Original Article

Seminal Oxidative Stress Markers, Calcium, Magnesium, and Semen Profile of Infertile Diabetic and Nondiabetic Nigerian Men

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Abstract

Context: Oxidative stress, vitamin, and macroelement deficiencies have been implicated in male infertility. It is unknown if diabetes mellitus with its attendant increased oxidative stress makes the seminal quality of the diabetic infertile men worse compared to their nondiabetic counterparts. **Aims:** The study investigated semen parameters, seminal plasma calcium, magnesium, Vitamins C and E, and total antioxidant capacity (TAC) in diabetic and nondiabetic infertile men. **Settings and Design:** This was a cross-sectional study involving 30 infertile men with type 2 diabetes, 30 infertile nondiabetic men and 30 fertile men. **Subjects and Methods:** Fasting plasma glucose, glycated hemoglobin, seminal plasma calcium, magnesium, TAC, Vitamin C, Vitamin E, semen analysis, and cultures carried out using the standard procedures. **Statistical Analysis Used:** Data were analyzed by the analysis of variance and Student's *t*-test; the level of significance was set at $P < 0.05$. **Results:** Both infertile groups had significantly lower ($P < 0.0001$) sperm count, percentage motility, TAC, Vitamin E and C, magnesium and calcium when compared to the fertile group. However, there was no statistically significant difference ($P > 0.05$) in the mean values of these parameters among the two infertile groups. The infertile men had a significantly higher ($P = 0.034$) frequency of bacterial isolates compared to the fertile men. *Staphylococcus aureus* was the most frequent organism isolated. **Conclusions:** Seminal calcium, magnesium, TAC, and Vitamin E and C were lower in both infertile diabetic and nondiabetic men as compared to that of fertile men, but the levels of these analytes were comparable in the infertile diabetic and nondiabetic men.

Keywords: *Antioxidants, calcium, magnesium, male infertility, seminal plasma, type 2 diabetics*

Introduction

Nigeria is the most populous country in Africa with about 160 million people and 400 ethnic groups and languages and there is a burden of disease with the rising incidence and prevalence of diabetes mellitus (DM) among the population. The World Health statistics indicate that Nigeria has the highest number of diabetics in sub-Saharan Africa.[1] There are about 3.1 million people with diabetes in the country,[2] and the current prevalence of 4.9%[3] has more than doubled the prevalence of 2.2% in the National Survey Report of 1997.[4] The incidence and prevalence of DM have continued to increase in Nigeria, despite a great deal of research and resources.[5]

DM is characterized by hyperglycemia due to abnormality either of insulin secretion, insulin action, or both.[6] Type 2 diabetes is a nonimmune disorder with varying degrees of insulin resistance and impaired insulin secretion usually associated with obesity.[7] It is the most common type of DM in Nigeria and accounts for over 95% of diabetes worldwide) results from a complex gene-environment interaction for which several risk factors, such as age, sex, ethnicity, family history, obesity, and hypertension, are well documented.[8]

DM is known to cause many systemic complications, including male infertility.[9] However, male infertility caused by impotence, retrograde ejaculation, and hypogonadism, is not widely recognized to be one of them.[10] However, a recent report has shown that DM induces subtle molecular changes that are important for sperm quality and function.[9] In a retrospective analysis, a very high prevalence of subfertility (51%) was found among patients with diabetes.[11] In another study, the prevalence of infertility among men with type 2 DM

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was 35.1%, with 16% being primary and 19.1% being secondary cases of infertility.[12] Ali *et al*. showed a highly significant increase in total sperm count and sperm concentration in patients with type 1 and type 2 DM. However, sperm motility and semen volume were lower than those of controls, but sperm morphology and the quality of sperm motility were unaffected.[13] Delfino *et al*. evaluated patients with both type 1 and type 2 DM. Semen analysis of these patients showed qualitative differences. The most important effects concerned the kinetic properties, especially progressive motility. Sperm morphology was also significantly compromised. On the other hand, sperm concentration did not show significant differences.[14] In summary, these data suggest that DM alters the conventional sperm parameters and influences semen quality. It is, therefore, necessary to evaluate the impact of DM on infertility.

