Original Research Article

Pattern of some Sperm Proteins, Anti-oxidants, Testosterone and Prostate-Specific Antigen of Infertile Males with Sperm cells Deformities in Port Harcourt, Rivers State

ABSTRACT

Aims: To investigate the pattern of some sperm proteins, antioxidants and prostate-specific antigen in the seminal plasma of infertile males with sperm cells deformities.
Study design: The study is a case-control study design to investigate semen parameters and sperm proteins in infertile males with sperm cells deformities in Port Harcourt, Rivers State.
Place and Duration of Study: The study was carried out in the Department of Medical Laboratory Science, Rivers State University, Port Harcourt. However, some of the Laboratory investigations were done in the Chemical Pathology Unit and Medical Microbiology Unit of University of Port Harcourt Teaching Hospital, Port Harcourt. The Study was between the periods of September, 2019 and Feb., 2022.
Methodology: A total of 276 males indicated interest to participate in the study of which 193 male subjects were recruited. Of the 193, there were 100 normospermic, 40 azoospermic, 53, oligospermic, 40 asthenozoospermic, 48 oligoasthenozoospermic, 26 teratozoospermic, 32 asthenoteratozoospermic, and 22 oligoasthenoteratozoospermic. HSP70, PKA, and MDA, TAC and GPX (in seminal plasma), PSA and testosterone (in serum) were analysed using ELISA while TAC was done using spectrophotometric methods. Results obtained were analyesd using GraphPad Prism and SPSS.
Results: Sperm motility, TSC, and normal sperm morphology were poor in oligospermic subjects but very severe in OAT subjects. Sperm proteins are clearly altered in individuals with abnormal sperm cells morphologies. HSP70, PKA were severely increased while OPN, TAC, and GPX were severely decreased in the seminal plasma of infertile males with abnormal sperm cells. Testosterone and PSA in blood plasma were significantly low and high respectively..
Conclusion: Oxidative stress plays significant role in sperm cell deformities and fertility. OAT subjects were the most affected.

Keywords*: Sperm Proteins, Sperm cell deformities, Male infertility, Port Harcourt, Niger Delta*

1. INTRODUCTION

Infertility is a globally problem affecting both males and females. Infertility is a condition with psychological, economic, medical implications resulting in trauma, stress, particularly in a social set up like ours, with a strong emphasis on child bearing [1]. According to the international committee for Monitoring Assisted Reproductive Technology and the World Health Organization (WHO), infertility is a disease of the reproductive system defined by failure to achieve clinical pregnancy after 12 months or more of regular unprotected sexual intercourse [1]. However, due to cultural and socio-economical as well as religious influence male infertility contributing to couple's inability to reproduce has been undermined and usually attributed to the female especially in developing countries [2]. It was reported by Uadia & Emopkae [2], that in Nigeria, male factor infertility accounts for up to 50% of all infertility cases and yet the female partners are being blamed for every form of childlessness. They also indicated that if proper diagnosis and checks are put in place, male infertility might cause over 70% of all infertility cases in Nigeria with rapid decrease in

normospermia (14.1%) and increasing cases of severe oligospermia (44.1%) and azoospermia (74.7%) in Nigeria.

Several factors are associated with infertility/male-infertility which includes genetic factors, environmental factors due to exposure to chemical contaminants cum endocrine disruptors, life style (e.g. smoking, alcohol consumption, etc), amongst others [3, 4, 5]. Major established causes of male infertility include absent of spermatozoa (azoospermia), reduced number of spermatozoa (oligozoospermia), reduced sperm motility (asthenozoospermia), reduced sperm vitality (necrozoospermia), abnormal sperm morphology (teratozoospermia) or any combination of these morphological distortions [6, 7]. However, male factor infertility has also been associated with sperm proteins expression or absence of these proteins in sperm or seminal plasma.

Therefore, there is a need to go beyond routine semen or sperm analysis, thus, the need to analysis the sperm proteins associated with male infertility/fertility such as osteopontin (OPN), Heat Shock Protein 70 (HSP*70*), Protein kinase A (PKA) and antioxidants such Glutathione Peroxidase (GPX) and Total Antioxidant Capacity (TAC) as well as lipid peroxidation marker like malonaldehyde (MDA). If some of these sperm proteins are not expressed or poorly expressed or probably over-expressed, it could also result in male infertility [2, 7]. Therefore, the aim of the study is to evaluate some sperm proteins, antioxidants capacity, testosterone and PSA in infertile with sperm cell deformities in Port Harcourt, Rivers State, Nigeria.

