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Seminal Plasma concentrations of Semenogelin and Zinc Among infertile males and their association with Asthenozoospermia.

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ABSTRACT

Poor semen liquefaction is one of the causes of male infertility which has become a public health challenge in Nigeria. Abnormal sperm motility is one of the commonest abnormalities detected in infertile males. The pathogenesis of poor liquefaction and solidification of semen are modulated by Semenogelin and some other secretions from male accessory organs. To evaluate the concentrations of Semenogelin and zinc in seminal plasma and to correlate the levels with percentage sperm motility. Four hundred men, mean age 36.4±6.8 years who were evaluated for infertility and 100 men, mean age 40.2±6.6 years of proven fertility were consecutively recruited for the study. Following routine semen analysis, seminal plasma Semenogelin-1 and zinc were determined by ELISA technique and Atomic Absorption spectrophotometry techniques respectively. Data generated were compared between groups and association between Semenogelin, zinc and percentage sperm motility was determined. Seminal plasma Semenogelin level was higher ($p<0.001$) in infertile males irrespective of the sperm concentration than controls. On the other hand, seminal plasma zinc level was lower ($p<0.001$) among oligozoospermia and azoospermia than controls. The mean Semenogelin level was significantly higher ($p<0.001$) among asthenozoospermia than those with normal percent motility while mean seminal plasma zinc level in asthenozoospermia was lower ($p<0.001$) than those with normal percentage motility. Seminal plasma Semenogelin ($R=-0.217$; $p<0.005$) correlated negatively with asthenozoospermia and zinc ($R=-0.148$;$p<0.05$) but zinc correlated (R-0.150;$p<0.05$) positively with asthenozoospermia. Alterations in the levels of Semenogelin and zinc are associated with asthenozoospermia among infertile males.

Keywords: Male infertility, Sperm motility, Semenogelin, zinc.

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INTRODUCTION

An estimated 15% of couples of reproductive age fail to achieve pregnancy within one year of regular unprotected sexual intercourse all over the world. Male infertility associated factors are responsible for about 50% of these cases and abnormal levels of seminal fluid proteins and trace elements could be implicated. Seminal plasma Semenogelin and zinc levels have been associated in male fertility potentials [1]. They have been reported to offer a wide range of information on the functional status of the entire male reproductive system. Semen analysis still forms the bedrock of the early investigations of male infertility, but the procedure remains imperfect in predicting fertility potentials [2]. There may be infertile men in whom there is a defect in one of the tasks that are very vital for spermatozoa to transverse the female reproductive tract to reach and fertilize an ovum. This is may not be detected by routine semen analysis. Such group consists of up to 40-50% of subfertile men [3]. It is therefore imperative to determine their levels in seminal plasma of men with infertility and association with sperm motility.

Semen liquefaction is one of the causes of male infertility and it refers to the inability of ejaculated semen to liquefy within one hour[4]. The pathogenesis of abnormal semen liquefaction is not completely understood, but it is generally believed that semen liquefaction and solidification are modulated by Semenogelin [5], a secretion of the seminal vesicles. The abnormal semen liquefaction can occur due to increased levels of Semenogelin, seminal vesiculitis, deficiency of zinc and magnesium as well as congenital deficiencies of the prostate and reduced prostate secretion [6,7]. Semenogelins are components of human semen coagulum produced by seminal vesicle and are made up two related proteins (Semenogelin 1 and Semenogelin 2). The proteins are responsible for sperm immobilization in the seminal coagulum[8]. It represents the most abundant seminal proteins and play vital role in sperm physiology, probably at different time point as the spermatozoa travel down the female genital tract. The physiological function ascribed to Semenogelin are regulation of sperm motility and capacitation, a series of transformations that spermatozoa undergo as they migrate in the female genital tract in order to reach, bind and fertilize the ovum)[1].

