Original Article

Alteration of neutral-alpha glucosidase in seminal plasma and correlation with sperm motility among men investigated for infertility Nigeria: a cross-sectional study

Muyiwa Adeleye Moronkeji[1,2], Mathias Abiodun Emokpae[1]

[1]Department of Medical Laboratory Science, School of Basic Medical Sciences, University of Benin, Benin City
[2]Department of Chemical Pathology, Ladoke Akintola University of Science and Technology Teaching Hospital, Osogbo, Nigeria

Abstract

Introduction: Proteomic studies are becoming popular lately among reproductive biologists in the diagnosis and management of several diseases including male infertility. **Objective:** To evaluate the level of Neutral-alpha Glucosidase (NAG) in the seminal plasma of men being investigated for infertility in and to correlate its activity with sperm motility. **Materials and Methods:** Four hundred men age range 23–60 years were consecutively recruited in the study. After physical and clinical evaluation, the semen was collected by self or assisted masturbation and analysis was done according to the World Health Organization guidelines. The semen was then centrifuged and seminal plasma separated for the assay of NAG activity by ELISA technique using reagents supplied by Melson Medical Co, Shangai, China within one week of collection. The participants were categorized in to normozoospermia (191), oligozoospermia (98), severe oligozoospermia (70) and azoospermia (41) based on sperm count. **Results:** The mean levels of NAG activity decreased with decreasing levels of sperm count with values lowest among azoospermia and highest among normozoospermic subjects. The comparison of NAG activity between the various categories and control subjects was significantly different ($P<0.001$) except for normozoospermia which was not significantly different ($P>0.05$) when compared with controls. The mean NAG activity level was significantly lower among the asthenozoospermia ($P<0.001$) compared with normozoospermia. The NAG activity correlated positively with percentage sperm motility (r=0.126; $P<0.02$) and percentage sperm count (r=0.107; $P<0.05$). **Conclusion:** The findings indicate that NAG can be used for the assessment of semen quality as an adjunct to traditional semen analysis.

Keywords: Astenozoospermia, male infertility, neutral α-glucosidase, semen analysis

Address for correspondence: Prof. M.A Emokpae, Department of Medical Laboratory Science, School of Basic Medical Sciences, University of Benin, Benin City, Nigeria. ORCID ID:0000-0002-6266-1774.
E-mail: mathias.emokpae@uniben.edu
Submission: 22–5–2020, **Revised:** 11–6–2020, **Accepted:** 19–6–2020, **Published:** 30–6–2020

INTRODUCTION

Infertility has become a public health challenge all over the world and the increasing decline in semen quality is worrisome especially among men in the so called infertility belt of sub-Saharan Africa. Proteomic studies are increasingly been used in the diagnosis and management of several diseases including male

Access this article online	
Quick Response Code:	Website: www.fertilityscienceresearch.org
	DOI: 10.4103/2394-4285.288724

How to cite this article: Moronkeji MA, Emokpae MA. Alteration of neutral-alpha glucosidase in seminal plasma and correlation with sperm motility among men investigated for infertility Nigeria: a cross-sectional study. Fertil Sci Res 2020;7:111-6.

infertility. Semen analysis is one of the initial assessments conducted in the investigation of men undergoing fertility evaluation.[1] Since semen analysis was introduced in the 1950s, it has remained the bedrock of male infertility evaluation. It involves detail evaluation of semen volume, pH sperm number, motility, morphology and viability of the ejaculate in the clinical laboratory. Ejaculates are mixture sperm cells, stored in the paired epididymis mixed with and diluted by fluid produced from the accessory sex organs.[2] But semen analysis does not provide sufficient evidence or insight in to the subcellular alterations that might occur in the spermatozoa and its micro-environment. This has necessitated the introduction of other assays that are detail and thorough that could make for clearer understanding at molecular level.[3,4]

The spermatozoa obtain their fertility potential during epididymal maturation phase prior to ejaculation.[5] It is at this final stage that seminal fluid is produced by the seminal vesicles, prostate gland and other accessory organs of the male reproductive system. The seminal fluid is comprised of proteins, fructose, mucus, vitamin C, flavins among others.[6] The ejaculated semen thus contains cellular (spermatozoa) and non-cellular components.[7,8] The non-cellular components provide energy, protection, aid capacitation and acrosome reaction which are very important for the reproductive success of spermatozoa.[9,10]

