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Pattern Of Semen Analysis, Seminal Testosterone And Prostate Specific Antigen (Psa) In Symptomatic Hiv Infected Subjects On Antiretroviral Therapy (Art) Among South Eastern Nigeria Men

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Abstract:

Background *This study was designed to evaluate the pattern of semen analysis, seminal Testosterone and PSA levels, in symptomatic HIV subjects in South Eastern Nigeria Men., 111 participants aged between 17 and 55 (41±12) years were randomly recruited. Based on WHO criteria for HIV staging, the participants were grouped as follows: Symptomatic HIV on ART (n=43), symptomatic HIV not on ART (n=39). Similarly, 39 HIV seronegative participants served as control.*

Methods *Blood samples were collected from the participants for the determination of HIV status. Also semen samples were collected from all the participants. The WHO recommended standard routine method were used to analyzed semen in an ejaculate. Seminal Testosterone and PSA were estimated using Enzyme Linked Immune Sorbent Assay (ELISA) method.*

Results *The result showed that the mean percent motility is significantly lower in symptomatic HIV participants not on ART and symptomatic HIV seropositive on ART compared with the HIV seronegatives ($p< 0.05$). The mean percent viable and semen volume also showed significantly lower values in symptomatic HIV not on ART and on ART compared with the HIV seronegatives ($p< 0.05$). The mean semen count x 10^6/ml showed higher count in symptomatic HIV on ART compared with symptomatic HIV not on ART($p< 0.05$). But maintained similar value compared with the HIV seronegative control ($p>0.05$). The PH, liquefaction time and viscosity showed similar values amongst the groups. Testosterone showed similar values in symptomatic HIV on ART and HIV seronegative participants ($p>0.5$). But showed higher significant values in both symptomatic HIV and HIV seronegative participants compared with symptomatic HIV participant not on ART ($p< 0.05$). The PSA showed no significant difference amongst the groups ($p>0.05$).*

Conclusions *The study concludes that HIV infection grossly affect the semen quality which was evidenced by the presence of hypospermia and sthenozoospermia on symptomatic HIV positive male participants.*

Keywords: *HIV, semen analysis, Testosterone and PSA*

Introduction

The male reproductive systems functions to produce normal mature sperm, which aids in the fertilization of female ova, for conception[1]. Sperm and male sex hormone production are under the control of gonadotropic hormones produced by the anterior pituitary[2]. Human Immunodeficiency Virus could be sexually transmitted[3]. HIV/AIDS infection has a far reaching effect on almost all organs of the body, the gonad organ inclusive[4].

Low levels of Testosterone have been observed in HIV male subjects[5]. This hormone plays an important role in spermatogenesis[6]. Semen analysis evaluates the number and quality of sperm in an ejaculate. It is measured using both physical examination and microscopy[7]. The analysis involves motility test, the morphology test, consistency, pH, volume, viability and sperm population[8].

The PSA is produced with the semen ejaculate, where it liquefies the semen and allows the sperm to swim freely into the uterine cervice[9]. PSA levels are affected in the presence of an infection and prostate enlargement[10,11]. The essence of this study is to evaluate the effect of HIV infection on the Testosterone levels, PSA status and semen analysis in symptomatic HIV/AIDS infected subjects on ART amongst Nigerian men.

Materials And Methods

Subjects

A total of 111 male participants were enlisted in this study. They aged between 17 and 55 (41 ±12) years. They were randomly recruited at both Antiretroviral Therapy (ART) Clinic and Voluntary Counseling and Testing (VCT) Centre in Nnamdi Azikiwe University Teaching Hospital (NAUTH), Nnewi, Nigeria. Based on World Health Organisation (WHO) criteria for HIV staging, the participants were staged and grouped as follows: 43 symptomatic HIV (stage 11) participants on ART. They were placed on Lamivudine, 150 mg twice daily, Stavudine, 40mg twice daily and Nevirapine, 200 mg twice daily, 39 symptomatic HIV (stage 11) participants on ART and 29 HIV seronegative participants who served as control. The subjects were given informed consent, while the study design was approved by the ethical committee of Nnamdi Azikiwe University Teaching Hospital, Nnewi, Nigeria. 2 ml of blood sample was collected from the participant in the groups. The participants were screened for HIV infection using Immunoassay and Immunochromatographic method. Semen samples were also collected from the participants and analysed by WHO[11,12] recommended