2. MATERIAL AND METHODS

2.1 Sample Size Determination

Sample size was calculated as 93 using the equation as described by Claran & Biswas [8] using the formula, sample size $=Z^2 P(1-P)/d^2$. Where Z= Standard normal variance =1.96, p= expected proportion of diseases in the population based on previous study, d = absolute error at 95% confidence interval = 0.05. The proportion of male-factor infertility was 6.5% as reported Sule et al. [9], a study carried out in Yenagoa, Bayelsa State. Therefore, a total of 93 semen and blood samples were collected from infertile males and 100 samples from fertile males (control) summating to 193 semen and blood samples.

2.2 Selection Criteria

A well-structured questionnaire was issued to all the participants to obtain demographic information, medical history and lifestyle after obtaining consent of participants.

2.2.1 Inclusion Criteria

Participants included in this work are those attending urology/fertility clinics, without history of hypertension, cardiovascular disorders, osteoporosis, diabetes mellitus or smoking. Omron digital blood pressure kit (Omron healthcare co., Ltd, Japan) was used to check the blood pressures of the subjects while glucose oxidase method was used to determine their fasting glucose state. Also, the control subjects recruited were males that established pregnancies within the period of this study with total sperm count of ≥ 20 x 10^6 cells/ml, normal sperm morphology and without bacterial infection of the semen at the time of investigation according to WHO criteria [10], while the infertile males were those that have failed to establish pregnancies after years and been clinically diagnosed of such. These subjects also have sperm count between 0 – 19 x 10^6 cells/ml.

2.2.2 Exclusion Criteria

Those excluded from the study were individuals that did not give their consent, semen leukocytes >1 x 10^6/ml, semen specimen collected without masturbation, no abstinence for at least 72 hours, and lifestyle like smoking, and alcohol consumption. In addition, participants with history of hypertension, cardiovascular disorders, osteoporosis, diabetes mellitus and prostate specific antigen (PSA) of >10ng/ml were also excluded.

2.3 Experimental Design

The study design is a case-controlled randomized study in which semen and blood samples were collected from case and control groups randomly amongst males visiting urology/fertility clinics/hospitals in Port Harcourt.

2.4 Subjects Characterization

The collection of semen and blood specimens was done for a period of 18 months (Nov., 2019 – April, 2021). A total of 276 males indicated interest to participate in the study of which 192 male subjects were recruited. Of the 193 subjects, 100 were fertile, normospermic (control) subjects while 93 were infertile males. In addition, the 93 infertile were further re-grouped into azoospermic and oligospermic subjects based on their respective total sperm counts. The azoospermic and oligospermic subjects were 40 and 53 in number respectively. The fertile males were those that established pregnancy within the period of the study. The infertile males were further classified in azoospermic and oligospermic groups depending on their total sperm count based and were re-grouped into asthenozoospermia (AZS), oligoasthenozoospermia (OAS), teratozoospermia (TZS), asthenoteratozoospermia (ATS) and oligoasthenoteratospermia (OAT) based on the morphology of their sperm cells under investigation using the WHO [10] criteria of classification.

2.5 Subjects Classification

Those classified as normozoospermia were control subjects with sperm concentration of $\geq$ 20 x 10^6/ml, progressively motile sperms $\geq$ 50% and normal sperm morphology $\geq$ 50%. Subjects with sperm concentration of ≤ 19 x 10^6/ml were grouped as oligozoospermia, those with <1.0 x 10^6/ml were grouped as azoospermia, those with progressively motile sperms ≤ 50% and normal sperm morphology > 30% ≤ 50% were classified as AZS while those with progressively motile sperms ≤ 50%, normal sperm morphology > 30% ≤ 50% and total sperm count ≤19 x 10^6/ml were grouped as OAS. More so, subjects with normal morphology of ≤ 30%, progressively motile sperms of $\geq$50% were grouped as TZS while those subjects with normal sperm morphology of ≤ 30% with progressively motile sperms of ≤ 50% were grouped as ATS. Finally, subjects with total sperm count of <19 x 10^6 cells/ml, normal sperm morphology of < 30% with progressively motile sperms of <50% were grouped as OAT. The classifications is based on WHO [10] criteria of classification.

