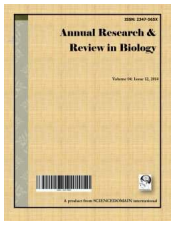

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Seminal Plasma Levels of Neutral Alpha Glucosidase Activity and Its Interactions with Spermiogram in Nigerian Males

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Author's contribution

This work was carried out in collaboration between all authors. Author AA conceptualized the study, managed the analyses of the study and wrote the draft of the manuscript. Author JE was involved in funding, laboratory analysis and literature searches. Author AOE reviewed the drafting of the manuscript. Author BSB designed and performed laboratory analysis. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: Seminal neutral alpha glucosidase activity is a useful parameter in determination of the cause of azoospermia, This has often been reported to have seasonal variation and has not been extensively studied in sub Saharan Africa.
Materials and Methods: The was a cross sectional study conducted on 122 male subjects attending a fertility clinic in Lagos, Nigeria. Semen samples were collected from subjects by masturbation into a sterile universal bottle. The samples were analysed after one hour to allow for complete liquefaction and the results categorized according to the WHO guidelines.
Results: The mean age, standard deviation (SD) and age group of the study subjects were 44

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(5.3) years and 30 - 56 years respectively. The mean ± 2SD of neutral alpha glucosidase activity in subjects with normozoospermia (20.42±14.55 mIU/ml) is comparable with other categories (f = 1.430, p = 0.236). The number and proportions of the categorized spermiogram in this report is oligoasthenozoospermia 18 (15%), azoospermia 18 (15%), oligozoospermia 18 (15%) and normozoospermia 64 (53.3%). Normozoospermic subjects had a significantly increased sperm concentration (61.19± 38.91), % active motility (56.56±16.92%), (f = 43.959, p = 0.000) and % non progressive motility (34.94±25.22%), (f = 8.203, p = 0.000) compared with other categories; while asthenozoospermic subjects had a significantly increased % abnormal sperm cells (60.00±0.00) compared with other categories (f = 21.448, p = 0.000). Neutral alpha glucosidase activity correlated positively with sperm concentration (r = 0.806, p = 0.005) in normozoospermic subjects.
Conclusion: We conclude from our results that the determination of neutral alpha glucosidase activity in seminal plasma may not give additional information of the fertility status, but shows premise in conjunction with other seminal biochemical parameters, clinical manisfestations and spermiogram.

Keywords: Infertility; neutral alpha glucosidase; seminal plasma; spermiogram.

1. INTRODUCTION

Fertility in the male is dependent on the proper production of sperm cells. Male factor infertility is seen as an alteration in sperm concentration and/or motility or morphology in at least one sample of two sperm analyses, collected between 1 and 4 weeks [1]. Male reproductive health has declined markedly globally in the recent year with attendant decrease in the quality of semen produced and consequently increased rate of infertility in men. As advances in medicine are made; new tests are being introduced to delve further into the specific causes of male infertility, one of which is neutral alpha glucosidase activity. Alpha-glucosidase is a carbohydrate -hydrolase that release alpha-glucose. This breaks down 3,1,4 linked oligossaccharides (disaccharides and starch) to glucose, which can be utilized as a source of energy. Alpha-glucosidase is a normal constituent of human semen produced mainly in the epididymis. The bulk of the alpha glucosidase activity in semen, and more particularly that of its neutral iso – enzyme, depends on secretion by the epididymis [2]. In patients with azoospermia and normal androgen levels in peripheral blood, neutral alpha glucosidase activity in seminal plasma is a reliable marker of the epididymal contribution of the ejaculate. A study in United Kingdom in 1998 by Comhaire, reported a significant correlation between neutral alpha-glucosidase activity with sperm count [3]

Neutral alpha-glucosidase (NAG) activity is also considered a functional epididymal marker in other several species [4,5]. The epididymis plays a crucial role in the maturation of spermatozoa and their acquisition of progressive motility and fertilizing capacity. Other available markers of epididymal function include L-carnitine, glycerylphosphocholine, though their use has not been universally implemented. One of the reasons for this is the diversity and relative complexity of some test procedures, and the debatable clinical meaning of the results for patient management. In the era of assisted reproduction, more attention is given to the possible use of epididymal and testicular spermatozoa for intracytoplasmic sperm injection (ICSI), and this has revived the interest in correct classification of cases with azoospermia. The determination of alpha glucosidase activity in semen, particularly of its neutral isoenzyme, has been claimed to be a rapid, sensitive and non-invasive method to differentiate secretory azoospermia from the excretory type, to localize the site of obstruction in the male genital tract, and to identify partial obstruction at the epididymal level [6,7]. In another study by Cooper et al., NAG was shown to be a reliable parameter to examine epididymal patency. As a result, the World Health Organisation considered seminal NAG activity a useful parameter to determine the cause of azoospermia, obstructive or non-obstructive with testicular origin and suggest a cut-off value of 20 mU/ejaculate [8]. However, a previous study had shown that this cut off value is seasonal dependent [9], and may affect the predictive power of the clinical impact of the assay. The reason for the seasonality of the seminal NAG activity remains speculative.