Unfavorable changes in the levels of some macroelements have been associated with secondary complications of DM, which affects many organs including the gonads. The deficiency of these essential elements in men may result in low testosterone, sperm count, and reduced libido.[15] The level of magnesium is an indicator of the state of health the prostate gland. Some workers suggest the measurement of magnesium, rather than zinc, as markers for infection because it was found that seminal magnesium levels were markedly decreased during prostate infection.[16] In addition, reduced seminal magnesium is associated with increased thromboxane levels which may cause increase nitric oxide and vasoconstriction that results in premature ejaculation.[17] 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. Calcium is also necessary for maximum motility of sperm cells. Bassey *et al*. showed that calcium and magnesium levels were significantly lower in the seminal plasma of infertile males compared to that in fertile males.[18]

La Vignera *et al*.in 2009 suggested that DM is associated with increased oxidative stress, probably due to the presence of high levels of advanced glycation end products (AGE). This may result in sperm DNA damage and impaired reproductive function.[11] Another study by Mohasseb *et al*. showed that antioxidant agents, such as Vitamins E and C, alpha-lipoic acid reduce germ cell apoptosis and restored testicular enzymatic activities to almost the control levels in the diabetic rats.[19] To the best of our knowledge, studies comparing these analytes in diabetic infertile men and in nondiabetic infertile men are scarce. A question that remains unanswered is that does the presence of DM with the attendant increased oxidative stress make the seminal quality of the diabetic infertile men worse compared to the nondiabetic infertile men? This study will hopefully provide data that will provide some answers.

This study investigated the baseline semen parameters, seminal plasma calcium, magnesium, and Vitamins C and E in diabetic and nondiabetic infertile men and fertile men and their values compared. The microbial pattern of the participants in the study was also examined.

Subjects and Methods

This study was a cross-sectional study and the participants were consecutively recruited for the study. A total of 90 men of Nigerian origin consented to participate in this study. Thirty of them were infertile men with type 2 diabetes and 30 were infertile nondiabetic men attending the diabetic and urology clinics of the University of Calabar Teaching Hospital, Calabar. Thirty apparently fertile men who had fathered at least one child were recruited as controls from the Calabar metropolis. The sample size was determined using the Leslie Kish formular.[20] Ethical clearance was obtained from the University of Calabar Teaching Hospital ethical committee before the commencement of the study. The Health research protocol number was UCTH/HREC/33/342. Informed consent was also obtained from all the participants.

Inclusion criteria were all the participants 30 years and above and exclusion criteria were men with type I diabetes, terminally ill diabetics as well as anyone who did not consent to participate in the study were excluded from the study.

For the assessment of diabetes, blood specimens were taken from the participants between 8 and 10 a.m. after an overnight fast. Blood glucose was measured by glucose oxidase method using Randox kit, Britain. Any participant with a fasting glucose level ≥6.7 mmol/L or a clinical diagnosis of diabetes with either dietary, oral, or insulin treatment was classified as diabetic. Glycated hemoglobin was also estimated to determine glycemic control. It was estimated using kits by Teco Diagnostics, Anaheim, USA. The kit employed a weak binding cation-exchange resin for the rapid separation of glycohemoglobin (fast fraction) from nonglycated hemoglobin with a normal range of <6%.

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 volume, motility, morphology, and count of spermatozoa using a microscope and an improved Neubauer counting chamber. At least 200 cells were examined, the sperm count, percentage motility, and morphology determined according to the standard guidelines.[21] The semen samples were centrifuged at 4000 rpm for 15 min and seminal plasma separated and kept in trace element-free containers and frozen until analyses carried out.

All semen samples were cultured using standard procedures[21] to determine the presence of pathogenic organisms in the samples. Semen samples were cultured on blood, chocolate, and CLED agar. They were incubated

at 37°C for 48 h. Colonies were identified using standard procedures.