Several seminal plasma proteins have been identified and characterized as potential biomarkers of not just male infertility, but differential diagnosis of pathologies associated with male infertility.[1] Neutral alpha glucosidase (NAG) hydrolyzes the α-1,4 glycosidic bonds of maltooligosaccharides and maltodextrines obtained from the hydrolysis of glycogen by α-amylases. It hydrolyzes α-1,2, α-1,3; and α-1,6 glycosidic bonds to a smaller extent.[11] Low NAG activity has been associated with functional deficiency of epididymis in infertile males.[12] Even though the exact role played by NAG in sperm function is not completely understood, some authors have reported that it may be related with sperm maturation through protein modification.[13] Some have observed a lower NAG activity as sperm abnormality increases,[14] and the spermatozoa from these subjects showed poor ability to bind zona pellucida; indicating that NAG may have a role in sperm- egg binding[13] In the light of the above reports, evaluation of NAG activity as part of laboratory assays in the evaluation of male infertility cannot be ignored. In addition, evaluation of NAG activity may be helpful for identifying epididymal patency and sperm abnormality. Unfortunately, conflicting reports exist in literature as to the usefulness of the assay in male infertility. Whereas Krause and Bohring[15] failed to provide any additional relevant information compared to data from routine semen analysis, other authors have suggested that NAG is an important marker for assessing seminal plasma quality in addition to routine semen analysis which are vital for diagnosis and treatment of male infertility.[16,17] This study seeks to evaluate the activity level of NAG in the seminal plasma of men investigated for infertility and its association with sperm motility.

MATERIALS AND METHODS

Study design

This is a cross-sectional study of males investigated for infertility between November 2017 and July, 2019 and the study participants were between the ages of 23-60 years. They comprised of males evaluated for infertility because their partners were unable to conceive after one or more years of unprotected intercourse. The control group was males without chronic clinical illnesses and had their baby within the last one year.

Ethical consideration

Ethical clearance was obtained from 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 were enlightened on the nature of the study and informed consent was obtained before specimens were collected.

Inclusion criteria

All male subjects aged 23–60years evaluated for infertility and consented to be enrolled 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 semen according to World Health Organization (WHO) criteria[19] were included and used as controls.

Exclusion criteria

Individuals with known pathological or congenital conditions such as 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 collection

Semen samples were collected in a sterile container by self or assisted masturbation after at least 3days of sexual abstinence (without the use of spermicidal lubricants). The specimens were delivered to the laboratory within 30 minutes of ejaculation. Two specimens were collected at different visits within two months for analysis and mean value of the determinations was used.

Laboratory analysis and techniques

Routine semen analysis

Semen analyses were done according to WHO protocol.[2] Following liquefaction the semen specimens, volume, appearance, pH, and viscosity were assessed. Routine semen analysis was performed microscopically with special interest in the sperm concentration, percentage motility and percentage morphology. Based on the sperm concentration/count according to WHO criteria,[19] the overall samples were therefore categorized into: normospermia; $\geq 15 \times 10^6$ cells/mL, oligozoospermia; $<15\text{-}5 \times 10^6$cells/mL, severe Oligospermia; $<5 \times 10^6$ cells/mL and azoospermia; absence of sperm cells in the ejaculate.

Neutral Alpha Glucosidase (NAG) This was assayed using competitive enzyme immunoassay assay technique by Melson Medical Corporation, Shanghai, China.

Principle: Neutral alpha Glucosidase (α-Glu) deconstructs disaccharides into glucose, and increase the blood glucose level. The stop solution changes the color from blue to yellow and the intensity of the color is measured using spectrophotometer at 450nm. In order to measure the concentration of α-Glu in the sample, the α-Glu ELISA kit includes a set of calibration standards. The calibration standards are assayed at the same time as the samples and allowed the operator to produce a standard curve of optical density versus α-Glu concentration. The activities of α-Glu in the samples are then determined by comparing the O.D of the sample to the standard curve.

STATISTICAL ANALYSES

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

RESULTS

The results from this study are presented in Tables 1 and 2, and Figure 1. Of the 400 men investigated for infertility, 191 were normozoospermia, 98 oligozoospermia, 70 severe oligozoospermia and 41 azoospermia [Figure 1].

