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COMPARATIVE ANALYSIS OF THE IMPACT OF HUMAN IMMUNE-DEFICIENCY VIRUS ON FERTILITY VARIABLES OF COMMERCIAL MOTOCYCLISTS AND NON-CYCLISTS IN NNEWI, SOUTH-EAST NIGERIA

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ABSTRACT

Background: Human immunodeficiency virus infection (HIV), a global epidemic especially in high-risk occupational groups, impacts negatively on fertility parameters and male- fertility. **Methodolgy:** A case-control study of thecomparative analysis on the impact of Human Immune-deficiency Virus on fertility variables of commercial motorcyclists and non-cyclists in Nnewi, South-East Nigeriawas conducted. A total of 152 males consisting of 49 commercialmotorcyclists and 103 non-cyclists of age-ranges of 19-79 years old was used. Pre-tested questionnaires and tools were used for data collection for variables; hormones were assessed by immunoassay technique, HIV virus was screened immune-chromatographically using parallel testing, positive samples confirmed with Western-blot technique, semen biomarkers (FSH,LH, prolactin, progesterone, testosterone) by Enzyme Linked Imunosorbent Assay (ELISA), semen qualities with new WHO methods, modified to suit sperm; fertility index and observed fertility index was mathematically-derived. Biologically important semen quality parameters were calculatedwith standard WHO mathematical-deduction method. **Results:** Results revealed a total of 4(2.6%) were HIV-1 infected; 3(6.1%) from tests and 1(1.0%) from controls. Positive HIV status impacted negatively significantly on observed fertility status (U^2 = 136.500; P = 0.049) in all males andsome semen quality variables; (teratozoospermia <50%) in test groups (U^2 = 20.500; P = 0.039), sperm concentration in both test and control groups,(U^2 = 21.000; P = 0.044); (U^2 = 0.000; P = 0.020)and non-significantly though with changes in mean rank values of semen biomarkers and serum hormones in HIV -1 $^+$ categories in both groups. **Conclusion:** HIV- 1 status significantly reduced and affected mean rank of testosterone hormones, teratozoospermia cells and sperm concentration semen qualities and observed fertility status incommercial motorcyclists than in non-cyclists reducing their fertilitypotential. Awareness is needed on the need for early detection and care as it can reduce male fertility.

KEYWORDS: HIV, Commercial motorcyclist, Non-cyclists, Semen quality, Hormones, Biomarkers, Fertility index.

INTRODUCTION

This is a research study on the impact of HIV on semen qualities, hormones, biomarkers and fertility index of occupational motor -cyclists in Nnewi, Nigeria. Commercial motor-cycling as an occupation is fraught with dangers and they have a high risk of contracting STD and HIV because they are "bridge-populations" with a risk for promiscuity because of the night- shift nature of the profession. Dangerous exposures of different kinds, trauma, stress and toxins experienced during work affects their reproductive capacity by affecting certain fertility parameters. An increased rate of HIV and sexually transmitted infections has been recently noticed in Nigerian males in the past decade, with Nigeria ranking second globally in 2014, while improving in 2018 to the second worst hit globally in a HIV/AIDS indicator and impact survey (NAIIS). With close to 2 million persons infected with HIV, Nigeria continues to rank among the top three countries with the highest burden of HIV in the world. Highest prevalence was observed in the South-South zone and the lowest prevalence in the North West zone. The new survey of prevalence estimated by state was categorized that seven

states including Anambra state are considered to have high prevalence of 2.0% and above, (NACA, 2019).

Commercial motor-cycling is a major source of transportation in Nnewi metropolis where most of the spare parts are assembled and sold. Almost all males rides a motorcycle in the town. The main source of public and private transportation in the city is through motorcycle, despite the terrible neglected road due to governmental negligence. These groups are exposed to people from all works of life as well to high risk of contracting STD and HIV because they are" bridge-populations "and to night- life, and most live separated from their spouses as established by (Society for Family Health and Action-aid Nigeria (2002). Dangerous exposures of different concentrations of chemicals, rays, and trauma, diseases in occupational environments andwork places can affect males and females' reproductive capacity, hormones and semen qualities, Schraderand Marlow, (2014). These can also increase risks for bacterial susceptibility to different microbial infections, as well as changes in biochemical markers and hormonal imbalance. Most outdoor occupation are association an increased rate of HIV and sexually transmitted infections which has been recently noticed in Nigerian males in the past decade, which ultimately affects semen production and has an adverse impact on male fertility. FMoH, (2007) in an IBBS national survey noted commercial motorcyclists as high-risk bridge populations. Most researches on occupational impact assessment was previously carried out in animals, especially with regards to male fertility, studies are rare. With the introduction of WHO new semen analytical method, studies on semen involving humans stands better chance of being monitored well, excluding some confounding variables that may arise.

The work aims toconduct a comparative analysis on the prevalence and impact of Human Immune- Deficiency Virus on fertility variables of commercial motorcyclists and non-cyclists in Nnewi, South-East Nigeria.

MATERIAL AND METHODS

Study area

The study area encompasses of Nnewi metropolitan commercial city in Anambra state, Nigeria.

Study design

This is a case-control study.

Sample size

The sample size was calculated using the formula by Naing *et al., (*2006)

$n= Z^2 X P (1-P)/d^2$

Where n= sample size.

P= prevalence percentage rate of male infertility in area of study (42.4%) (Ikechebelu et *al.*, 2003).