Zinc is second most abundant element in human tissues and is excreted from the prostate gland as a low-molecular-weight complex. It is estimated that the zinc levels in seminal plasma may entirely represent prostatic secretory function. After ejaculation, about 50% of the amount in the complex is redistributed and linked to medium- and high-molecular-weight compounds such as Semenogelin generated from the seminal vesicles [9]. In the human reproductive system, Zn plays an essential role in spermatogenesis, from its primitive stage to maturation, contributes to the ultrastructural stabilization of chromatin compaction, regulation of the

mitochondria-dependent processes, such as cell respiration and apoptosis [10,11]. Zinc is also acts as cofactor for DNA-binding proteins with Zn fingers. It is a part of copper (Cu)/zinc superoxide dismutase, and several proteins that are involved in the repair of damaged DNA [12]. The objective of this study was to evaluate the concentrations of seminal plasma Semenogelin-1, zinc and their correlation with asthenozoospermia among infertile males.

MATERIALS AND METHOD

Study participants and study design:

This is a cross sectional study of males who were investigated for infertility. They consist of 400 men, mean age 36.4±6.8 years (rage;21-60) who were consecutively recruited between November 2017 and July 2019 from infertility clinics in Osogbo, Osun State, Nigeria. The control group was 100 men, mean age 40.2±6.6 years (range; 22-59) who have had babies within the past one year. All males investigated for infertility were grouped according to semen parameters as normozoospermia (spermatozoa count ≥15 x 10^6/mL), oligozoospermia (spermatozoa count 0.1-14.9 x 10^6/mL) and azoospermia (absence of sperm cells) as well as percent asthenozoospermia and percent teratozoospermia.

Ethical Consideration

The study protocol was reviewed and approved by the Health Research Ethics Committee of Osun State Ministry of Health, Abere, Osogbo, Osun State (Ref. OSHREC/PRS/569/149) dated 30th November, 2017. All study participants gave informed consent to participate in the study before they were enlisted.

Inclusion Criteria

All male subjects aged 21-60years who were evaluated for infertility, gave consented, without physical abnormalities or chronic illnesses were included in the study. Subjects without chronic clinical illnesses and had their babies within the last one year, whose seminal fluid analysis showed over 15 million sperm cells per milliliter according to WHO criteria [13] were included and used as controls.

Exclusion Criteria

Individuals with known pathological or congenital conditions such as severe hypertension, diabetes mellitus, sexually transmitted diseases, testicular varicocele and genital warts were excluded. In addition, individuals currently on antioxidant supplementation, smokers and alcoholics were also excluded due to their high seminal reactive oxygen species levels and possibly low antioxidant activity which might lead to decreased motility and abnormal sperm morphology.

Sample size:

The sample size (n) was calculated using estimated prevalence of 40% from previous study on male infertility in Nigeria (14) and sample size determination formula by Lwange and Lemeshow(15). $N= Z^2(1-P)P/d^2$. The calculated sample size was 369 which was increased to 400 for the purpose of this study.

Laboratory Analysis

Specimen collection:

Semen samples were collected by self or assisted masturbation after 3-5days sexual abstinence. Specimens were submitted to the laboratory within 30 minutes and were allowed to liquefy at room temperature. Manual semen analysis was done according to WHO criteria [13].

Seminal Plasma:

After liquefaction and semen analyses done, the sample was centrifuged at 3000 rpm for 5 minutes and the supernatant was separated into sterile vacutainer plain and store at -80^{o}C until biochemical analyses were performed.

Human Semenogelin-1 Assay (Cusabio Biotechnology, Wuhan, China)

Principle:

The assay employs the quantitative sandwich enzyme immunoassay technique. Antibody specific for SEMG1 has been pre-coated onto a microplate. After removing any unbound substances, a biotin-conjugated antibody specific for SEMG1 is added to the wells. After washing, avidin conjugated Horseradish peroxidase (HRP) is added to the wells. Following a wash to remove any unbound avidin-enzyme reagent, a substrate solution is added to the wells and colour develops in proportion to the amount of SEMG1 bound in the initial step. The color development is stopped and the intensity of the color is measured. The values were read from standard curve previously determined.

