**.

Estimation of GPx

Principle: GPx polyclonal antibodies, which have been pre-coated on the micro-well plates, are reacted with enzyme-labeled antibodies and patients' serum to form antigens and antibodies sandwich complex. The antibody-bound fraction is separated from an unbound antigen by decantation and washing, after which the enzyme substrate is added for color development. The intensity of the color produced is proportional to the concentration of the GPx present in the sample **(33)**.

Statistical Analysis

The results obtained were subjected to statistical analysis using SPSS 23 (SPSS Inc, Chicago, Ill, USA). All parameters were expressed as mean±SD. Analysis of variance (ANOVA) was the tool of choice in comparing means. A correlation analysis was also done to determine the relationship between parameters. P-values at <0.05 or <0.01 were taken to be statistically significant.

Results

The results of the Zn, Mg, Se, and GPx levels are represented in the tables and figures listed below.

Table 1 shows the parameters in infertile and control subjects. Zn and GPx were significantly lower (P<0.05) in both primary and secondary infertility compared with controls. Se and Mg showed a significant increase (P<0.05) in secondary infertility compared with both primary infertility and control subjects.

Table 1. Biochemical Parameters in infertile subjects and control subjects

Parameters	Primary infertility n= 25	Secondary infertility n=25	Control n=40	F values	P values
Age	30.84±5.96	29.36±4.32	30.70±4.35	0.752	0.474
Zinc	31.10±5.70^{c}	32.46±10.53^{c}	37.17±5.05	6.552	0.002**
Selenium	54.93±12.1^{b}	82.50±1.36^{ac}	64.25±0.27	11.029	0.000**

Parameters	Primary infertility n= 25	Secondary infertility n=25	Control n=40	F values	P values
Magnesium	2.1±20.16[b]	2.82± 29.97[ac]	2.16±37.80	8.296	0.001**
Glutathione peroxidase	2.88±0.83[c]	3.01±1.17[c]	4.32±.96	21.574	0.000**

Key: **= test significant at P-value<0.05
Results are represented as mean ± standard deviation (SD)
[a]*P*<0.05 when compared with primary infertility.
[b]*P*<0.05 when compared with secondary infertility.
[c]*P*<0.05 when compared with control subjects.

Table 2 shows the relationship between the parameters in subjects with primary infertility. There was a negative correlation between Zn and GPx in subjects with primary infertility at a P-value<0.05. There was no significant correlation (*P*>0.05) between other parameters in subjects with primary infertility.

Table 3 shows the relationship between age, Zn, Se, Mg, and GPx in secondary infertility. There was no significant correlation (*P*>0.05) between the parameters.

Table 4 shows the relationship between the parameters in control subjects; only Se and GPx showed a positive correlation at a P-value<0.01.

Table 2. Relationship between all the parameters in subjects with primary infertility

Parameters	Age		Zinc		Selenium		Magnesium		Glutathione Peroxidase	
	R	P	r	P	r	p	r	P	R	p
Age	1	-	0.006	0.979	0.117	0.578	0.141	0.501	0.125	0.402
Zinc	0.006	0.979	1	-	0.172	0.411	0.352	0.085	-0.434*	0.030
Selenium	0.117	0.578	0.172	0.411	1	-	-0.142	0.499	-0.143	0.496
Magnesium	0.141	0.501	0.352	0.085	-0.142	0.499	1	-	-0.202	0.334
Glutathione peroxidase	0.175	0.402	-0.434*	0.030	-0.143	0.496	-0.202	0.334	1	-

**Correlation is significant at the 0.01 level (2-tailed)
*Correlation is significant at the 0.05 level (2-tailed)

Table 3. Relationship between all the parameters in subjects with secondary infertility

Parameters	Age		Zinc		Selenium		Magnesium		Glutathione Peroxidase	
	R	p	r	p	R	P	R	P	R	p
Age	1	-	0.227	0.275	0.158	0.450	-0.205	0.325	0.057	0.788
Zinc	0.227	0.275	1	-	-0.276	0.181	-0.114	0.587	-0.152	0.467
Selenium	-0.158	0.450	-0.276	0.181	1	-	0.065	0.757	-0.021	0.920
Magnesium	-0.205	0.325	-0.114	0.587	0.065	-0.757	1	-	-0.035	0.869
Glutathione Peroxidase	0.057	0.788	-0.152	0.467	-0.021	0.920	0.035	0.869	1	-

Table 4. Relationship between all the parameters in control subjects

Parameters	Age		Zinc		Selenium		Magnesium		Glutathione Peroxidase	
	R	p	r	P	r	p	r	P	R	p
Age	1	-	0.151	0.353	-0.286	0.073	-0.017	0.916	-0.191	0.237

