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Seminal Plasma Selenium, Calcium, Magnesium and Zinc Levels in Infertile Men

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Infertility constitutes a major part of the health problems in our society. Many factors may be responsible for male infertility; this study was designed to determine the relationship between seminal plasma levels of selenium, zinc, calcium and magnesium and semen parameters in patients with male infertility. Eighty males of Nigerian origin with history of infertility provided standard semen specimens for fertility assessment while sixty two aged matched apparently healthy subjects were used as controls. The infertile men were classified as oligospermic, asthenospermic and azospermic, using the World Health Organization (WHO) criteria. The seminal plasma selenium and zinc concentrations were determined by flame atomic absorption spectrophotometry while calcium and magnesium concentrations were estimated by colorimetric methods. Descriptive seminal fluid analyses were performed on all the samples according to the World Health Organization standard. The concentration of seminal plasma calcium and magnesium were significantly higher ($p < 0.05$) in the fertile group than the infertile cohorts. The seminal plasma zinc and selenium levels were not significantly different ($p > 0.05$) between the control and the infertile groups. There was a significant positive correlation between calcium and percentage motility ($r = 0.529$, $p < 0.05$), sperm count and percentage motility ($r = 0.266$, $p < 0.05$), selenium and sperm count showed a weak positive correlation. Seminal plasma calcium and magnesium levels were significantly higher in the fertile than the infertile cohorts. These findings suggest that seminal plasma calcium and magnesium play a very important role in male fertility and should be considered as part of management plan for male infertility.

Key words: Seminal plasma micronutrients, male infertility

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INTRODUCTION

Infertility constitutes a major part of the health problems in our society. Infertility can be defined as the failure to conceive (regardless of cause) within one year of unprotected intercourse (De Melo-Martin, 2002). The cause of infertility most of the time remains idiopathic despite comprehensive investigations; however, 40% of all cases of failure to conceive are due to male factors (Croxford *et al.*, 2011). In the past decade numerous assays have been performed on both fertile infertile subjects in an effort to elucidate factors that may contribute to male infertility. The levels of trace elements, cations and toxic metals and their effects on spermatogenesis, sperm characteristics are being investigated. There is evidence that alteration in seminal plasma levels of Zn, Mg, Ca, Se, Pb, Cd, Cu, Ni, Co affect sperm quality with decreased fertility potential. Trace elements are essential for successful fertilization (Fraser, 1987). This is possibly due to deficiencies in the semen of inorganic micronutrient required for successful fertilization. This study determined the levels of Selenium, Zinc, Calcium and magnesium in seminal plasma of both fertile and infertile males and their association with infertility.

MATERIALS AND METHODS

Sixty-two fertile and eighty infertile males of Nigerian origin attending Infertility Clinics in the University of Calabar Teaching Hospital were studied. The mean age of investigated patients was 36±1.4 years. Semen samples were obtained by “self help” into a wide mouthed 20 mL sterile container after 3-5 days of abstinence from sex. The samples were analyzed for liquefaction time, volume, motility, morphology and count of spermatozoa using an improved Neubauer counting chamber. At least 200 cells were examined, the sperm count, percentage motility and morphology determined by a well trained scientist. The samples were classified as normospermic (n = 62)

indicated by sperm count >20 millions/mL, motility >50% and normal morphology >40%, asthenospermic (n = 40) indicated by sperm count >20 millions/mL and motility <50% irrespective of morphology, oligospermic (n = 18) indicated by sperm count <20 millions/mL irrespective of motility or morphology and azospermic (n = 22) indicated by the complete absence of spermatozoa. The semen samples were centrifuged at 400 rpm for 15 min and seminal plasma separated and kept in trace element free containers at -70°C until analyses carried out at room temperature in accordance with World Health Organization Criteria (WHO, 2010).

Calcium and magnesium levels measured by colorimetric method; with a kit obtained from Giesse diagnostics Rome. Total seminal plasma selenium and Zinc concentrations were determined by flame atomic absorption spectrophotometry using the Buck Model 205. The data generated were analyzed using SPSS statistical package version 18.