2.6 Blood and Semen Sampling

Semen specimens were collected into universal sterile plastic containers by masturbation after an abstinence period of 72 hours while 5ml of venous whole blood samples were collected into plain bottles.

2.6.1 Sample Preparation

Semen specimens collected were placed on the bench at room temperature of 25^{0}C and allowed for 40 minutes to liquifaction before analyses. The liquefied semen samples were centrifuged at 4500rpm for 15 minutes as described by Conqer et al.[11] to obtain seminal plasma. The seminal plasma specimens were aliquoted into plain tubes and kept frozen at -70^{0}C prior to laboratory analysis. Whole blood samples were collected into plain bottles, allowed to stand for 30 minutes to clot, retracted and spun at 3500rpm for 10 minutes to obtain serum for the estimation of testosterone and prostate-specific antigen.

2.7 Equipment and Reagents

Equipment used includes Olympus binocular microscope, and neubauer haemocytometerwere used for sperm quality analysis, while Auto Elisa P microplate reader (Labtech) was used for the quantitative analysis of sperm proteins. Digital Olympus microscope with camera (Olympus, Japan) was used. Other equipment used include mermert oven (at 37^0C), universal bucket centrifuge model 320, Omron digital blood pressure kit (Omron healthcare co., Ltd, Japan), pipettes and microscopic glass slides and cover slips. Commercially available Osteopontin (OSP), Heat shock protein *70* (HSP70), and Protein-kinase A(PKA), Malondialdehyde (MDA),Prostate-specific Antigen (PSA), and testosterone (Testo) ELISA kits were purchased from Bioassay Technology Laboratory (Shangai, China).Glutathione peroxidase (GPX) ELISA kits and total antioxidant capacity (TAC) spectrophotometric kits were purchased from Elabscience (Houston, Texas, USA)and Fortress Diagnostics (Antrim, United Kingdom) respectively. Other reagents used include Haier thermocool deep freezer (China). Chemical of analytical grade (AR) were used in the preparation of some reagents.

2.8 Laboratory Analysis of Sperm Parameters

The semen volume was determined using graduated glass pasteur pipette while the pH was determined using combi-9 strip. Determination of viscosity was done as described by Vasan [12]. Sperm viability was determined as described by WHO [13] and Moratti et al. [14] using 10µL of 0.5% eosin Y in 0.9% aqueous sodium chloride solution. Total sperm count (TSC) was determined using Neubauer cell counter as described by WHO [13] and Ochei [15]. The estimate and quantitation of pus cells, epithelial cells, and motility of the sperm cells were done as described by WHO [15]. The morphology of the sperm cells was done using the methylene blue-eosin staining technique after incubation at 25^0C with trypsin for 10minutes described by WHO [15].

2.9 Assay of Biochemical Parameters

The specimens used were seminal plasma and serum specimens. All the parameters were assayed using seminal plasma except testosterone and PSA that were assayed in serum. HSP*70*, OPN and PKA, GPX, testosterone, and PSA were determined as described by Engvall & Perlmann [16] ELISA quantitative assay method while MDA was determined using ELISA method as described by Moron et al. [17]. More so, Total Anti-oxidant Capacity (TAC) was done using Colorimetric Method as Described by Mencci et al. [18].

2.10 Statistical Analysis

Statistical package used for data analysis were Graphpad Prism 8.0.2 (California, USA), and Statistical Package for Social Science (SPSS) version 23.0. Descriptive statistics used were mean and standard deviation while inferential statistics used include students't-test, Chi-Square, and One-Way ANOVA with Post-Hoc done with Tukey's multiple comparison analysis tests. Results were presented as Mean±Standard Deviation. Statistical significance was set at $p<0.05$.

3. RESULTS

3.1 Prevalence of Abnormal Sperm cell Morphology

The results of prevalence of normospermic, azoospermic and oligospermic subjects indicated 52%, 20.8%, and 27.1% respectively. The comparison using chi-square indicated no significant difference. Likewise, no significant differences were seen in that of asthenozoospermia, oligoasthenozoospermia, teratozoospermia, asthenoteratozoospermia

and oligoasthenoteratospermia infertile subjects with prevalence of 20.8%, 25.0%, 13.5%, 16.6%, and 11.4% respectively (table 1).