standard routine method. Seminal Testosterone and PSA levels were also analysed using Enzyme Linked Immunosorbent assay (ELISA) method. Informed consent was obtained from those who participated in the study. The Nnamdi Azikiwe University Teaching Hospital Board of Ethical Committee approved the study design.

Methods

Semen Analysis

Semen samples were collected hygienically by masturbation into a sterile container and kept at 37°C for 15 – 30 minutes for liquefaction. The semen samples were analysed accordingly to WHO guidelines and methods [8]. The volume of the ejaculate was measured by aspiration into a graduated pipette. Semen pH was read onto a pH metre. A drop of well-liquefied semen was placed on a slide and covered with the condenser iris closed sufficiently; the field was assessed for motility. A total of 100 spermatozoa were counted and out of the hundred, the percentage of both motile and non-motile was noted. Eosin wet preparation was also done to assess percentage spermatozoa that are still viable. The values for motility and viability were expressed as percentage.

Determination Of Semen Count

The semen counts were analysed accordingly to WHO guidelines and methods [8].The semen count was done by diluting the semen 1 in 20 with sodium bicarbonate-formalin as diluting fluid. Using a Pasteur pipette, an improved Neubauer chamber was filled with the well-mixed diluted semen and covered with a 20 mm x 20 mm cover slip using x 40 objectives, the count was made in an area of 2 large squares. Calculation of number of spermatozoa in 1 in 20 dilutions was done by multiplying the number counted by 1000000.

Estimation Of Testosterone By Randox Laboratories Limited, U.K

Serum Testosterone was estimated by Enzyme Linked Immunosorbent assay (ELISA). The procedure was as described by the manufacturer of the kit (Randox Laboratories Limited, UK); 10 µl of serum sample was added to appropriately labeled microtitre wells and 100 µl Enzyme conjugated detection antibody was also added to the wells. Also, 5µl of rabbit anti-Testosterone reagent was added in each well. The same procedure was performed for the standard as well as HIV negative control serum samples. They were

incubated at 37°C for 90 minutes. The wells were washed 3 times with deionized water to removed unbound antibodies. 100µl TMB reagent was added and well incubated at room temperature for 20 minutes for the colour to develop. 100µl of HCL was added to the various wells to stop further development of colour. Absorbance was read at 450nm using ELISA machine and deionized water served as blank. The test and control samples concentrations were extrapolated from the standard curve. The standard curve was plotted from the optical density values and concentrations of series of FSH standards (0, 0.1, 0. 5, 2, 6 and 18 ng/ml) provided by the manufacturer of the kit

Estimation Of PSA By Randox Laboratories Limited, U.K

Serum PSA was estimated by Enzyme Linked Immunosorbent assay (ELISA). The procedure was as described by the manufacturer of the kit (Randox Laboratories Limited, UK); 50 µl of serum sample was added to appropriately labeled microtitre wells, 50 µl of Zero buffer and 100 µl Enzyme conjugated detection antibody were also added to the wells. The same procedure was performed for the standard as well as HIV negative control serum samples. They were incubated at room temperature for 20 minutes. The wells were washed 3 times with deionized water to removed unbound antibodies. 100µl TMB reagent was added and well incubated in the dark for 20 minutes for the colour to develop. 100µl of HCL was added to the various wells to stop further development of colour. Absorbance was read at 450nm using ELISA machine and deionized water served as blank. The test and control samples concentrations were extrapolated from the standard curve. The standard curve was plotted from the optical density values and concentrations of series of FSH standards (0, 2, 4, 15, 60 and 120 ng/ml) provided by the manufacturer of the kit.