A cut-off value of 12 mIu/ml was observed to distinguish ductal obstruction from primary testicular failure. The cut of value (12 mIu/ml) had 95% specificity in identifying obstructive azospermia. This suggests that test can predict

intra uterine insemination (IUI) response (higher pregnancy rate greater than 78u per ejaculate) as high level indicates better zona-binding capacity [10]. Intra uterine insemination depends on the number of sperms recovered after an enrichment method.

Assisted reproductive technologies (ARTs), which include in vitro fertilization (IVF), and intracytoplasmic sperm injection (ICSI), currently been used to treat infertility may not be successful when semen samples with low levels of neutral alpha glucosidase activity are used. Association of neutral alpha glucosidase activity with defective sperm maturation in the epididymis has been attested to in a previous study [11]. It is instructive to note that a high rate of miscarriage after ICSI possibly reflects the fact that compromised spermatozoa are sometimes used and they lead to irreparable DNA damage in the embryo [12]. Failure in ICSI depends on DNA integrity.

The measurement of alpha -glucosidase has proved to be useful for the differential diagnosis of azoospermia [13]. In other previous studies, neutral alpha- glucosidase activity was observed to be low in cases of epididymal obstruction [14,15]. This study is aimed at determining the levels of neutral alpha-glucosidaes activity in the seminal plasma of Nigerian men and its association with spermiogram.

2. METHODS

This was a cross sectional analytical study carried out for a period of four months from March 2015 to July 2015.The study population consisted of one hundred and twenty two male subjects between the ages of 30 and 56 years, attending the fertility clinic at the Obstetrics and Gynecology Department of the Lagos University Teaching Hospital, Lagos, Nigeria. Inclusion criteria included those with a pre- ejaculatory period of less than three days and not more than to seven days. Those excluded include subjects with pre - ejaculatory period longer than 7 days and those who used lubricants to aid masturbation. Ethical approval was obtained from Ethics and Research committee of the hospital and informed consent was obtained from the study subjects. Semen samples were collected from subjects by masturbation into a sterile universal bottle. The samples were physically examined after 60 minutes to ensure complete liquefaction for pH, volume, consistency. The pH was tested for using pH paper within the range of 6.1 – 10.0 and the accuracy was checked against known standards before use. Motility was determined and assigned active and non progressive, sperm concentration was determined using the improved neubauer counting chamber after dilution with formal – bicarbonate solution, vitality was determined by staining with eosin and spermatozoa were considered normal if there were neither defects of the head (shape, ratio of length to breadth), nor defects of the neck, tail or centre. The results of the spermiograms were categorized into four according to WHO guidelines [16], viz; normozoosperma, oligozoospermia, asthenozoospermia and oligoastneozoospermia. The semen samples were centrifuged for 5 minutes at 3000 revolutions per minute. The supernatant seminal plasma was carefully recovered and stored frozen. The activity of neutral alpha glucosidase was determined by a colorimetric method, using Episcreen Plus kit (Belgium).

2.1 Principle of Alpha Glucosidase Activity

Under specified conditions (p H = 6.8, temperature = 37℃), alpha-glucosidase catalyze the conversion of the substrate 4 (para) – nitrophenyl – α – D – glucopyranoside (PNPG) to α – D - glucopyranoside and 4 – nitro phenol (PNP). The yellow colour of the latter product is measured spectrophotometrically at 405 nm wavelength.

PNPG + α – glucosidase ⟶ α – D – glucopyranoside + PNP (yellow)

As the reaction buffer contains SDS, the acid form of α – glucosidase (originating from the prostate) is selectively inhibited. This allows specific determination of neutral enzyme activity.

2.2 Statistical Analysis

Data were analysed using SPSS version 17. Between group and within group analysis was carried out using one way analysis of variance. Independent student t – test was used to compare continous variables. Pearson correlation coefficient determination was performed to evaluate the degree of association between neutral alpha glucosidase acivity and the spermiogram. Multiple regression analysis was used to predict outcomes. Quantitative data are expressed as mean and standard deviation

(SD). Probability (P) values of less than 0.05 were considered to be statistically significant.