Magnesium was estimated using a test kit based on the magnesium-blue xilidile reaction for quantitative determination of Mg in biological fluids[22] obtained from Giesse Diagnostics, Rome-Italy. Calcium was estimated using the modified O-cresolphthalein complexone based colorimetric method[23] with a kit obtained from Giesse diagnostics, Rome, Italy. Seminal plasma Vitamin E was analyzed using a Bioassay ELISA kit by United States Biological, Salem, USA. Seminal plasma Vitamin C was estimated using the modified reduction colorimetric method[24] with a kit obtained from Giesse diagnostics, Rome. Seminal plasma total antioxidant capacity (TAC) measurement was done using the total antioxidant status assay kit from Rel Assay Diagnostics, Turkey. The assay is based on the measurements of the reduction of 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid; ABTS) radical. The assay was calibrated with Trolox and the results expressed as mM trolox equivalent/L (mmol trolox Equiv/L).

Statistical analysis was performed using the PAWstatistic 18, a statistical package from SPSS Inc., California, USA. The results were expressed as a mean ± standard deviation. The data were analyzed using the Student's *t*-test and analysis of variance followed by a *post hoc* test using the least significant difference. The level of significance was set at 95% of confidence interval, where value of $P < 0.05$ was considered as statistically significant.

Results

A comparison of the mean values of age, semen volume, sperm count, percentage motility, TAC, ascorbic acid, Vitamin E, Calcium, Magnesium, fasting plasma glucose, glycated hemoglobin levels in fertile and infertile diabetic and nondiabetic men is shown in Table 1. There was a statistically significant variation ($P < 0.0001$) in the values of sperm count, percentage motility, TAC, ascorbic acid, Vitamin E, Magnesium, fasting plasma glucose, glycated hemoglobin, and calcium ($P = 0.013$) among the groups. There was, however, no statistically significant statistical variation ($P > 0.05$) in the age and semen volume among the groups [Table 1].

Both infertile groups had significantly lower ($P < 0.0001$) sperm count, percentage motility, TAC, ascorbic acid, Vitamin E, magnesium and calcium compared to the fertile controls. However, there was no statistically significant statistical difference ($P > 0.05$) in the mean values of these parameters among the two infertile groups. The diabetic infertile men had significantly higher ($P < 0.0001$) fasting plasma glucose, glycated hemoglobin compared to the nondiabetic groups [Table 2].

A comparison of the frequency of bacterial isolates from semen cultures from participants in the study showed that the infertile men had a significantly higher ($P = 0.041$) frequency of bacterial isolates compared to the fertile men. *Staphylococcus aureus* was the most frequent organism isolated in both groups [Table 3].

Discussion

In this study, both sperm count and percentage motility were significantly lower in both diabetic and nondiabetic infertile men compared to that of the controls. This is because both parameters are measures of the seminal quality and hence determine the probability of the occurrence of fertilization. It is interesting to note that these two parameters were comparable in both infertile groups.

Seminal plasma TAC, Vitamin E and C were lower in the infertile group compared to the fertile men. A lot of studies[25-28] have shown that seminal plasma TAC and antioxidant levels are generally decreased in infertile men whether they are diabetic or not. This is because of the high levels of oxidative stress observed in the seminal plasma of

Table 1: Comparison of semen volume, sperm count, percentage motility, seminal plasma total antioxidant capacity, Vitamins C and E, calcium and magnesium levels in infertile diabetic and nondiabetic men and fertile men

Parameter	Fertile men (*n*=30)	Infertile men		Calculator *F*	Critical *F*	*P*
		Diabetics (*n*=30)	Nondiabetics (*n*=30)			
Age (years)	37.7±8.10	35.2±7.55	36.2±7.53	0.794	3.101	0.455
Semen volume (ml)	2.67±1.20	2.31±1.00	2.40±1.04	0.850	3.101	0.431
Sperm count (m/cm^3)	96.11±42.95	6.53±5.51	6.58±5.40	126.304	3.101	0.0001*
Percentage motility	71.83±6.82	26.50±15.32	25.33±14.02	132.709	3.101	0.0001*
TAC (mmol/L trolox equivalent)	0.60±0.18	0.30±0.20	0.27±0.18	26.973	3.101	0.0001*
Vitamin C (mg/dl)	2.19±0.42	1.55±0.85	1.46±0.71	10.072	3.101	0.0001*
Vitamin E (µmol/L)	0.49±0.10	0.24±0.12	0.26±0.11	47.402	3.101	0.0001*
Calcium (mg/dL)	13.38±4.94	9.03±7.14	8.70±7.31	4.775	3.101	0.011*
Magnesium (mg/dL)	5.90±0.74	3.52±1.86	3.38±1.77	25.030	3.101	0.0001*
Fasting plasma glucose (mmol/L)	4.26±0.55	8.58±3.85	4.35±0.56	35.520	3.101	0.0001*
Glycated haemoglobin (%)	5.06±0.63	8.12±1.59	5.18±0.70	79.297	3.101	0.0001*