RESULTS

Table 1 shows that there was no significant variation (p>0.05) in age and seminal volume, Zinc and selenium among the four groups. However, percentage motility, sperm count, Ca²⁺ and Mg²⁺ all showed significant variation among the four groups (p<0.05).

As expected, the normospermics had a mean sperm count which was significantly higher (p<0.05) than those of the other groups (Table 1). The oligospermics and normospermics had both had a mean percentage motility which were both significantly higher (p<0.05) than that of the oligoasthenospermics and azospermics. There was no significant difference (p>0.05) in the percentage motility between the normospermic and oligospermic men.

The mean level of Mg²⁺ of the normospermic control group was significantly higher than those of all the infertile groups (p<0.05). However, there was no significant difference (p>0.05) in the mean level of Mg²⁺ in the oligospermic men when compared to that of

Table 1: Comparison of age, semen volume, percentage motility, sperm count, seminal plasma calcium, magnesium, selenium and zinc levels in normospermic fertile and infertile male groups

Parameters	Fertile normospermic	Infertile male			Calculated-F	Critical-F	p-value	Remarks
		Oligoasthenospermic	Oligospermic	Azospermic				
Age (years)	36.77±1.03	36.32±1.13	36.89±1.66	34.30±1.65	0.575	2.671	>0.05	NS
Vol. (mL)	2.26±0.09	2.42±0.08	2.33±0.09	2.63±0.16	1.869	2.671	>0.05	NS
Sperm count (m cm ⁻³)	46.60±3.39*##	11.0±0.83	15.78±0.61	0	46.191	3.074	<0.05	S
Motility (%)	68.36±2.06*	13.45±2.11	64.40±3.09	0	176.195	3.074	<0.05	S
Ca ²⁺ (mg dL ⁻¹)	13.37±0.61*##x	9.34±1.25x	9.67±1.21x	4.43±1.02	12.755	2.671	<0.05	S
Mg ²⁺ (mg dL ⁻¹)	5.92±0.72*##x	3.88±2.15x	3.85±1.38x	2.62±1.59	34.330	2.671	<0.05	S
Se (µg dL ⁻¹)	2.00±0.08	2.02±0.09	1.91±0.09	1.90±0.07	0.263	2.671	>0.05	NS
Zn (µg dL ⁻¹)	8.79±1.66	9.50±1.90	8.55±2.03	8.58±1.81	1.880	2.671	>0.05	NS
n	62	40	18	20	-	-	-	-

Values are Mean±SD, *Higher than that in Oligoasthenospermics, #: Higher than that in Oligospermics, x: Higher than that in azospermics, y: Higher than that in Normospermics

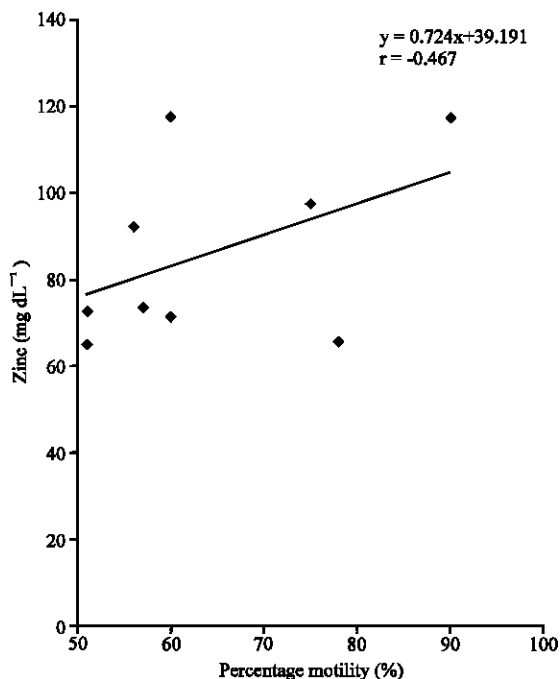


Fig. 1: A correlation plot of zinc against percentage motility in oligospermic infertile men, $p < 0.05$, $n = 18$

oligoasthenospermic men. The oligospermics had a mean level of Mg^{2+} which was significantly higher ($p < 0.05$) than that of the azoospermics.