Table 1: Prevalence of Morphological Abnormal sperm cells in infertile males

Classification	No	Prev.	% Prev.	X^2	Pvalue
Sperm count					
a. Normospermic	100	0.520	52.0	1.00	0.607
b. Azoospermic	40	0.208	20.8		
c. Oligospermic	53	0.271	27.1		
Sperm Morphology					
a. Asthenozoospermic (AZS)	40	0.208	20.8	3.333	0.504
b. Oligoasthenozoospermic (OAS)	48	0.25	25.0		
c. Teratozoospermic (TZS)	26	0.135	13.5		
d. Asthenoteratozoospermic (ATS)	32	0.166	16.6		
e. Oligoasthenoteratospermic (OAT)	22	0.114	11.4		

Prev. = Prevalence

3.2 Results of Sperm Parameters in Infertile Males

The result of age and sperm parameters such as the ejaculatory sperm volume (ml) in the azoospermic, oligospermic, and normospermic subjects indicated no significant difference in the age of the subjects recruited. However, the results of motility, active motility, normal sperm morphology, and total sperm count indicated significant higher values in normospermic subjects compared against oligospermic subjects. On the other hand, the results of abnormal sperm morphology indicated significantly higher values in oligospermic subjects compared against normospermic subjects at $p<0.05$ (Table 2).

In addition, when AZS, OAS, TZS, ATS, and OAT subjects were considered, again, the age, pH, and sperm volume of the subjects were not significantly different. However, significantly higher values were observed in the results of motility, active motility, normal sperm morphology, and total sperm count in normospermic subjects compared against other groups with sperm cells deformities. On the other hand, significantly higher values of abnormal sperm morphology were observed in oligospermic subjects compared against normospermic subjects. However, significantly higher values were seen in the abnormal sperm morphology of AZS and OAS subjects compared against other forms of sperm cell deformities at $p<0.05$ (Table 3).

Table 2: Results of Sperm parameters of Azoospermic, oligospermic, Normospermic Subjects

Parameters	Azoo	Oligo	Normo	F(t)value	pvalue	Remark
Age (years)	40.80±5.39	39.30±7.199	39.22±7.03	0.4151	0.6615	NS
Sperm volume (mL)	2.47±0.96	2.48±1.33	2.63±1.51	F=0.1386	0.8707	NS
pH	7.85±0.43	7.92±0.27	7.96±0.29	F=0.8327	0.4382	NS
Motility (%)	-	$32.20±20.04^b$	$60.51±17.33^c$	t=6.302	<0.0001	S
Active Motility (%)	-	$20.68±18.47^b$	$45.86±21.59^c$	t=4.973	<0.0001	S
Norm. Morph. (%)	-	$68.88±34.89^b$	$82.55±25.21^c$	t=2.022	0.0469	S
Ab.Morph (%)	-	$32.00±30.06^b$	$17.49±15.71^c$	t=1.997	0.0496	S
TSC (10^6cells/mL)	-	$9.564±5.882^b$	$42.53±19.75^c$	t=8.141	<0.0001	S

KEY: Azoo= Azoospermic, Oligo= Oligospermic, Normo=Normospermic, Norm. Sperm Morph = Normal Sperm Morphology, Ab. Sperm Morph= Abnormal Sperm Morphology, TSC=Total Sperm Count. Tukey's Post Hoc: Within same row, values with different superscripts differ significantly at p=.05

3.3 Results of Sperm proteins, anti-oxidants Enzymes, MDA, Testosterone and

PSA in Azospermic, Oligospermic and Normospermic Subjects

The ANOVA results of sperm proteins in seminal plasma indicated significantly higher values were seen in HSP70 of oligospermic subjects compared azoospermic and normospermic subjects. The normospermic subjects also had higher values of HSP70 compared against azoospermic subjects. In addition, significantly higher values of OSP and MDA were observed in Azoospermic subjects compared against oligopermic and normospermic subjects while oligospermic subjects had higher values of OSP compared against normospermic subjects. PKA showed significantly lower value in azoospermic subjects compared against oligospermic and normospermic subjects. No significant difference was observed between normospermic and oligospermic subjects. More so, significantly lower values were in GPX, TAC and testosterone of azoospermic subjects compared against oligospermic and normospermic subjects. In addition, significantly lower values were seen again in oligospermic subjects compared at normospermic subjects. Finally, the results of PSA in azoospermic, oligospermic, and normospermic subjects were not significantly different.