3. RESULTS

The mean age, standard deviation (SD) and age group of the study subjects were 44 (5.3) years and 30 – 56 years respectively. The mean ± 2SD of neutral alpha glucosidase activity in subjects with normozoospermia (20.42±14.55 mIU/ml) is comparable with other categories (f = 1.430, p = 0.236). The number and proportions of the categorized spermiogram in this report is oligoasthenozoospermia 18 (15%), azoospermia 18 (15%), oligozoospermia 18 (15%) and normozoospermia 64 (53.3%). Normozoospermic subjects had a significantly increased sperm concentration (61.19±38.91), % active motility (56.56±16.92%), (f = 43.959, p = 0.000) and % non progressive motility (34.94±25.22%), (f = 8.203, p = 0.000) when compared with other categories; while asthernozoospermic subjects had a significantly increased % abnormal sperm cells (60.00±0.00) (f = 21.448, p = 0.000). These and other results are shown in Table 1. Multiple regression analysis showed that neutral alpha glucosidase acivity predicted seminal pH (beta value of -0.349, p = 0.032) and percentage normal sperm morphology (beta value of 2.647, p = 0.013), see Table 4.

4. DISCUSSION

Before starting any medical reproductive measures, the reason for male infertility must be precisely diagnosed. The activity of neutral alpha glucosidase in seminal plasma gives additional information to the reason for male infertility. This biochemical entity has been proven to distinguish between obstructive from non obstructive azoospermia.

In this study, levels of neutral alpha-glucosidaes activity in the seminal plasma of Nigerian men were evaluated as well as its association with spermiogram. We observed no significant differences in the seminal volume and pH in the different categories of semen in this study. The levels of neutral alpha glucosidase activity showed no significant differences in all the categories, though, oligoasthenozoospermic men had the lowest values. Our observation is at variance with a previous study by Levrant et al. [17] which showed that the activity of neutral alpha glucosidase in azoospermic ejaculates is lower than non – azoospermic. This is aptly explained by the fact that our cohort of participants may be free of obstruction of the reproductive tract; since other studies have been able to link neutral alpha glucosidase activity with obstructive azoospermia when measured with other seminal biochemical variables such as fructose, and carnithine. A previous study by Guerin et al, reported that azoospermic males with bilateral obstruction between the epididymis and the ejaculatory duct have low alpha glucosidase in their seminal plasma.

It is interesting to note in this study a positive relationship between NAG with pH in

Table 1. Levels (Mean ± SD) of seminal biophysical parameters and Neutral alpha glucosidase activity in different categorized semen

Parameters	Oligoasthenozoospermia	Azoospermia	Oligozoospermia	Asthenozoospermia	Normozoospermia	F values	P values
pH	7.59±0.59	7.07±1.73	7.62±0.55	7.60±0.00	7.67±0.84	1.769	0.148
Active motility (%)	18.33±6.61	0.00±0.00	20.78±7.61	22.50±3.54	56.56±16.92	43.959	0.000*
Non progressive motility (%)	6.94±2.43	0.00±0.00	14.44±12.61	16.50±2.12	34.94±25.22	8.203	0.000*
Immature sperms (%)	23.06±28.44	0.00±0.00	25.00±27.39	6.00±1.41	19.59±15.46	2.777	0.036*
Normal sperm morphology (%)	51.25±39.35	0.00±0.00	51.25±31.60	77.50±3.54	43.33±22.38	7.505	0.000*
Abnormal sperm morphology (%)	51.43±3.78	0.00±0.00	57.50±13.89	60.00±0.00	59.76±22.28	21.448	0.000*
Sperm vitality (%)	51.43±3.78	0.00±0.00	44.29±9.76	40.00±0.00	30.33±19.32	19.260	0.000*
Sperm concentration/ml	2.67±3.69	0.00±0.00	2.61±3.73	22.65±7.71	61.19±38.91	15.422	0.000*
NAG (m IU /ml)	47.51±29.30	27.19±27.85	36.93±31.18	78.75±27.93	47.07±33.74	1.430	0.236
Volume (ml)	2.77±1.02	3.12±1.53	2.66±0.99	3.90±1.27	3.23±1.55	0.573	0.684

**significant*

asthenozoospermic men. Azoospermic ejaculates had the lowest pH values. This suggests the fact that sperm cells are more viable at a particular pH range, however the viability of sperm may become affected at excessively higher pH values. Multiple regression analysis was carried out to establish the predictive ability of neutral alpha glucosidase on seminal biophysical variables. In this model, neutral alpha glucosidase served as the dependent variable, while other seminal biophysical variables served as the independent variables. It was observed in this study that neutral alpha glucosidase activity predicted seminal pH (beta value = -0.349, p = 0.032) and percentage normal sperm morphology (beta values = 2.647, p = 0.013). Our findings on seminal pH corroborates with an earlier study conducted on the correlation between the secretory function of the male accessory glands and sperm parameters in normospermic controls and infertile patients in which seminal alpha-glucosidase levels were significantly correlated with semen volume, and pH [18].