Z=Confidence Interval of 95% which is equivalent to confidence coefficient of 1.96.

d=desired level of precision or significance = 0.05

Using the formula, a total minimum samples size of 196 is required but a total sample size of 456 of urine, blood and semen samples were used though 210 volunteers registered for the research, only 152 men submitted the three samples and their questionnaires and were ready to provide more samples if necessary.

Study population/controls

A total 210 men willingly volunteered but 152 of them participated completely, gave full consent, adhered to all abstinence rules and submitted all the necessary samples and questionnaires including a second semen sample, while the rest dropped out half-way due to several reasons. The volunteers consisted of apparently healthy males of different occupational groups residing in Nnewi commercial metropolitan city. All participants (test and control groups) were of age-range of between 19-79 years old and were residing in Nnewi. Out of the 152 men, forty-nine (49) were registered commercial motorcyclists and were considered as the test groups while one hundred and three (103) men who were not in "Okada" business, and do not ride motor-cycles were considered as controls. Fifteen (15) of the men in test groups were recruited from Okada point in Otolo village, fourteen (14) from Umudimkwa (Umudim) point and ten (10) each were from Uruagu and Nnewi-ichi centers each, representing the four villages in the town. Thirty-eight (38) men from each of the four villages were recruited as controls.

Sampling technique: Consecutive non-probability sampling technique was used to collect samples from volunteers. Samples were collected from subjects based on being a commercial motorcyclist or non- motorcyclist and adult reproductive age irrespective of fertility statuses of the time of study. For the test groups, only registered commercial motorcyclists plying in Nnewi were invited to their headquarters in Okada white-house at Otolo, Nnewi through an oral announcement made by a town announcer through the Chairman of the association. Willing participants were recruited from volunteers ensuring that selection was made from four main commercial motorcyclist's parks from each of the four villages where they habitually ply to ensure equal distribution of men representing each village. Recruited participants were educated on the reason for the research and nature of samples needed. Control participants were also selected from each villages excluding any commercial motor-cycle operator and anyone who drives a motor-cycle. Any willing male residing in each of the four villages and belonging to the selected age category were recruited. The eligibility of each of the control respondent could be a father, non-father, married, single, widowed, divorced or separated; occupations varied like traders, artisans, civil servants, or student, All willing participants were recruited after explanations were made

on the reason for the research and nature of samples needed. The respondents were carefully selected and educated on the study reasons before questionnaires were distributed.

Inclusion criteria

Selection was based on their being apparently healthy fertile males who fulfilled the requirements listed;

1. Adult males who were between 19 and 79 years of age, sexually active as at the time of study and of different occupational category for controls and only commercial motorcyclists for test groups.
2. They must be living in Nnewi.
3. They must not be on antibiotic or herbal therapy 5- 7 days prior to sample collection or during the study.
4. They must be willing to participate and must submit all necessary required samples and questionnaires.
5. They must have no history of vasectomy or prostate removal.

Exclusion criteria

1. Unwillingness to participate and did not submit all necessary required samples and questionnaires.
2. Males of different profession who were below 18 years.
3. Males with history of vasectomy or prostate removal.
4. Those living outside the vicinity of the study area.
5. Those on antibiotic or herbal therapy as at the time of study.

Ethical clearance: Ethical approval for the study was obtained from the Ethical committee of Department of Medical Laboratory Science, Faculty of Health Sciences and Technology, Nnamdi Azikiwe University, Nnewi, and an official authorization permit for sample collection from Nnewi Okada Amalgamated Association. Procedures and methods were followed in accordance with the ethical standards of the Helsinki declaration of 1975, revised in the year 2000, (WHO, 2001). Informed consent was obtained from all study participants.

MATERIAL AND METHODS

Data collection: Prior to sample collection, all the volunteers were educated on the reason and nature of the research. Willing participants were given a copy of informed consent to fill and return. Questionnaire was used for data collection. It was completed by each volunteer as he submits his sample. English language as well as vernacular was used for data collection by oral interview. The questionnaire was used to gather data from the respondents.

Samples / sample collection: The samples used in the study include semen samples and blood serum). Samples were coded with a unique identification number as well as with the patient's name. Results were collated using this unique identification number to maintain subject's confidentiality. Semen samples were collected by masturbation method. Prior to that, all protocols regarding sample collection was explained to the volunteers. Abstinence for at least three days, avoidance of antibiotic and herbal therapy use for at least 7 days, sterility maintenance and adherence to sample collection rules and submission time of within 30 minutes was strictly adhered to. All precautions were according to WHO (2010) specification. Semen sample was collected with sterile pre-weighed and personal identity-labeled wide-necked plastic stericon containers and submitted immediately. Subjects who had to collect their sample at home preserved them and kept it at near body temperature in their pockets, until they arrived in the laboratory within 30 minutes. If any delay in processing was expected, they were kept in the incubator at 37 ^{o} c, otherwise they were cultured first, before microscopic analysis. Samples were properly labeled with name, sex, number, and weight of sample containers, time of collection and arrival, method of semen collection and indication of any spills or contamination noted for other necessary activities. Sampling was done in batches. As they submitted their samples, questionnaires were filled out.

Blood: Eight (8) milliliters of blood was collected aseptically from the cubital fore-arm after cleansing the area with 70% alcohol using a sterile 10ml syringe (BD India.) Five (5mls) were allowed to clot and serum extracted after centrifugation at 1000rpm for 5 minutes and stored frozen at -4 ^{0} C for hormonal assays, (Follicle stimulating hormone (FSH), Luteinizing hormone (LH), Prolactin, Progesterone, and Testosterone), and Human Immunodeficiency Virus (HIV) screening as described by (Cheesbrough, 2006). The serum was used within 2 weeks. Whole blood was used for CD4 count to confirm HIV status when positive.