Table 3: Results of Sperm Proteins, Anti-Oxidants, MDA, Testosterone and PSA in Azoospermic, Oligospermic and Normospermic Subjects

Parameters	Azoospermic	Oligospermic	Normospermic	Fvalue	pvalue	Remark
HSP70 (ng/mL)	8.30±4.41[a]	19.60±7.73[b]	12.17±4.59[c]	25.15	<0.0001	S
OPN (ng/mL)	7.45±2.09[a]	5.36±1.83[b]	4.05±1.90[c]	22.50	<0.0001	S
PKA (ng/mL)	5.35±1.56[a]	8.04±3.21[b]	7.54±3.04[b]	5.698	0.0047	S
GPX (ng/mL)	4.83±1.56[a]	6.73±1.82[b]	8.32±1.07[c]	45.17	<0.0001	S
MDA (ng/mL)	111.5±34.03[a]	91.87±33.64[b]	52.15±17.29[c]	42.53	<0.0001	S
TAC (mmol/L)	1.72±0.89[a]	2.07±0.88[a]	7.49±2.0[b]	147.4	<0.0001	S
‡TESTO (ng/mL)	1.26±0.65[a]	2.48±1.09[b]	4.91±1.41[c]	76.54	<0.0001	S
‡PSA (ng/mL)	5.89±3.14	4.91±2.57	4.17±3.07	2.472	0.0901	NS

Tukey Post-Hoc: Within same row, values with different superscripts differ significantly when various groups were compared. ‡Parameters were assayed in serum. S=Significant, NS=Not Significant @ p<0.05

When some sperm proteins in seminal plasma were considered in subject with sperm cells deformities, the ANOVA results of sperm proteins in seminal plasma indicated significantly lower values were seen in HSP70, PKA, MDA, and PSA of normospermic subjects compared against subjects whose sperm cells were deformed physiologically or morphologically. Furthermore, significantly lower values were seen in MDA of AZS, OAS, and TZS subjects compared against ATS and OAT subjects. More so, a significantly higher value was observed in PSA of OAT subjects when compared against normospermic subjects as well as AZS, OAS, TZS, and ATZ subjects. However, significantly lower values were seen in OSP, GPX, TAC, and testosterone of AZS, OAS, TZS, and ATS subjects when compared against normospermic subjects at p<0.05 (Table 5).

Table 4: Results of One-Way ANOVA of Age, Sperm parameters of Infertile Males with Sperm Cells Deformities

Parameters	Norm	AZS	OAS	TZS	ATS	OAT	Fvalue	pvalue	Remark
Age (years)	39.22±7.03	39.60±7.09	39.58±7.59	36.46±5.39	39.0±6.88	38.0±6.54	0.4646	0.8020	NS
Sperm volume (ml)	2.63±1.51	2.54±1.39	2.59±1.38	2.52±1.27	2.23±1.47	2.02±1.15	0.474	0.7950	NS
pH	7.96±0.29	7.95±0.22	7.89±0.25	7.92±0.18	7.94±0.25	7.91±0.30	0.233	0.9474	NS
% Motility	60.51±17.33^{a}	26.75±14.05^{b}	27.38±16.53^{b}	29.0±18.48^{b}	26.69±17.0^{b}	24.27±17.46^{b}	24.76	<0.0001	S
% Active Motility	45.86±21.59^{a}	15.60±10.96^{b}	15.92±12.46^{b}	12.69±11.83^{b}	11.75±11.33^{b}	12.55±13.05^{b}	23.83	<0.0001	S
% Norm Morph.	82.55±25.21^{a}	74.70±31.63^{a}	68.21±34.19^{a}	21.08±6.56^{b}	25.50±18.71^{b}	22.82±6.030^{b}	26.06	<0.0001	S
% Ab. Morph	17.49±25.71^{a}	25.30±31.63^{a}	31.71±34.08^{a}	79.69±6.13^{b}	74.50±18.71^{b}	77.18±6.030^{b}	25.99	<0.0001	S
TSC (10^{6}cells/ml)	42.53±19.75^{a}	10.11±5.92^{b}	9.34±5.92^{b}	16.19±19.68^{b}	16.62±18.43^{b}	8.36±6.65^{b}	25.18	<0.0001	S

Turkey's Post Hoc: Within same row, values with different superscripts differ significantly at p<0.05. Norm=Normospermic, AZS=asthenozoospermic, OAS=oligoasthenozoospermic, TZS=Teratozoospermic, ATS=Asthenoteratozoospermc, OAT=Oligoasthenoteratozoospermic, TSC=Total Sperm Count.