Table 1 shows that the mean levels of NAG activity decrease with decreasing levels of sperm count with values lowest among azoospermia and highest among normozoospermic subjects. The comparison of NAG activity between the various categories and control subjects was significantly significant ($P < 0.001$) except for normozoospermia which was not significantly significant ($P > 0.05$). The mean NAG activity level was significantly lower among the asthenozoospermia ($P < 0.001$) compared with normozoospermia. The difference in the mean NAG activity levels among the teratozoospermia was lower but not statistically significant ($P > 0.05$). Table 2 indicates the correlation of NAG activity with measured sperm indices. The NAG activity correlates significantly with percentage sperm motility ($r = 0.126$; $P < 0.02$) and percentage sperm count ($r = 0.107$; $P < 0.05$). The correlation between NAG activity and percentage sperm morphology was not statistically significant ($r = 0.103$; $P > 0.05$).

Table 1: Levels of neutral-alpha glucosidase activity based on semen quality among study participants

Categories/Parameters	Number of subjects (n)	NAG activity (μU/mL)mean±SD	*P*-value
Sperm count			
Normozoospermia(>15 × 106cells/mL)	191	30.60±4.21	0.50
Oligozoospermia (>5–<15 × 106cells/mL)	98	28.20±3.82	0.001
Severe Oligozoospermia (<5 × 106cells/mL)	70	25.61±3.60	0.001
Azoospermia (absence of sperm cell)	41	23.12±3.41	0.001
Control group (>15 × 106cells/mL)	100	31.46±9.46	
Sperm motility (%)			
≥40%	283	31.82±5.2	
<40%	76	24.69±4.6	0.001
Morphology (%)			
≥4%	260	28.78±9.21	
<4%	99	27.82±8.60	0.09

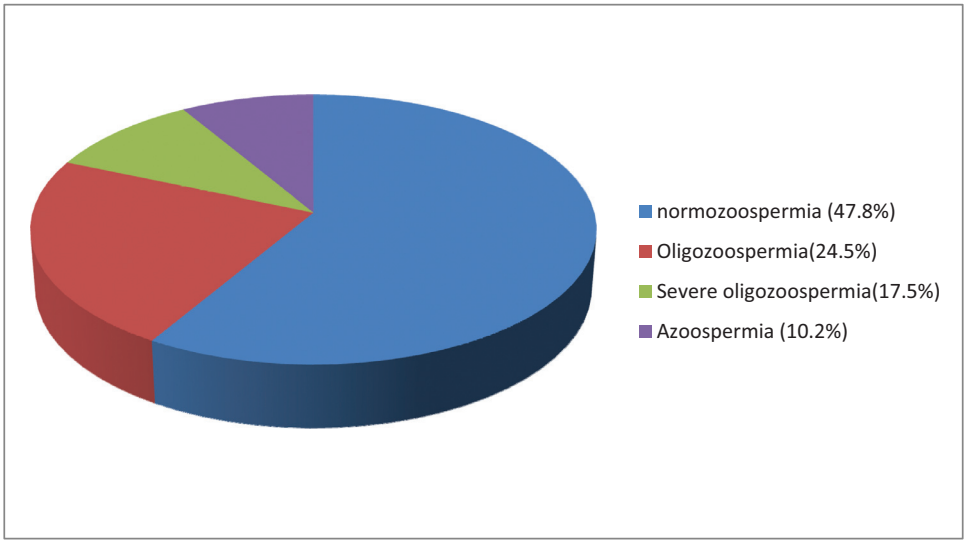

Figure 1: Categories of infertility among the study participants

Table 2: Correlation of neutral-alpha glucosidase activity with sperm indices

Correlation	R-value	*P*-value
Neutral alpha glucosidase activity vs percentage sperm count	0.107	0.05
Neutral alpha glucosidase activity vs percentage sperm motility	0.126	0.02
Neutral alpha glucosidase activity vs percentage sperm morphology	0.103	0.06

DISCUSSION

The proteomic approach to the laboratory work-up in the evaluation of male infertility is increasingly popular among reproductive biologists. It is useful in identifying the molecular factors associated with the aetiologies of male infertility.[1] The sperm cells depend on the non-cellular components of semen fluid for their normal biological function, since the non-cellular components contain factors needed for the provision of energy and protection of sperm cells during their movement within the female reproductive tract and subsequent fertilization process.