Sample processing (semen plasma extraction and storage)

As soon as samples arrived, semen samples were placed on the bench for liquefaction and observations noted within 15 minutes. Other analysis was carried out immediately within 1 hour of collection. Semen plasma was extracted by spinning in a centrifuge at 10 000 g /min for 10 minutes in sterile well -cleaned plain glass tubes immediately after cultures were performed to avoid contamination of samples. It was decanted in aliquots using a sterile disposable plastic pipette and sperm-free plasma stored in sterile Eppendorf plastic tubes in the freezer at -4°C. Samples for zinc analysis was stored in clean polystyrene containers previously ensured to be zinc free by proper cleaning in cleaning fluids according to methods specified by (WHO, 2010).Semen plasma was analyzed within one week. All semen analysis were carried out at the post-graduate laboratory of the Department of Medical Laboratory Science, Nnamdi Azikiwe University, Nnewi Campus, semen plasma zinc analyzed at Springboard Research Laboratories, Awka andsemen citric acid, semen fructose, alpha-neutral glycosidase assay, all hormonal assays analyzed at Mega Diagnostic Laboratories, Nnewi. Retracted clotted blood

was spun in a centrifuge at 10 000 g /min for 10 minutes and serum obtained and stored in clean tubes in a freezer at – 4° C and male hormones analyzed within one week. CD 4 counts were carried out the same day at human virology unit of Nnamdi Azikiwe University, Awka, Nnewi.

Analysis of samples: The parameters analyzed in this research include:

HIV screening test: HIV 1 and 2 antibodies were screened in serum samples to assess presence or absence of antibody to the virus which may result to male infertility.Method used was the three panel parallel testing method recommended by (CDC, 2014) using qualitative immune-chromatographic method as described by Ariah*et al.* (1999) with Determine kit (Determine™ Alere kit, Alere Medical Co. Ltd, Chiba, Japan), Unigold and Stat pack immune-chromatographic kits methods.

HIV Confirmation- This was carried out using CD4+ T - cell western-blot Cyflowcytometry method with cyflow counter (Model CY-s-3022, Partec, Germany), Towbin*et al.(*1979) usingCD4 easy count kit, (Partec, Germany).Solid state laser, green filter 532nm and 3 optical parameters for detecting side scatter (SSC), orange (FL2) and red (FL3) fluorescence signals.

Semen plasma biomarker tests

Fructose concentration- Photometric quantification of fructose in semen plasma was carried out to assess for the level of fructose in semen plasma as a marker of secretory function of epididymis function with fructose kit (Fertipro N.V, Belgium) ref number; FPO9 129 ROI B.5 and assay carried out according to photometric kit resorcinol method as described in (WHO, 2010). Result was expressed as (mg/ml). Biologically important variables like total semen fructose (mg/ml ejaculate), corrected semen fructose (mg x 10^6/ml), true-corrected semen fructose concentration (mg/ml) were obtained by mathematical-deduction methods, (WHO, 2010).

Semen plasma citric acid concentration: Citric acid concentration in semen plasma was assessed to determine the prostate function. This was done usingspectrophotometric method described in (WHO, 2010) using (Agilent Technologies 200 AA 240fs AA series, USA) and expressed as (mg/ml).Total citric acid concentration(mg or more /ml ejaculate) was obtained by mathematical-deduction methods.

Seminal zinc concentration: All glassware and plastic wares were rinsed with 10% nitric acid overnight and thoroughly washed with deionized distilled water before use. Zinc concentration (Zn-C) in seminal fluid plasma was determined by flame atomic absorption spectrophotometer, (Agilent Technologies 200 AA 240fs AA series, USA.). The method used was flame atomic absorption spectrophotometric method (WHO, 2010).Zinc concentration was expressed in (µg/ml) and total zinc concentration in (µg/ml) calculated by mathematical-deduction methods.

Alpha (ὰ)-neutral glucosidase enzyme: This was performed to assess the secretion contributory status of the epididymis to the ejaculate, and differentiates the major causes of azoospermia, (obstructive and non-obstructive) types using Episcreen Plus™ Enzyme ὰ-neutral glucosidase kit, (Fertipro, Belgium). This was carried out according to methods described in WHO (2010). Result was expressed in (mU/ejaculate). Incubation time and dilution factor was corrected as corrected factor- (WHO,2010).Result was expressed in (mU/ejaculate).

Seminal acid-phosphatase assay: Seminal plasma acid- phosphatase was determined to assess prostate function using Kind and King (1954) colorimetric method modified by Hillman's colorimetric method (1971) as described in Tiez (1995) to suit sperm. Samples were later tested on the automated biochemical analyzer. Since semen contains a high concentration of acid phosphatase, it was diluted 1:2000-1:4000 to fall within parameter with buffer.

Hormonal assessment assays: Serum FSH, LH, Testosterone, Prolactin, and Progesterone assays were carried out to assess male fertility using enzyme linked imunosorbent assay (ELISA) quantitative immunoassay method with kits according to methods described byTietz (1999) following the manufacturer's instructions. Kitswere from (Immunodiagnostics, Cortez, Inc., Calabasas, California; Accu diagnostic™ C.A) and expressed in (ng/ml and mg/ml).

Semen analysis: All assays were according to (WHO, 2010).