Table 2. Levels of Neutral alpha glucosidase activity (mean ± 2SD) in categorized seminal plasma

Categories	Neutral alpha glucosidase activity Mean ± 2 SD
Oligoasthenozoospermia	11.09±106.11
Azoospermia	33.51±77.9
Oligozoospermia	25.43±99.29
Asthenozoospermia	22.89±34.61
Normozoospermia	20.41±114.55
	f = 1.430, p = 0.236

We also noted that NAG is positively associated with increased immature spermatozoa and inversely related to abnormal sperm cells as observed in both normozospemic and asthenozoospermic ejaculates. Presence of immature spermatozoa may suggest active spermatogenesis, in which a complex interdependent population of germ cells produces spermatozoa by mitosis and meiosis. Although no relationship of NAG with motility was observed, which disagrees with a previous report; it is pertinent to note that seminal plasma

Table 3. Pearson correlation coefficient of neutral alpha glucosidase activity with biophysical semen parameters in different categories of seminal plasma

Seminal biophysical variables	Oligozoo-spermia r (p)	Azoospermia r (p)	Oligoastheno-zoospemia r (p)	Astheno-zoospermia r (p)	Normo-zoospermia r (p)
p H	0.129 (0.481)	- 0.824 (0.006)*	0.106 (0.651)	0.750 (0.020)*	0.763 (0.010)*
% active	-0.136 (0.459)	N/A	-0.227 (0.335)	-0.141 (0.717)	-0.060 (0.868)
% non progressive	-0.323 (0.071)	N/A	-0.363 (0.116)	0.212 (0.584)	-0.380 (0.279)
% immature	0.220 (0.227)	N/A	-0.030 (0.899)	0.701 (0.035)*	0.826 (0.003)*
% normal	0.437 (0.048)*	N/A	0.533 (0.050)*	0.329 (0.426)	0.451 (0.224)
% abnormal	-0.195 (0.398)	N/A	-0.455 (0.102)	-0.799 (0.017)*	-0.827(0.006)*
% sperm vitality	0.189 (0.500)	N/A	0.484 (0.182)	0.599 (0.155)	0.528 (0.178)
Sperm concentration	0.113 (0.539)	N/A	0.060 (0.801)	0.654 (0.056)	0.806 (0.005)*
Volume	0.252 (0.163)	0.043 (0.913)	0.200 (0.399)	0.397 (0.290)	0.450 (0.192)

**significant; N/A – Not applicable*

Table 4. Multiple regression analysis of neutral alpha glucosidase activity with the spermiogram

Spermiogram	Standard error	Beta values	t values	Significance
pH	4.992	-0.349	-0.246	0.032*
% active motility	0.526	0.648	1.243	0.223
% non progressive motility	0.463	-0.508	-1.104	0.280
% immature sperm cells	0.967	0.040	0.163	0.871
% normal sperm morphology	0.167	0.521	2.647	0.013*
% abnormal sperm morphology	0.334	0.269	-0.748	0.460
% sperm vitality	0.442	-0.024	-0.070	0.945
Sperm concentration	0.270	0.138	0.639	0.528
Volume	3.266	0.295	1.881	-0.070

**significant*

NAG increased significantly with increase in sperm concentration and normal spermatozoa. This was noticed in normozoospermic group and both oligozoospermic and oligoasthernozoospermic groups respectively.

We have also shown that the mean ±2 standard deviation (at 95% confidence interval) of neutral alpha glucosidase activity in seminal plasma of normozoospermic ejaculates from Nigerian men to be 20.41±114.55, which may serve as a cut – off value for NAG in our locale.

5. CONCLUSION

We conclude from our results that the determination of neutral alpha glucosidase activity in seminal plasma may not give additional information of the fertility status, but showed premise in conjunction with other seminal biochemical parameters, clinical manisfestations and spermiogram. Other causes of insufficient epididymal secretion, such as hypo – androgenism or severe testicular atrophy must however be excluded.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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