*Significant at $P<0.05$. Mean±SD. TAC: Total antioxidant capacity; SD: Standard deviation

Table 2: Comparison of sperm count, percentage motility, seminal plasma total anti-oxidant capacity, Vitamins C and E, calcium, and magnesium levels in infertile diabetic and nondiabetic men and fertile men using *post hoc* analysis

Parameter	Groups		Mean difference	*P*
	Fertile men (*n*=30)	Infertile diabetics (*n*=30)		
Sperm count (m/cm^3)	96.11±42.95	6.53±5.51	89.573	0.0001*
Percentage motility	71.83±6.82	26.50±15.32	45.333	0.0001*
TAC (mmol/L trolox equivalent)	0.60±0.18	0.30±0.20	0.300	0.0001*
Vitamin C (mg/dl)	2.19±0.42	1.55±0.85	0.637	0.001*
Vitamin E (µmol/L)	0.49±0.10	0.24±0.12	0.252	0.0001*
Calcium (mg/dL)	13.38±4.94	9.03±7.14	4.350	0.012*
Magnesium (mg/dL)	5.90±0.74	3.52±1.86	2.379	0.0001*
Fasting plasma glucose (mmol/L)	4.26±0.55	8.58±3.85	−4.313	0.0001*
Glycated haemoglobin (%)	5.06±0.63	8.12±1.59	−3.057	0.0001*
Parameter	**Groups**		**Mean difference**	***P***
	Fertile men (*n*=30)	**Infertile nondiabetics (*n*=30)**		
Sperm count (m/cm^3)	96.11±42.95	6.58±5.40	89.530	0.0001*
Percentage motility	71.83±6.82	25.33±14.02	46.500	0.0001*
TAC (mmol/L trolox equivalent)	0.60±0.18	0.27±0.18	0.325	0.0001*
Vitamin C (mg/dl)	2.19±0.42	1.46±0.71	0.730	0.0001*
Vitamin E (µmol/L)	0.49±0.10	0.26±0.11	0.233	0.0001*
Calcium (mg/dL)	13.38±4.94	8.70±7.31	4.687	0.007*
Magnesium (mg/dL)	5.90±0.74	3.38±1.77	2.513	0.0001*
Fasting plasma glucose (mmol/L)	4.26±0.55	4.35±0.56	−0.084	0.887
Glycated haemoglobin (%)	5.06±0.63	5.18±0.70	−0.113	0.682
Parameter	**Groups**		**Mean difference**	***P***
	Infertile diabetics (*n*=30)	**Infertile nondiabetics (*n*=30)**		
Sperm count (m/cm^3)	6.53±5.51	6.58±5.40	−0.433	0.995
Percentage motility	26.50±15.32	25.33±14.02	1.167	0.721
TAC (mmol/L trolox equivalent)	0.30±0.20	0.27±0.18	0.025	0.608
Vitamin C (mg/dl)	1.55±0.85	1.46±0.71	0.093	0.599
Vitamin E (µmol/L)	0.24±0.12	0.26±0.11	−0.019	0.508
Calcium (mg/dL)	9.03±7.14	8.70±7.31	0.337	0.843
Magnesium (mg/dL)	3.52±1.86	3.38±1.77	0.134	0.738
Fasting plasma glucose (mmol/L)	8.58±3.85	4.35±0.56	4.230	0.0001*
Glycated haemoglobin (%)	8.12±1.59	5.18±0.70	2.947	0.0001*

*Significant at $P<0.05$. TAC: Total antioxidant capacity

Table 3: Frequency of bacterial isolates from semen cultures from subjects in the study