Initial macroscopy and microscopy: Appearance, liquefaction, pH., viscosity (gravity-fall method), cell observations, agglutination grading, motility, sperm count, motility, immobility, non-progressive and progressive motility (%), manual motility classification and characterization, all biologically important calculations deducted from standard derived formula (TM, TNPM, TMSC,SC, and TSC).

Vitality (%): Membrane integrity of the cells was assessed using one-step dye -exclusion eosin-nigrosin staining method, Bjorn-Dahl et *al.* (2003), within 30mins-1hr post ejaculation.

Sperm morphology (%): Feathering method for slide preparation and modified Papanicolaou staining method for staining and total number of morphologically normal spermmathematically calculated according to (WHO, 2010).

Normal forms (%): Two criteria was used, .Kruger's criteria and WHO criteria,(WHO, 2010).

Abnormal morphology (%): All sperm part defect was assessed.

Fertility index: Calculating a fertility index from the observed density, morphology, and motility of sperm in human semen. Fertility index was derived mathematically from semen analysis according to method and formula described by (Harvey, 1953).

Statistical analysis: Results obtained were analyzed using statistical package- SPSS version 21 after being subjected to normality test. Data in the research were not normally distributed, therefore, the non -parametric Mann -Whitney (U) test and Kristal –Wallis (K) tests, as well as prevalence percentage and chi-square association used where necessary and P values of <0.05 were considered significant at 95% confidence interval (CI).

RESULTS

Comparative frequency distribution and chi square of HIV-1 infected subjects in test and control in relation to age range.

Three, 3(6.1%) commercial motorcyclists and 1(0.97%) non-cyclists tested positive to HIV-1 out of a total of 49 and 103 males sampled (100%) in table 1. Age range of 45- 64 years old had the highest prevalence 3(7.1%) and 65-79 years old category 0(0.0%) the least in all sampled. No statistical significance was observed in test groups (X^2= 0.664; P = 0.718) and controls(X^2 =4.191; P=0.123) among the age groups.

Table 1: Comparative frequency distribution and chi square of HIV-1 infected subjects in test and control in relation to age range.

Occupation	Age category (years)	HIV-1	HIV-1	Total	X^2	P-value
		Negative status %	Positive status %			
Motorcyclists	19-44	24	1	25		
		96.0%	4.0%	100%		
	45-64	20	2	22	0.664	0.718
		90.9%	9.1%	100.0%		
	65-79	2	0			
		100.0%	0.0%			
	TOTAL	46	3	49		
		93.9%	6.1%	100.0%		
Non-cyclists	19-44	81	0	81		
		100.0%	0.0%	100.0%		
	45-64	19	1	20	4.191	0.123
		95.0%	5.0%	100.0%		
	65-79	2	0	2		
		100.0%	0.0%	100.0%		
	TOTAL	102 99.1%	1 0.97%	103 100.0%		
Total	19-44	105	1	106		
		99.1%	0.9%	100.0%		
	45-64	39	3	42	4.623	0.099
		92.9%	7.1%	100.0%		
	65-79	4	0	4		
		100.0%	0.0%	100.0%		
	TOTAL	148	4	152		
		97.4%	2.6%	100.0%		

In figure 1 below, commercial motorcyclists had more HIV-1 positive status 3(6.1%) than non-cyclists 1(0.97%) and highest occurrence was in the age- range of45-64 years old in all males. None was infected in the oldest age groups 3(7.1%).

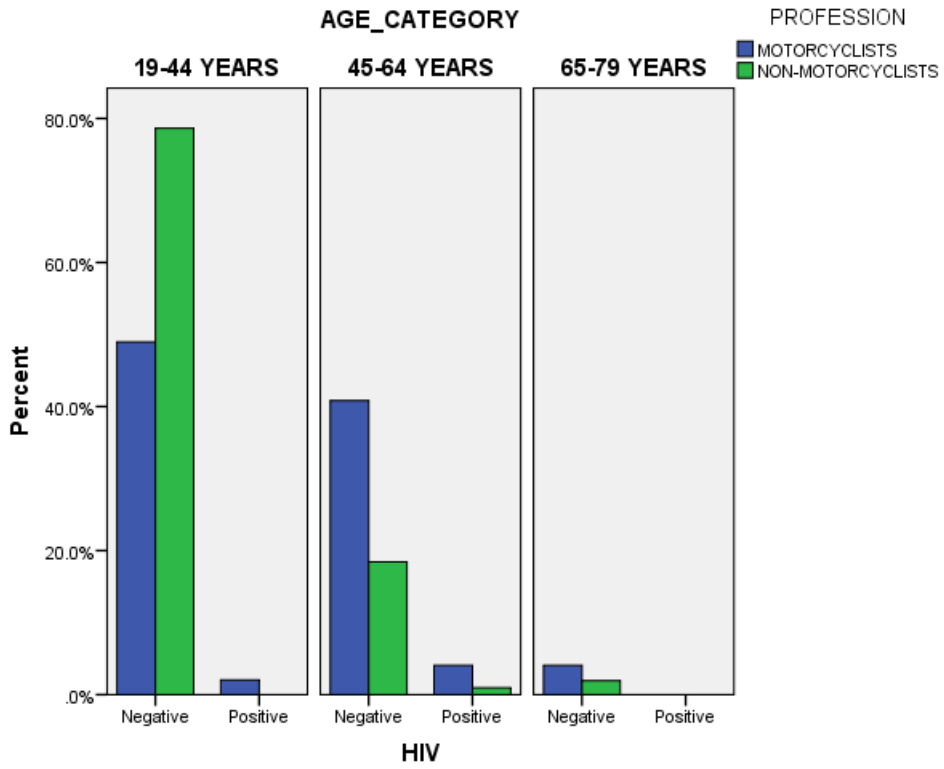

Figure 1: Comparative bar chart of frequency distribution of HIV-1 status in age categories of tests and controls.