The mean calcium level of the fertile group was significantly higher ($p < 0.05$) than those of all the infertile groups. In addition, the mean calcium levels of the azoospermic group was significantly lower ($p < 0.05$) than those of the other infertile groups. However, there was no significant difference ($p > 0.05$) between the asthenoligospermics and oligospermics.

There was also a positive correlation between Zinc and percentage motility in oligospermic group ($r = 0.463$, $p < 0.05$; Fig. 1). There was a significant positive correlation between calcium level and percentage motility ($r = 0.728$, $p < 0.05$; Fig. 2) and selenium and sperm count ($r = 0.529$, $p < 0.05$; Fig. 3) in oligospermic infertile males. This shows that increased seminal zinc and calcium levels is associated with increased percentage motility of sperm cells while increased selenium levels is associated with better sperm count in oligospermic infertile males.

DISCUSSION

This study showed that the seminal plasma calcium level was significantly lower in the oligospermic, azoospermic and asthenoligospermic infertile men compared to the normospermic controls ($p < 0.05$). Wong *et al.* (2001) in his study in Netherland reported

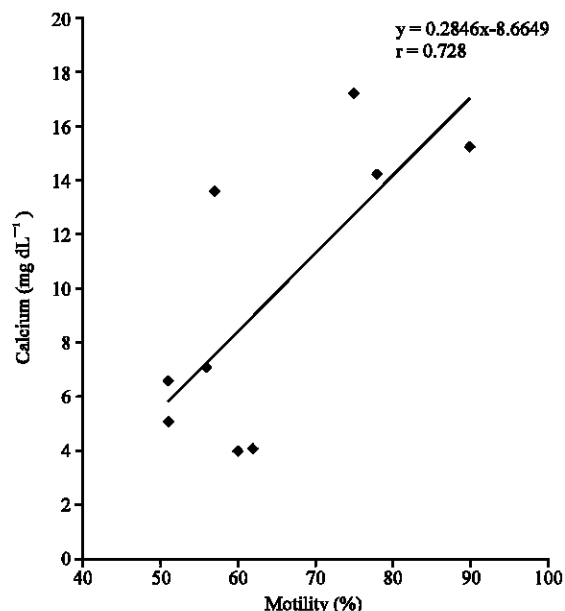


Fig. 2: A correlation plot of percentage motility against seminal plasma calcium in oligospermic infertile men, $p < 0.05$, $n = 18$

similar findings of low Ca^{2+} in the seminal plasma of infertile men which accounts for hypomotility. This has demonstrated the importance of calcium in sperm physiology, including motility (Lindemann *et al.*, 1987; Sorensen *et al.*, 1999) metabolism (Peterson and Freund, 1976), acrosome reaction and fertilization (Yanagimachi, 1981). It is known that calcium is required to initiate the acrosome reaction with its attendant release of enzymes and membrane alterations needed for successful egg-sperm interaction. This accounts for the significantly higher seminal plasma calcium level in normospermic compared to the oligospermic, azoospermic and asthenoligospermic subjects. Calcium is also necessary for maximum motility of sperm cells.

Although the study showed no significant difference in the seminal plasma selenium levels of the subjects and controls, Selenium is thought to be essential for normal spermatogenesis of mammals and the critical role it plays is principally mediated by two selenoproteins, namely Phospholipids Hydroperoxide Glutathione Peroxidase (PHGP_x) and selenoproteins P. These function as anti-oxidants preventing effects of oxidative stress on sperm cells by free radicals (Boitani and Puglisi, 2008). The glutathione peroxidase also forms part of the membrane structure of sperm cells conferring structural integrity on the cells. Laboratory evidence have shown that PHGP_x is the major selenoproteins expressed by germ cells in the testes, having multiple functions and representing an important link between selenium, sperm