In this study NAG activity was decreased with decreasing concentration of sperm cells while percentage sperm motility was significantly lower ($P < 0.001$) among men with asthenozoospermia than normozoospermia. This findings are consistent with previous studies.[16,17] It was reported that among Chinese infertile men, the level of NAG in normozoospermia was significantly higher than that in subfertile and infertile men ($P = 0.0001$), while the level of NAG in subfertile men was significantly greater than that in infertile men ($P = 0.0001$). In addition, a significant difference was reported in normozoospermia, teratozoospermia, asthenospermia, severe oligozoospermia, asthenoteratozoospermia, oligoasthenospermia, oligoasthenoteratozoospermia and azoospermatism ($P < .05$).[16] The authors concluded that NAG is a crucial marker for assessing seminal plasma quality in Chinese men, which might assist in the diagnosis of male infertility. In the same vein, Mankad *et al.*[17] observed that the mean NAG activity was lowest among the azoospermic group compared with oligozoospermic and normozoospermic groups. Karthikeyan and Manian[18] reported that NAG was significantly lower among infertile Indian men with azoospermia than controls. The evaluation of NAG in human semen has been suggested to be sensitive, non-invasive and with a short turn-around time for identifying obstructive and non-obstructive azoospermia.[19] In the present study, we did not seek to identify the type of azoospermia rather it was designed to know the activity level NAG among infertile men investigated for infertility.

Male infertility has become a major health problem that requires urgent attention but appears to be neglected in Nigeria.[20] Asthenozoospermia characterized by reduced sperm motility is often reported as part of the aetiologies of male infertility in several studies. The physiology and molecular basis of asthenozoospermia is not fully understood.[21] The causes of poor sperm motility include abnormal metabolism in the testicular tissue or epididymis, structural deficiency in the sperm tail and functional deficiency of the epididymis or other accessory sex glands.[22-24] Even though traditional semen analysis is

one of the first assays done in the evaluation of male infertility, it does however reveal the reason for defects associated with asthenozoospermia. It is plausible that individual protein defects in spermatozoa or seminal plasma might be responsible for poor sperm motility and/or fertilization failure.[21] Proteomic studies on asthenozoospermia are increasingly conducted with new proteins and pathways involved in sperm motility identified.[21] The ability of the sperm cells to move forward is crucial for successful fertilization of the oocytes.[25]

Although a significant correlation between NAG and sperm concentration was reported by Levrant *et al.*,[13] they concluded that routine determination of NAG activity is not practical; but when an epididymal pathology leading to a physiological or anatomical functional abnormality is suspected, the evaluation of NAG activity may be useful in the diagnosis, and aid in the prognosis of the condition. Among the draw backs associated with NAG determination include duration of sexual abstinence and storage. It was reported that NAG activity was significantly lower after two to three days of sexual abstinence when compared with both four to five days of abstinence and six to seven days of abstinence respectively.[26] The participants in our study had approximately the same duration of sexual abstinence. In the same vein, storage at −20°C may affect the NAG activity as it was observed that the equine α-glucosidase activity was lower by 10% during one week and subsequently declined to 10%, 30% and 40% after 10, 20 and 30days respectively. Storage for over three months period caused NAG activity to decay by 60%–70% of the initial activity level.[27] It was suggested that azoospermia ejaculates with low NAG activity may suggest a complete post Caput epididymal occlusion, since in humans, most of the enzyme activity is observed in or above this anatomical region.[14] Subjects with low serum testosterone concentration are likely to present with low NAG activity.[28]

CONCLUSION

The mean seminal plasma levels of NAG activity decrease with decreasing levels of sperm count with values lowest among azoospermia and highest among normozoospermic subjects. The mean NAG activity levels was significantly lower among the asthenozoospermia ($P < 0.001$) than normozoospermia and NAG activity correlated positively with percentage sperm motility and percentage sperm count. The findings show that NAG can be used for the assessment of semen quality as an adjunct to traditional semen analysis.