Significant influence of HIV-1 virus positive status on semen quality, fertility index and observed fertility status in tests and control groups

Significant difference was observed among the mean rank values of semen qualities of the HIV -1 categories in relation to those with teratozoospermia in the test groups (HIV $^{-}$ (51.67); HIV $^{+}$ (34.50)(U = 20.500; P = 0.039) in table 2abelow ; sperm numbers in test groups (HIV $^{-}$ (26.04); HIV $^{+}$ (9.00)(U = 21.000; P = 0.044); and in controls (HIV $^{-}$ (51.00); HIV $^{+}$ (102.00)(U = 0.000; P = 0.020) and inobserved fertility in the total males sampled (HIV $^{-}$ (77.58); HIV $^{+}$ (36.63) (U= 136.500; P = 0.049).

Fertility index (FI) was not significant (P>0.05), butin those who tested HIV -1 positive (test =19.17; controls= 96.00; total=79.00) and HIV negative (test =25.38; controls= 51.06; total=76.43), the husband's sub fertility alone in test groups in both statuses may be enough to account to wives' fate to conceive during the period and in controls and total males in both statuses, males were fertile, and any sub fertility is almost entirely due to the wife.

Table 2a: Significant influence of HIV- 1 virus status on semen quality, fertility index and observed fertility status in tests and control groups.

Variables	Categories	Total Mean rank	Test Mean rank	Control Mean rank	Total (2 tailed) UP – value		Test (2 tailed) UP – value		Control (2 tailed) UP – value	
Semen qualities										
<50%(teratozoospermia)	Negative	77.49	26.05	51.67	149.500	0.086	**20.500**	**0.039***	33.500	0.667
	Positive	39.88	8.83	34.50						
Sperm number	Negative	76.78	26.04	51.00	255.000	0.637	**21.000**	**0.044***	**0.000**	**0.020***
	Positive	66.25	9.00	102.00						
	Positive	88.25	28.67	89.50						
Fertility Index	Negative	76.43	25.38	51.06	286.000	0.908	51.500	0.485	6.000	0.137
	Positive	79.00	19.17	96.00						
Observed fertility status	Negative	77.58	25.04	51.92	**136.500**	**0.049***	67.000	0.953	8.500	0.176
	Positive	36.63	24.33	9.50						

Non-significant influence of HIV-1 virus status on semen quality andsemen characterization in tests and control groups.

None-significant statistical impacts were observed on the mean rank values of several semen qualities and semen characterization inall groups sampled P > 0.05 with lower values in class of progressive motility, non-progressive motility, abnormal forms, immotile forms (asthenozoospermia), mean rank of fructose, citric acid, concentration in all groups were lower in HIV^+ groups in all tested groups, zinc concentration in (controls)only, corrected alpha neutral glycosidase (test groups) only and acid phosphatase in (total males and controls) groups. In HIV^+ groups, higher values were obtained in total semen fructose concentration in all groups, corrected and true corrected semen fructose in all except controls, total citric acid concentration, total zinc and alpha neutral glycosidase in all, corrected alpha neutral glycosidase and acid phosphatase in test groups.

Table 2b: Non- significant influence of HIV-1 virus status on semen quality, semen characterization and observed fertility status in tests and control groups.

Sperm variables	Categories	Total	Mean Rank (frequency) Test	Control	Total (2 tailed) X^2P – value		Test (2 tailed) X^2P – value		Control (2 tailed) X^2P – value	
SEMEN QUALITIES										
Volume (ml)	Negative	75.88	24.63	51.13	203.500	0.285	52.000	0.511	13.000	0.275
	Positive	99.63	30.67	89.00						
19% (> 6 – 10%)	Negative	76.19	24.39	22.56	250.000	0.594	41.000	0.265	12.430	0.133
	Positive	88.00	34.33	38.66						
Class of progressive motility	Negative	76.88	25.22	51.64	248.000	0.525	59.000	0.708	36.000	0.725
	Positive	64.50	21.67	37.00						
< 50% Forward progression (a + b)/ < 25% with a	Negative	76.99	26.05	51.67	224.000	0.277	50.400	0.489	45.300	0.332
	Positive	58.50	8.83	34.50						
Non progressive motility Interpretation	Negative	19.67	12.87	15.55	30.000	0.728	10.435	0.655	15.987	0.474
	Positive	16.50	11.56							
Normal forms	Negative	76.19	24.74	51.21	250.000	0.596	57.000	0.650	21.500	0.431
	Positive	88.00	29.00	80.50						
Abnormal forms	Negative	76.61	25.21	51.71	279.500	0.849	59.500	0.708	29.500	0.588
	Positive	72.38	21.83	30.50						
Middle piece	Negative	75.77	24.27	51.56	260.000	0.689	57.000	0.687	44.000	0.882
	Positive	84.50	28.00	45.00						
Tail	Negative	76.35	25.19	51.39	243.000	0.545	36.500	0.198	39.000	0.784
	Positive	63.25	14.17	63.00						
Immature forms	Negative	75.99	24.16	22.02	292.500	0.983	52.000	0.543	45.003	0.233
	Positive	76.38	29.67	30.01						
Viable forms	Negative	76.77	25.59	51.29	256.000	0.603	42.000	0.284	29.500	0.588
	Positive	66.50	16.00	72.50						
Non-viable forms	Negative	76.55	25.26	51.29	288.500	0.922	57.000	0.650	29.500	0.588
	Positive	74.63	24.00	72.50						
Total no of intact membranes	Negative	76.96	25.73	51.35	228.000	0.377	35.000	0. 172	35.500	0.706
	Positive	59.50	13.83	66.50						