Seminal plasma Zinc Determination:

The seminal plasma zinc was determined using Atomic Absorption Spectrometry (AAS) [16]. The mean of duplicate testing was used for data analysis.

Statistical Analysis:

The data generated from the study were compared between the groups using unpaired Students-t-test and One way analysis of variance (ANOVA) as appropriate by statistical software SPSS version 21 (SPSS Inc, Chicago, IL, USA). A p-value ≤ 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

The sperm quality results indicate that 170(47.4%) had normal percentage motility while 189(52.6%) had percentage motility <40% (asthenozoospermia). The percentage motility was higher ($p<0.001$) among those with percentage motility ≥40 than those with

asthenozoospermia. Those with teratozoospermia 99(27.6%) had percentage morphology lower ($p<0.001$) than those with normal percentage morphology. Similarly those with semen volume ≥1.5mL was significantly higher than those with volume <1.5mL. Table 2 shows the comparison of seminal plasma Semenogelin and zinc levels among asthenozoospermic subjects and normal percentage motility. Seminal plasma mean Semenogelin level was significantly higher ($p<0.001$) among asthenozoospermia than those with normal percent motility. Conversely, mean seminal plasma zinc level in asthenozoospermia was significantly lower ($p<0.001$) than those with normal percentage motility. Seminal plasma Semenogelin level was higher ($p<0.001$) in infertile males irrespective of the sperm concentration when compared with controls. On the other hand, seminal plasma zinc level was lower ($p<0.001$) among oligozoospermia and azoospermia than controls. The level in normozoospermia was not significantly different from controls. Seminal plasma Semenogelin ($R= -0.217$; $p<0.005$) correlated negatively with asthenozoospermia and zinc ($R= -0.148$; $p<0.05$) but zinc correlated (R-0.150;$p<0.05$) positively with asthenozoospermia.

Table 1: Classification Sperm Indices among males investigated for infertility based on WHO Criteria 2010

Sperm Indices	Frequency (%)	Mean(SD)	P-value
Sperm Concentration (x 10^6/mL)			
Normozoospermia(≥15 x10^6/mL)	191(47.8)	67.2±3.49	
Oligozoospermia (0.1-14.9 x 10^6/mL)	168(42)	7.56±2.81	0.001
Total motility (%)			
Normal (≥40%)	170(47.4)	44.2±0.5	
Asthenozoospermia (0-39%)	189(52.6)	21.2±0.6	0.001
Sperm morphology (%)			
Normal (>4%)	260(72.4)	6.7±0.2	
Teratozoospermia(0-3%)	99(27.6)	3.1±0.5	0.001
Volume (mL)			
>1.5mL	386(96.5)	3.02±1.6	
<1.5mL	14(3.5)	1.2±0.02	0.001

Table 2: Comparison of Seminal Plasma Semenogelin and zinc levels among infertile males and Control subjects

Parameters	Asthenozoospermia		Control subjects	P-value
	0-39%	≥40%		
Age (Years)	37.2±4.6[a]	36.9±5.2[a]	40.2±6.6	0.05
Number of subjects	189	170	100	
Semenogelin(ng/mL)	3.78±0.5	1.47±0.28[a]	1.42±0.3	0.001
Zinc (µg/mL)	0.81±0.01	2.06±0.08[a]	2.08±0.02	0.001

a=p>0.05.