Acknowledgements

We appreciate the contributions of all Physicians, Nursing and Medical Laboratory Scientists towards the completion of this study.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Martins AD, Selvam MKP, Agarwal A, Alves MG, Baskaran S. Alterations in seminal plasma proteomic profile in men with primary and secondary infertility. Scientif Reports (Nature Research) 2020;10:7539.
2. WHO laboratory manual for the examination and processing of human semen. 5th ed. WHO Press, World Health Organization, 20 Avenue Appia, 1211 Geneva 27, Switzerland 2010. p. 7-113.
3. Oehninger S, Ombelet W. Limits of current male fertility testing. Fertil Steril 2019;111:835-41.
4. Wang C, Swerdloff RS. Limitations of semen analysis as a test of male fertility and anticipated needs from newer tests. Fertil Steril 2014;102:1502-7.
5. De Jonge C. Biological basis for human capacitation-revisited. Hum Reprod Update 2017;23:289-99.
6. Purvis K, Magnus O, Morkas L, Abyholm T, Rui H. Ejaculate composition after masturbation and coitus in the human male. Inter J Androl 1986;9:401-6.
7. Druart X, de Graaf S. Seminal plasma proteomes and sperm fertility. Animal Reprod Sci 2018;194:33-40.
8. Maxwell W, De Graaf S, Ghaoui R, Evans G. Seminal plasma effects on sperm handling and female fertility. Soc Reprod Fertil Supplement 2007;64:13.
9. Samanta L, Parida R, Dias TR, Agarwal A. The enigmatic seminal plasma: a proteomics insight from ejaculation to fertilization. Reprod Biol Endocrinol 2018;16:41.
10. Rodríguez-Martínez H, Kvist U, Ernerudh J, Sanz L, Calvete JJ. Seminal plasma proteins: what role do they play? Am J Reprod Immunol 2011;66:11-22.
11. Kotońska-Feiga J, Dobicki W, Pokorny P, Nowacki W. The activity of neutral α-glucosidase and selected biochemical parameters in the annual cycle of breeding carp (Cyprinus carpio L.). PLoS ONE 2015;10:e 0142227.
12. Comhaire F, Mahmoud A, Schoonjans F, Kint J. Why do we continue to determine a-glucosidase in human semen? Andrologia 2002;34:8-10.
13. Levrant S, Watanabe M, Land S, Sauer R, Jeyendran RS. The relevance of neutral glucosidase activity in andrology. Syst Biol Reprod Med 2009;55:116-9
14. Pena P, Risopatron J, Villegas J, Miska W, Schill WB, Sanchez R. Alpha-glucosidase in the human epididymis: Topographic distribution and clinical application. Andrologia 2004;36:315-20.
15. Krause W, Bohring C. Why do we determine alphaglucosidase activity in human semen during infertility work-up? Andrologia 1999;31:289-94.
16. Qiu Z, Chu Q, Zhang W, Luo C, Quan S. Level of neutral alpha-1,4-glucosidase in seminal plasma of Chinese men. Andrologia 2018;50: e12948.

17. Mankad M, Sathawara NG, Doshi H, Saiyed HN, Kumar S. Seminal plasma zinc concentration and alpha-glucosidase activity with respect to semen quality. Biol Trace Elem Res 2006;110:97-106.
18. Karthikeyan V, Manian R. Evaluation of fructose and α-glucosidase and its correlation with human semen parameters for better prognosis of male infertility. Drug Invention Today 2019;12:1294-8.
19. Garcia Diez LC, Esteban Ruiz PF, Villar E, Corralez Hernandez JJ, Burgo R, Delgado M, *et al*. Enzyme and hormonal markers in the differential diagnosis of human azoospermia. Arch Androl 1992;28:181-94.
20. Uadia PO, Emokpae MA. Male infertility in Nigeria: a neglected Reproductive Health issue requiring attention. J Basic Clin Reprod Scis 2015;4:45-53.
21. Cao X, Cui Y, Zhang X, Lou J, Zhou J, Bei H, *et al*. Proteomic profile of human spermatozoa in healthy and asthenozoospermic individuals. Reprod Biol Endocrinol 2018;16:16.
22. Ryder TA, Mobberley MA, Hughes L, Hendry WF. A survey of the ultrastructural defects associated with absent or impaired human sperm motility. Fertil Steril 1990;53:556-60.
23. Comhaire FH, Mahmoud AM, Depuydt CE, Zalata AA, Christophe AB. Mechanisms and effects of male genital tract infection on sperm quality and fertilizing potential: the andrologist's viewpoint. Hum Reprod Update 1999;5:393-8.
24. Infante JP, Huszagh VA. Synthesis of highly unsaturated phosphatidylcholines in the development of sperm motility: a role for epididymal glycerol-3-phosphorylcholine. Mol Cell Biochem 1985;69:3-6.
25. Turner RM. Moving to the beat: a review of mammalian sperm motility regulation. Reprod Fertil Dev 2006;18:25-38.
26. Elzanaty S. Association between age and epididymal and accessory sex gland function and their relation to sperm motility. Arch Androl 2007;53:149-56.
27. Dias AJB, Maia MS, Retamal CA, Lopez ML. Identification and partial characterization of a-1,4-glucosidase activity in equine epididymal fluid. Theriogenology 2004;61:1545-58.
28. Gonzales GF. Basal serum testosterone as an indicator of response to clomiphene treatment in human epididymis, seminal vesicles and prostate. Andrologia 2002;34:308-16.