Sperm concentration	Negative	77.19	26.02	51.38	194.000	0.240	22.000	0.050	38.000	0.765
	Positive	51.00	9.33	64.00						
Total sperm number	Negative	76.39	25.33	51.20	279.500	0.849	54.000	0.565	20.000	0.412
	Positive	80.63	20.00	82.00						
Total no of progressively motile cells	Negative	76.67	25.63	51.12	271.500	0.778	40.500	0.248	12.000	0.255
	Positive	70.38	15.50	90.00						
Manual motility classification	Negative	76.95	25.70	51.30	229.000	0.377	37.000	0.200	30.000	0.608
	Positive	59.75	14.33	72.00						
Sperm concentration characterization	Negative	76.15	24.58	51.53	244.000	0.426	49.500	0.435	47.500	0.941
	Positive	66.25	31.50	48.50						
Sperm concentration classification	Negative	76.15	24.01	51.76	215.500	0.313	23.500	0.050	24.000	0.490
	Positive	98.50	40.17	25.00						

Mann-Whitney U- test analysis of the influence of HIV-1 virus status on semen biomarkers in commercial motorcyclists (tests) and non-cyclists (controls).

No significant statistical impact was observed on the mean rank values of all semen biomarkers, in all groups sampled P > 0.05 though mean rank of fructose, citric acid, concentration in all groups were lower in HIV^+ groups in all tested groups, zinc concentration in (controls) only, corrected alpha neutral glycosidase (test groups) only and acid phosphatase in (total males and controls) groups. In HIV^+ groups, higher values were obtained in total semen fructose concentration in all groups, corrected and true corrected semen fructose in all except controls, total citric acid concentration, total zinc and alpha neutral glycosidase in all, corrected alpha neutral glycosidase and acid phosphatase in test groups in table 3 below.

Table 3: Mann-Whitney U- test analysis of the influence of HIV-1 virus status on semen biomarkers in commercial motorcyclists (tests) and non-cyclists (controls).

Variables	Categories	Total Mean rank	Test Mean rank	Control Mean rank	Total (2 tailed) UP – value		Test (2 tailed) UP – value		Control (2 tailed) UP – value	
BIOMARKERS										
Fructose concentration	Negative	76.6	25.28	51.85	274.500	0.805	56.000	0.621	15.000	0.314
	Positive	71.13	20.67	16.00						
Total semen fructose	Negative	76.40	24.93	51.73	281.000	0.863	66.000	0.922	27.500	0.549
	Positive	80.25	26.00	28.50						
Corrected semen fructose	Negative	75.62	24.91	51.00	165.500	0.451	65.000	0.891	26.354	0.523
	Positive	94.83	26.33	51.26						
True corrected Semen fructose	Negative	76.52	25.00	51.83	293.500	0.977	69.000	1.000	17.000	0.353
	Positive	75.88	25.00	18.00						
Citric acid concentration	Negative	76.88	25.48	51.88	239.500	0.514	47.000	0.388	12.000	0.255
	Positive	63.38	17.67	13.00						
Total citric acid	Negative	76.11	24.92	51.25	239.000	0.511	65.000	0.891	25.000	0.510
	Positive	90.75	26.17	77.00						
Zinc concentration	Negative	76.49	24.97	51.54	295.000	0.991	67.500	0.953	46.000	0.922
	Positive	76.75	25.50	47.00						
Total zinc concentration	Negative	76.03	24.60	51.33	226.500	0.424	50.500	0.460	33.000	0.667
	Positive	93.88	31.17	69.00						
Total corrected α- neutral glucosidase	Negative	75.70	25.11	50.56	249.000	0.588	64.000	0.860	6.000	0.139
	Positive	87.00	23.11	95.00						
Acid phosphatase	Negative	76.73	24.70	51.96	262.000	0.686	55.000	0.593	4.500	0.098
	Positive	68.00	29.67	5.50						

In table 4 below, there was no significant difference in the mean rank values of all hormones sampled in all groups P>0.05, though in some values, they were raised or lowered.

Table 4: Mann-Whitney U- test analysis of the influence of HIV-1virus status on serum hormonesin commercial motorcyclists (tests) and non-cyclists (controls).

Variables	Categories	Total Mean rank	Test Mean rank	Control Mean rank	Total (2 tailed) UP – value		Test (2 tailed) UP – value		Control (2 tailed) UP – value	
FSH	Negative	75.44	24.03	51.26	133.500	0.070	24.500	0.062	26.000	0.529
	Positive	115.88	39.83	76.00						
Testosterone	Negative	76.78	25.71	51.14	255.000	0.637	36.500	0.185	14.000	0.294
	Positive	66.25	14.17	88.00						
LH	Negative	75.95	24.15	51.67	215.000	0.351	30.000	0.113	33.000	0.667
	Positive	96.75	38.00	34.00						
Prolactin	Negative	75.45	24.16	51.22	141.000	0.074	30.500	0.113	22.000	0.451
	Positive	115.25	37.83	80.00						
Progesterone	Negative	76.86	25.18	51.64	248.000	0.580	60.500	0.738	36.500	0.725
	Positive	64.50	22.17	37.50						

DISCUSSION

More men from test groups (commercial motorcyclists) had more positive status for HIV -1 antibody 3 (6.1%) than in controls 1 (1.0%), (P>0.05) in table 1. Probable reason could be because these occupational groups in the geographical area may sub-optimal information onsexually transmitted disease (STD) preventive health education, or a defective perception on the topic and health education with poor knowledge and perception of the disease, the night- shifts nature of their profession which makes them prey to commercial sex-workers " (bridge-populations)" STD and garbage life with frivolous activities, may have had habit of exchanging "free-ride" for sex, multiple-sexual partners or do not wear condom.