Statistical Analysis

The result of the analysis was statistically analysed. Students't-test and one way analysis of variance (ANOVA) were used to compare means. The analysis was performed with the use of Statistical Package for Social Sciences (SPSS) statistical software package, version 13.0. $P < 0.05$ was considered statistically significant.

Variables	% motility	% non-motility	% viable	% non-viable	pH	Lique-faction time (min)	viscosity	Semen count x 10^6/ml	Semen volume (ml)
(A) Symptomatic male participants on ART (n=43)	34.56± 24.50	65.21± 24.50	36.09 ± 21.16	63.42± 22.46	7.17± 0.44	39.49± 29.30	1.19± 0.50	24.93± 20.06	2.22± 1.03
(B) Symptomatic male participants not on ART (n=39)	15.92± 21.64	84.08± 21.04	16.00 ± 20.38	83.41± 20.27	7.47± 0.67	43.33± 13.59	1.59± 0.50	12.08± 16.08	1.42± 1.03
(C) HIV Seronegative Control participants (n=29)	51.52± 29.95	41.59± 28.74	50.97 ± 27.57	42.14± 28.94	7.35± 0.48	28.76± 13.55	1.28± 0.45	27.07± 22.98	3.02± 1.35
F (p) value	17.02 (<0.05)	24.53 (<0.05)	18.44 (<0.05)	25.53 (<0.05)	6.51 (<0.05)	3.31 (>0.05)	7.46 (>0.05)	6.29 (<0.05)	17.01 (<0.05)
A V B	(<0.05)	(<0.05)	(<0.05)	(<0.05)	-	-	-	(<0.05)	(<0.05)
A V C	(<0.05)	(<0.05)	(>0.05)	(<0.05)	-	-	-	(>0.05)	(<0.05)
B V C	(<0.05)	(<0.05)	(<0.05)	(<0.05)	-	-	-	(<0.05)	(<0.05)

Table 1: Mean (±SD) semen analysis in symptomatic HIV (stage 11) male participants on ART, symptomatic HIV (stage 11) male participants not on ART and HIV seronegative control group

Key: F (p) value = symptomatic HIV male on ART, symptomatic HIV male not on ART and HIV seronegative control compared (using ANOVA)

A V B = symptomatic HIV male on ART compared with symptomatic HIV male not on ART (using student's t-test)

A V C = symptomatic HIV male on ART compared with HIV seronegative control (using student's t-test)

B V C = symptomatic HIV not on ART compared with HIV seronegative control subjects (using student's t-test)

Variables	Testosterone(ng/ml)	PSA (ng/ml)
(A) Symptomatic male participants on ART (n=43)	3.09± 2.42	4.12± 3.67
(B) Symptomatic male participants not on ART (n=39)	2.27± 1.40	3.84± 2.62
(C) HIV Seronegative Control participants (n=29)	3.78± 2.47	4.95± 3.40
F (p) value	4.25 (<0.05)	0.70 (<0.05)
A V B	(>0.05)	(>0.05)
A V C	(>0.05)	(>0.05)
B V C	(<0.05)	(>0.05)

Table 2: Mean (±SD) seminal Testosterone and PSA levels in symptomatic HIV (stage 11) male participants on ART, symptomatic HIV (stage 11) male participants not on ART and HIV seronegative control group

Key: F (p) value = symptomatic HIV male on ART, symptomatic HIV male not on ART and HIV seronegative control compared (using ANOVA).

A V B = symptomatic HIV male on ART compared with symptomatic HIV male not on ART (using student's t-test).