Bacterial isolates	Frequency (%)		Critical *t*	Calculator *t*	*P*
	Fertile men (*n*=30)	Infertile men (*n*=60)			
Staphylococcus aureus	2 (6.7)	9 (15.0)			
Coagulase negative *Staphylococcus*	1 (3.3)	2 (3.3)			
Escherichia coli	0	3 (5.0)			
Citrobacter spp.	0	1 (1.7)			
Proteus mirabilis	0	1 (1.7)			
Total	3 (10.0)	16 (26.7)	2.080	1.99	0.041*

*Significant at $P<0.05$

these groups of subjects which depletes the antioxidants. However, it would have been expected that with the increased burden of oxidative stress caused by DM, which includes glycation of seminal proteins, the levels of the antioxidants would have been lower in the diabetic infertile men. This is because DM is associated with increased oxidative stress, probably due to AGE. They are important triggers of oxidative stress and cell dysfunction in numerous diabetic complications.[10] Mallidis *et al*. (2011), in their study hypothesized that these compounds could also be present in the male reproductive tract.[9] However, this was not observed in this study, could this be that there may be a compensatory rise in the levels of enzyme antioxidant systems as observed by Omu *et al*., 2014 whose work showed a reduction in the levels of seminal TAC, but no changes in glutathione peroxidase and Anya, 2016 who

observed that though serum TAC levels were reduced in the diabetics there was a compensatory rise in superoxide dismutase levels compared to nondiabetic controls.[25,29]

A similar trend was observed with seminal plasma magnesium and calcium. Their levels were lower in both the diabetic and nondiabetic infertile groups compared to that of the fertile men. Among the infertile groups, the levels of calcium and magnesium were comparable. The decreased seminal magnesium may be a result of defective transportation of magnesium from blood to semen.[30] This indicates that magnesium might play a role in male infertility in general. Decreased seminal magnesium may also be an indication of prostatic infection. Studies by Ghasemi *et al*. and Najim *et al*. also reported lower magnesium levels in the seminal plasma of diabetic men.[31,32] The reason for the low calcium levels in the infertile groups is unclear. Sperm cell motility and function have been observed to be negatively influenced by reduced seminal plasma calcium which may result in infertility.[33] A Nigerian study showed that calcium and magnesium levels were significantly lower in the seminal plasma of infertile males compared to that in fertile males,[18] whereas another showed no significant differences in seminal plasma total calcium but reported lower ionized calcium level in infertile males compared to controls.[33] However, Sağlam *et al*. reported no differences in either seminal plasma calcium and magnesium levels between fertile and infertile men.[34]

The presence of a higher frequency of bacterial isolates shows that infection of the reproductive system apart from being directly responsible for fertility problems may also be responsible for an increase in the oxidative stress levels leading to a decrease in antioxidant levels. Their direct effects include adhesion phenomena and sperm agglutination as well as the release of virulence factors causing changes to cell morphology and motility.[35] The indirect way being through the inflammatory pathway and increase in oxidative stress. Reports of a strong association between male infertility and inflammation of the male reproductive system have been made. One of the factors responsible for the alteration of semen quality through inflammation is the presence of genito-urinary infections.[35,36] It is believed that reactive oxygen species (ROS) overproduction associated with inflammatory reactions are caused by pathological bacterial strains that colonize or infect the reproductive tract.[35,36] Subsequently, this results in oxidative imbalance as well as a buildup of leukocytes and phagocytosis initiation, leading to the generation of pro-inflammatory cytokines which promote bursts of ROS. Consequences of this include spermatozoon peroxidative damage and persistence of oxidative stress response in the semen long after the infectious agent has been eradicated, causing further damage to spermatozoa[37] and resulting in infertility.

A limitation of this study is that we did not evaluate the enzyme antioxidant levels which may have helped explain why there was no significant difference in the antioxidant status of the diabetic infertile and nondiabetic infertile men.

Conclusions

Seminal TAC, ascorbic acid, Vitamin E, Calcium, and magnesium levels are decreased in both infertile diabetic and nondiabetic men compared to fertile men, but there is no significant difference in the levels of these analytes in the infertile diabetic and nondiabetic men.

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Conflicts of interest

There are no conflicts of interest.

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