In a study by FMoH (2007), Anambra state had a higher than average HIV prevalence for three categories of male occupational groups sampled (armed forces (7.6%), police and transport workers (5.8%), of which commercial motor cyclist belong to. Many studies in Nigeria as well as globally have proved that mobile transport workers including commercial motorcyclists are at high risk HIV/STI exposure. High prevalence of risk behaviors for HIV among commercial motor cyclists was noted by (Abiodun, 2013) in a research in Shagamu, Lagos, where polygamous relationships, lack of proper education on STIs, high prevalence of multiple sexual partners, non-condom use, predominated and educational level attained, not being involved in in (voluntary counseling and testing) were consistently associated with its presence. This incidence was consistence also with findings by Mohammed *et al.*, (2007) who found a prevalence of 32(8.7%) sera-positive HIV -I antibody positive out of a total of 379 men in Abuja commercial motorcyclist.

Highest prevalence was found in the middle aged 45-64years old category in test 2(9.1%), controls 1(5.0%), and total men, 3(7.1%).The probable reasons for this observation in this age category in this research could be because at that age, there is more probability of their having had multiple sexual relationships, could be facing marital crisis, spousal separation, death of spouse or divorce, renewed dating with experimental sex-habits due to the introduction of Viagra or they may not have been involved in the target catchment age-group in the last HIV –awareness intervention in the study area.

In Nigeria, FMoH (2012) conducted HIV risk-factor surveillance survey using samples from 4,882 transport workers including long distance drivers (47%), short distance drivers (29%) and commercial motorcycle riders (24%) aged between 15-49 years. Risk factors include being sexually experienced(90%), long distant driving (83%), short distant driving (82%), had sexual intercourse with female sex workers in the 12 months preceding the survey (76%) and 66% were married and lived with their spouse. In Kampala, Uganda, more HIV positive males 7.5% (95%, CL5.2-10.0) were observed in age- range of males of median age of 26 years sampled (n=683 males) in motorcycle taxi ('boda-boda') drivers Linda*et al.* (2015), similar to of 6.1% in the present research.Kirk (2017) noted that in Ireland, more middle-aged men and women were dating, having radical sex habits without mid-life crisis or looking after their sexual health, with consequent rise in STI. Monsell and Laluskey (2016) reviewed research papers on the factors influencing STI transmission in middle-aged population of 45-65 years heterosexual individuals and found they were at increasing risk of contracting a sexually transmitted infection, similar tofindings in the present research. Identified risk factors includes, low condom use, middle age crisis, sexually behaviors like contact with sex workers and sexual encounters abroad, breakdown and formation of new relationships during middle-age, success in new drug for medications for erectile dysfunction e.g. Viagra and Cialis, enhancing an increase in sexual activity, increase in mid-life divorce rate, online dates, perceiving themselves as less likely to be at risk, lack of safe-sex education directed to their age groups.

There were significant differences in the changes in the mean rank values some semen qualities among the HIV status categories. HIV-1 positive status showed a

significant difference in mean rank values of teratozoospermia cells between HIV^+(8.83) and HIV^- (26.05) men in test groups only (U= 20.500 ; P =0.039) in the present research, agreeing with findings by Umpathy *et al*.(2001*).* Reason is probably related to repressed stage of the infection, inflammatory cell response, decrease in testosteronospermia with resultant gonadal changes, metabolic changes associated with taking HARRT drugs, oxidative stress created by physiological changes like leucocytospermia, trauma and increased scrotal temperatures due to saddle seat, reduced CD4 counts, HIV asymptomatic chronicity, individual immune status or increased viral load numbers in the infected cyclists. Barabazo *et al.* (2004) observed morphological and topographical changes in HIV/AIDs males receiving HAART proposed to be due to HARRT rather than HIV^+ status with decreased total sperm count (oligospermia) and progressive motility (PM), causing sperm quality change. Nicopoullos *et al.* (2009) also noted a significant relationship between CD4 count and a positive correlation with sperm parameters with those with full blown AIDs being less fertile than HIV^+ men, in line with findings in this work. Direct effects of HIV virus on hormones was proposed by Baraboza *et al.* (2009) to lead to gonadal failure, ochitis, hypogonadism, leucocytospermia, oligospermia and teratozoospermia, while Umapathy *et al.* (2001) noted leucocytospermia associated with sperm quality changes and low CD4 count giving room for opportunistic infections that causes ochitis and epididymal changes.