Table 3: Comparison of seminal plasma Semenogelin and zinc according to fertility subtypes

Fertility Subtypes	N	Semenogelin(ng/mL) mean±SD	Zinc(µg/mL) mean±SD)
Normozoospermia(≥15 x10^6/mL)	191	3.21±0.6	2.10±0.6
Oligozoospermia(0.1-14.9 x 10^6/mL)	168	3.82±0.5	1.98±0.5
Azoospermia(Absence of sperm cells)	41	3.62±0.8	1.73±0.6
Controls	100	2.47±0.2	2.16±0.4
P-value		0.001	0.001

Table 4: Correlation of Semenogelin, zinc with asthenozoospermia among infertile subjects

Correlation	R	P-value
Semenogelin vs Asthenozoospermia	-0.217	0.005
Semenogelin vs Zinc	-0.148	0.05
Zinc vs Asthenozoospermia	0.150	0.05

DISCUSSION

The ability of routine semen analysis to accurately predicts male fertility potential has been a subject of debates [3], especially among subjects with normozoospermia but infertile. This has led to the development of other biomarkers that could be used in the diagnosis of abnormal molecular mechanisms that control the function and competence of human spermatozoa. Ejaculated semen suddenly coagulates to form semi-solid gelatinous meshwork of narrow and long fibres of which semenogelins are the major components that immobilize spermatozoa [1]. This gelatinous scaffold is quickly degraded into low molecular mass proteins by prostate specific antigen (PSA) to allow spermatozoa to move [17]. This action is not however dependent on seminal hyperviscosity since semenogelins do not prevent sperm motility via viscosity but a fragment of the degradation product called Seminar plasma motility inhibition product [1]).

In this study, seminal plasma Semenogelin concentration was higher among oligospermia and azoospermia than controls. The concentration correlated negatively with asthenozoospermia among infertile males. This finding is consistent with previous study in which an inverse association was observed between Semenogelin and spermatozoa of infertile males with asthenozoospermia [18]. Several authors have reported the role play by Semenogelins in the physiological function of control of motility and capacitation [19-22]. Some have reported other potential uses of Semenogelin to include the choice of selection of a most suitable method of assisted reproductive technology and in the decision to use in-vitro fertilization (IVF) or intracytoplasmic sperm injection (ICSI) instead of intrauterine insemination. Several hypotheses have been postulated to explain how Semenogelins hinder spermatozoa movement within the female genital tract. Zinc ion instigates coagulation as it binds to Semenogelin and in the process brings about some conformation changes thereby making the spermatozoa to

bind to one another and hinder sperm motility [23]. It was also suggested that Semenogelin can bind to spermatozoa membrane via its phospholipids and thus allows the passage of zinc ions which ultimately immobilize spermatozoa [1]. It is possible Semenogelins bind to membrane receptors, a process that triggers several biological activities that hinder sperm motility.

Seminal plasma zinc level was significantly lower among infertile males than controls and correlated positively with asthenozoospermia. Some studies also reported that the seminal plasma zinc concentrations from infertile patients were significantly lower than those from normal controls [24-27]. One study reported that the zinc concentration in the seminal plasma from infertile men was significantly higher than that in normal men [28], and some other studies showed no significant difference between infertile and normal males [29-31]. An association between zinc and sperm motility was also reported by Sorenson et al [10]. It has been suggested that the high binding capacity of Semenogelin and its byproducts to zinc ion is vital for the regulation of PSA activity and enhances the binding of Semenogelin to spermatozoa proteins in cells free assays as well as the movement of zinc ion to sperm nucleus to support DNA stability [32,33]. Changes in semenogelin and zinc levels may impact on spermatozoa functions. Zinc is the most common inorganic ions present in seminal plasma and acts as cofactor or inhibitor for several proteolytic enzymes involved in the coagulation-liquefaction process

CONCLUSION:

Seminal plasma Semenogelin concentration was higher among infertile males than controls while zinc was lower among infertile males than control subjects. There was an inverse relationship between asthenozoospermia and Semenogelin while zinc level correlated positively with asthenozoospermia. It is possible that changes in Semenogelin and zinc concentrations might be partly responsible for asthenozoospermia among infertile males.

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CONFLICTS OF INTEREST:

None declared

An association was found between high zinc concentrations and low linearity of sperm movements as expressed by a decrease in

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