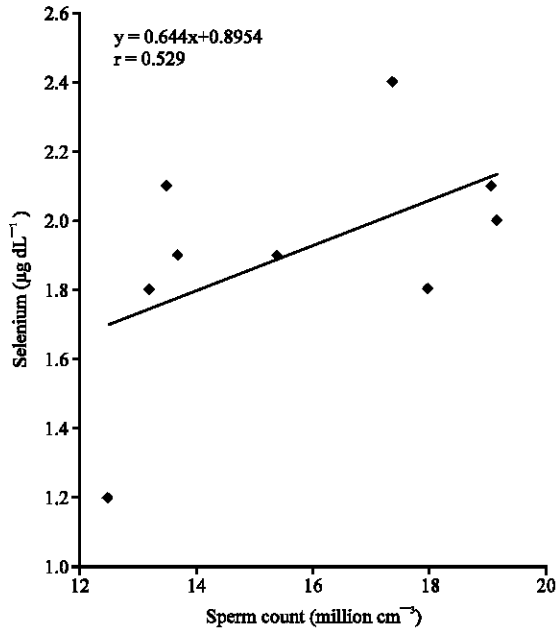


Fig. 3: A correlation plot of seminal plasma selenium against sperm count in oligospermic infertile men, $p < 0.05$, $n = 18$

quality and male fertility (Oguntibeju *et al.*, 2009). Roy *et al.* (1990) and Okada and Miyazaki (1987) had also reported no significant difference in the seminal plasma selenium levels of fertile and infertile men while Atig *et al.* (2012) reported a slight increase of seminal Se in controls compared to infertile groups and Saaranen *et al.* (1989) and Akinloye *et al.* (2005) observed a significant decrease of seminal Se levels in asthenozoospermics and oligozoospermics. However, the positive correlation between calcium and percentage motility and seminal plasma and sperm count in the oligospermics in this study seem to indicate that the oligospermics may benefit from calcium and selenium supplementation. This is supported by observations by Safarinejad and Safarinejad (2009). In a study on the efficacy of selenium and/or N-acetylcysteine for improving semen parameters in infertile men, they reported that selenium and N-acetyl-cysteine treatment resulted in decreased serum FSH, increased testosterone and inhibin B levels and a significant improvement in all semen parameters.

The significantly higher levels of magnesium in the fertile males when compared with the infertile groups indicate the healthy state of their prostate glands. Some workers suggested the measurement of magnesium, rather than zinc, as markers for infection because it was found that Mg levels were markedly decreased during prostate infection (Papadimas *et al.*, 1983). Also decreased magnesium is associated with increased thromboxane level which causes increase vasoconstriction and nitric

oxide that lead to premature ejaculation (Omu and Fernandes, 2001). Thus, prostate infection may result in low seminal plasma level of calcium, zinc and magnesium, premature ejaculation and infertility.

The mean levels of zinc among the four groups in this study showed no significant variation. Various studies have suggested that zinc plays an essential role in the physiology and development of gonads and spermatozoa (Awadallah *et al.*, 2003). It is important in sperm production and or viability and is important in the prevention of spermatozoa degradation, in addition to its antibacterial activity in seminal fluid (Carreras and Mendoza, 1990). Despite these facts, reports related to zinc levels in seminal plasma and its possible association with male infertility are conflicting. Earlier studies reported low zinc levels in seminal plasma in association with various conditions such as prostate infection (Colleen *et al.*, 1975), oligospermia, oligoasthenospermia and azoospermia (Fuse *et al.*, 1999) and infertile men regardless of semen quality (Chia *et al.*, 2000), while others reported no correlation with semen quality or with male infertility (Wong *et al.*, 2001). Our result is in agreement with those of previous investigators who reported no significant difference in the seminal plasma Zinc levels of the fertile and infertile men. The positive correlation between zinc and percentage motility oligospermics in this study also seem to indicate that the oligospermics may benefit from zinc supplementation.

CONCLUSION

These findings suggest that seminal plasma calcium and magnesium play a very important role in male fertility and should be considered as part of management plan for male infertility. Oligospermics may benefit from calcium, magnesium and zinc supplementation in their management.

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