The significant difference in the impact of HIV -1 virus on mean rank values of sperm numbers, between HIV^+ (9.00) and HIV^- (26.04) men in test groups (U=21.000; P=0.044) and controls (102.00 vs. 51.00) (U=0.000; P=0.020) in the present studying table 3 could be as a result of duration of the infection and toxic effects of anti-retroviral therapy. Anti-retroviral drugs has been shown to induce toxic effects on sperm, Carr *et al.* (2000) as possible consequences of treatment on reproductive function by causing metabolic and endocrine changes affecting testis dysfunctions, total reproductive function and gamete function. Sperm counts were decreased in HIV infected men but total sperm count was not affected by duration of HIV infection. Decrease in CD4 counts below $200/mm^3$ was also associated with decreased sperm concentration and total sperm count. Studies by Reinek*et al.* (2001) showed sperm concentration decreased significantly between the 1^{st} and 2^{nd} ejaculate fraction that split ejaculates in HIV^+ infected men, though fructose concentration did not increase. Other non-significant changes in semen quality like decreased progressively motile cells, viable forms etc. as a result of HIV-1 infection in the research could be attributed to confounding factors and biases inherent in some studies as also noted by Bujan*et al*.,(2007).

Statistical significant impact of HIV- 1 status was observed on the mean values among statuses of observed fertility in the total males sampled (U= 136.500; P = 0.049) where the infected participants had a lower mean value, and fertility index though not significant ($p<0.05$) showed that for those who tested HIV positive, (total=79.00; test =19.17; controls= 96.00) and HIV negative, (total=76.43; test =25.38; controls= 51.06) , the husband's sub fertility alone in test groups in both disease statuses may be enough to account to wives' fate not to conceive during the period, while in infected and non- infected control groups as well as in total males, for any fertile male, and any sub fertility is almost entirely due to the wife. Though in most cases, chances of conception depend solely on wives' fertility, these obtainable in sub fertile marriage. Hypothesized reasons for how HIV infection affected fertility in commercial motorcyclists in the present research could be by the toxic effects of ART on reproductive organs which may contribute to sub-fertility in HIV infected participants, effects of prior STD in the group especially as they were found to harbor more varieties of STD more than control groups, as well as risks for opportunistic infections. Again, psychosocial factors in patients with HIV infection may affect reproductive desires and outcomes, biological alterations in reproductive physiology caused by the virus in semen fluid compartment.

Carr *et al.* (2000) noted that consequences of treatment HIV with drugs onreproductive function could result to metabolic and endocrine changes which can in turn cause testis dysfunctions, affect total reproductive and gamete functions. Barboza *et al. (2009)* noted *viral* persistence in specialized male genital tract compartment which later induce toxic proteins by selective pressure, interactions among HIV virus, spermatozoa and their progenitor cells and testicular germ cell causing infertility in males.Sub-fertility in HIV positive men have been known to reduce over time if it is caused by STD acquired prior to HIV infection, but will persist if it is directly results from HIV infection (Galvin and Cohen, 2004).

HIV-1 infection statuses in test groups and controls were associated with changes in mean rank values of semen biomarkers, hormones and fertility index in groups studied, though no significant impact of HIV virus was observed on any of the semen biomarkers tested among all the biomarkers ($P>0.05$). This happened probably because HIV-1 is just a virus that causes the immune system to be affected in huge ways, and though transmitted only through body fluids, the male reproductive system can still function normally without any changes or just a slight reduction. The virus may not have infiltrated the epididymis or vesicular or prostatic gland secretions. They could be on ART which could have helped a great deal in boosting their immune status. Duration of the infection or stage of the infection as of the time may not have been enough to produce enough inflammatory cells to cause ROS changesin the male accessory tracts.

Decreased mean rank levels of semen citric acid and fructose concentration was observed in HIV positive

more than in HIV sera-negative males in test (17.67 vs. 25.48), controls (13.00 vs. 51.88) and total population (63.38 vs. 76.88) in the present research probably as a result of opportunistic bacterial and fungal infections which may have produced inflammatory cells that caused secretory dysfunctions and viral infiltration in the accessory glands (epididymis, prostate, seminal vesicles) Eggert - kruse (2003), or lowered CD4 causing viral spread. Shehu-Xhilaga*et al.* (2007) also noted decreased quantities of citric acid, phosphates, fructose concentration, zinc concentration, total zinc and alpha-glutamyltransferase activity in accessory glands in AIDs stage of HIV infections only, with reduced CD4 counts and increase viral load, in line with findings in this research. Umpathy *et al.* (2001) studied the impact of accessory gland functions on sperm characteristics of HIV-infected men in Zimbabwe and also failed to find any changes in all the biochemical markers affected in HIV seropositive men, in line with findings also in this research, though leucospermia was almost always found in significant association with the seropositive men causing impaired sperm motility.

Finally, HIV positive status had no significant impact on the mean rank values of all hormones tested in all participants in the present study, (P>0.05), though lowered mean rank values of testosterone was observed more in HIV^+ males in the test groups (14.17) and total males (66.25) than in HIV^- men in test groups (25.71); 148(76.48) in table 4 maybe signifying disease progression. Gonadal and extra gonadal effects are associated with HIV infection in the early stage when men have normal testosterone, but as the disease progresses, testosterone falls because of androgen deficiency common in AIDs wasting syndrome. Direct effects of HIV leading to gonadal failure have been proposed in men and women though proof of hypothesis remains elusive. HIV infected men are more likely to have ochitis hypogonadism and leucospermia which causes changes in sperm than the non-infected males (Barboza *et al*., 2004).

In conclusion, HIV-1 status affected semen quality (sperm concentration, shape and abnormal forms) and observed fertility status more significantly than biomarkers and hormones especially in test groups. It is therefore recommended that educative, early detection, treatment and preventive measures should be adapted with special consideration on older adults of 45-65 years old to avert fertility problems.

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