Zinc	0.151	0.353	1	-	0.138	0.394	-0.078	0.631	-0.096	0.557
Selenium	-0.286	0.073	-0.138	0.394	1	-	-0.214	0.185	0.413**	0.008
Magnesium	-0.017	0.916	0.078	0.631	-0.214	0.185	1	-	0.201	0.214
Glutathione Peroxidase	-0.191	0.237	-0.096	0.557	0.413**	0.008	-0.201	-0.214	1	-

**correlation is significant at the 0.01 level (2-tailed)
*correlation is significant at the 0.05 level (2-tailed)

Discussion

Infertility comes with a wide range of sociocultural, emotional, physical, and financial problems **(1, 34)**. The present research was designed to evaluate the levels of Zn, Mg, Se, and GPx in female subjects diagnosed with infertility (both primary and secondary infertility) compared with control subjects.

The serum Zn level was significantly lower in subjects diagnosed with infertility compared with controls. This agrees with the work of Jameson **(35)**, who stated that the deficiency of Zn is a cause of infertility because it could affect maturation by doubling up the number of degenerating oocytes and increasing abnormalities in a chromosome. No significant difference was observed in the level of Zn between primary and secondary infertile subjects, meaning that there is Zn deficiency in infertility irrespective of whether it is primary or secondary.

The serum GPx level was significantly lower in subjects with infertility compared with controls. This disagrees with previous work that found GPx to be significantly higher in infertile subjects compared with controls **(36)**. A lot of factors could be responsible for significantly low GPx; it could be a result of exposure to oxidative stress, which causes oxidative damage to the female's reproductive tract **(37–39)**, thereby obstructing the modeling of tissues, hormone signaling, maturation of the oocyte, development of follicles, functioning of tubes, ovarian steroid development, changes in the cyclical endometrium, and functioning of the germ cells. All these processes are involved in reproduction, hence causing the individual to be infertile. Also, GPx has been shown to be protective against oxidative damage, and the decreased level may be a causal factor experienced by women with infertility. There was no significant difference in GPx when primary and secondary infertile subjects were compared. In this research, there was a significant negative correlation between GPx and Se in subjects diagnosed with primary infertility (P<0.05), which supports previous findings **(40)**. However, there was a positive correlation between GPx and Se in control subjects. This association is important and might have contributed to fertility in control subjects.

The serum Se level was found to be significantly lower in primary infertile subjects compared with secondary infertility and control subjects. This is in line with a finding where Se was found to be significantly lower in primary infertility **(41)**. Other researchers also detected significantly low Se levels in sub-fertile women **(42)**. This may be a result of the presence of Se-binding protein-1, which is an ovarian autoantibody protein that causes premature ovarian failure (which is a cause of infertility) **(43)**. More so, it has been shown that deficiencies in Se may lead to complications in gestation, abortion, and damage to some systems of the fetus, such as the nervous and immune systems **(44)**. However, the study observed a significant increase in the level of Se in secondary infertility, which is in line with the findings of significantly higher levels of Se in infertile males **(45)**. There was no significant correlation between Se and other parameters in subjects diagnosed with infertility. This is also in line with a study **(41)**, where there was no significant correlation between Se and other parameters.

In the present study, the plasma Mg level was significantly lower in subjects diagnosed with primary infertility compared with secondary infertility and control subjects. This supports the findings that Mg was deficient in female subjects diagnosed with primary infertility **(46)**. This could be a result of a poor feeding lifestyle in the locality leading to a deficiency in Mg. However, no significant positive correlation was established between Mg and other parameters in subjects diagnosed with infertility.

Conclusion

The study revealed that female subjects with infertility had decreased levels of zinc, magnesium and glutathione peroxidase. However, there was an increased level of selenium in secondary infertility while significantly low level of selenium was reported in primary infertile subjects. There was no significant correlation between zinc, magnesium and other parameters but an inverse correlation was established between selenium and glutathione peroxidase in subjects diagnosed with primary infertility and a significant positive correlation between selenium and glutathione peroxidase in control subjects. Thus, the study concluded that the parameters may be used in the routine assessment, diagnosis and monitoring of cases of infertility. The study also suggests that infertile subjects could be treated with supplements containing these trace elements to take care of the deficiencies.

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Author's Contributions

AO and BO were responsible for the project design, survey, statistical calculations and writing of the manuscript. ES was responsible for experimental analysis, scientific and conceptual contributions to the manuscript.

Conflict of Interest

The authors declare no conflict of interest.

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