A V C = symptomatic HIV male on ART compared with HIV seronegative control (using student's t-test)

B V C = symptomatic HIV not on ART compared with HIV seronegative control participants (using student's t-test)

Result

The result showed that the mean percent motility is significantly lower in symptomatic HIV (stage 11) participants not on ART 15.92 (±21.64) and symptomatic HIV (stage 11) participants on ART 34.56 (±24.37) (in each case) compared with HIV seronegative control participants 51.52 (±29.95) ($p<0.05$). The mean (±SD) percent viable count 16.00 (±20.38) and serum volume 1.42 (±1.03) in symptomatic HIV (stage 11) participants not on ART and percent viable count 36.09 (±21.16) and semen volume 2.22 (±1.03) in symptomatic HIV participants on ART also showed significantly lower mean percent compared with HIV seronegative viable count 50.97 (±27.57) and semen volume 3.02 (±1.35) (in each case) ($p<0.05$).

The mean (±SD) semen count (x 10^6/ml) in HIV negative control participants 27.07 (±22.98) and symptomatic HIV infected participants on ART 24.93 (±20.06) showed higher values compared with the symptomatic HIV participants not on ART ($p<0.05$), but the symptomatic HIV infected participants on ART, semen count mean ((±SD), 24.93 ((±20.06) maintained similar values with the HIV seronegative control participants 27.07 ((±22.98) ($p<0.05$). However, the pH, Liquefaction and viscosity of semen in the three parameters showed no significant difference ($p<0.05$) (Table 1).

The result in table 2 showed significantly lower Testosterone concentration (ng/ml) in symptomatic HIV participants not on ART 2.27 (±1.40) compared with the HIV seronegative control participants 3.78 (±2.47) ($p<0.05$). However, there was similar values in Testosterone concentration when symptomatic HIV participants on ART was compared with the HIV seronegative control participants ($p>0.05$). There was no significant difference in PSA when compared amongst symptomatic HIV participants , on ART, symptomatic HIV participants not on ART and HIV seronegative control participants ($p<0.05$).

Discussion

In this study, the percent motility and percent viability were significantly reduced in symptomatic HIV positive participants on ART, suggesting that HIV infection has a prolonged damaging effect on the semen motility and viability. Nevertheless, the percent motility and percent viability were significantly improved on symptomatic HIV participants on ART. The implication of this finding is that the ART has a recovery effect on the host system[12].

The present study also showed that the semen count and semen volume were significantly improved on symptomatic HIV participants on ART. The semen count in this study falls within the WHO criteria for normal count which is 15 million sperm/ml[8]. This improvement might be as a result of the administration and intake of ART on the host. ART has been found to reduce viral load in individuals[13] .The reduction in viral load must have allowed the normal functioning of the system and hence the normalization of the semen count.

The study further showed insignificant mean differences in semen pH, liquefaction time and viscosity levels observed amongst the groups and between the groups studied. Although, the pH value in symptomatic HIV positive participants on ART were within

alkaline range[14]. This means that HIV infection may not have effect on these parameters and they will not have marked differences in the studied groups.

The seminal Testosterone level was significantly different amobgst the groups studied. But the level was significantly reduced in symptomatic HIV positive participants not on ART. This finding implies that HIV infection has a debilitating effect on the gonads. The HIV infection may also cause a peripheral conversion of Testosterone to Estrogen hence the low value of Testosterone observed in symptomatic HIV positive participants. Estrogen effect is seen during peripheral conversion of Testosterone to Estrogen by Enolase and Aromatase[15].

An insignificant seminal PSA level was observed amongst the groups and between the groups. It has been reported that physiological PSA level improves semen quality and it contributes to about 25 to 35% seminal fliud[16, 17]. But the studies showed no much difference between the group in the PSA levels. One might expect that the PSA level should reduced in symptomatic HIV positive participants not on ART. The discrepancy might be due to the fact that we limited ourselves with symptomatic HIV (stage 11).

The study concludes that HIV infection grossly affect the semen quality which was evidenced by the presence of hypospermia (low semen production) and sthenozoospermia (poor motility) on symptomatic HIV positive male participants.

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