Table 5: Results of one-way ANOVA of sperm proteins, anti-oxidants, MDA, Testosterone, and PSA in subjects with Morphological Sperm Deformities

Parameters	Normo	AZS	OAS	TZS	ATS	OAT	Fvalue	pvalue	Remark
HSP70 (ng/ml)	12.17±4.58^{a}	17.39±3.62^{b}	16.41±3.74^{b}	18.11±3.71^{b}	15.78±4.11^{b}	18.20±3.99^{b}	9.335	<0.0001	S
OSP (ng/ml)	4.39±2.14^{a}	2.31±0.68^{b}	2.67±0.61^{b}	2.52±0.72^{b}	2.59±0.62^{b}	2.27±0.86^{b}	11.28	<0.0001	S
PKA (ng/ml)	7.541±3.04^{a}	11.44±4.28^{b}	10.98±3.57^{b}	10.34±3.11^{b}	10.60±2.77^{b}	13.57±3.21^{b}	9.071	<0.0001	S
GPX (ng/ml)	8.32±1.07^{a}	5.46±1.82^{b}	6.34±1.71^{b}	4.79±1.25^{b}	4.56±0.97^{b}	4.35±0.86^{b}	37.50	<0.0001	S
MDA (ng/ml)	52.15±17.29^{a}	90.11±32.97^{b}	99.92±40.04^{b}	109.2±33.65^{b}	83.44±31.63^{b}	129.1±29.74^{c}	20.17	<0.0001	S
TAC (mmol/L)	7.48±2.00^{a}	2.12±0.92^{b}	2.98±2.05^{b}	2.21±1.14^{b}	2.43±1.23^{b}	1.33±0.27^{b}	62.25	<0.0001	S
‡ TESTO (ng/ml)	4.91±1.41^{a}	2.64±0.95^{b}	2.392±1.02^{b}	2.30±1.14^{b}	2.08±0.95^{b}	1.96±0.68^{b}	31.36	<0.0001	S
‡ PSA (ng/ml)	4.17±3.07^{a}	5.53±2.84^{a}	5.26±2.59^{a}	5.82±2.75^{a}	6.49±2.71^{a}	7.67±2.13^{b}	3.845	0.0028	S

Turkey's Post Hoc: Within same row, values with different superscripts differ significantly at p<0.05. Normo =Normozoospermic, AZS =Asthenozoospermic, OAS = Oligoasthenozoospermic, TZS = Teratozoospermic, ATS = Asthenoteratozoospermic, OAT = Oligoasthenoteratozoospermic. ‡Parameters were assayed in serum.S=Significant @ p<0.05

4. DISCUSSION

The results indicated 20.8% and 27.1% as prevalence of azoospermic and oligozoospermic males respectively summating as 47.1% of male infertily. Our findings are in accordance with the report of Uadia & Emokpe [2], who reported prevalence of 42.6% as cases of male factor infertility in Southern Nigeria. In addition, our findings with reference to oligospermic status are similar with the reports of Anaezichukwuolu [19] and Odunvbun et al. [20], who reported prevalence of 22.8% and 25% respectively as infertile males with oligozoospermic status in Edo and Delta State respectively. However, the reports of Anaezichukwuolu [19], concerning the prevalence of azoospermic subjects of 11% contradicts our findings of 27.1% as observed in our study. Furthermore, the prevalence of infertile males with asthenozoospermia of 20.8% and oligoathenozoospermia of 25%, contradicts the results of Anaezichukwuolu ([19] but were similar to the findings of Green & Nwachuku [21] conducted in Port Harcourt. Anaezichukwuolu [19] reported prevalence of 7.3% and 2% for asthenozoospermia and oligoathenozoospermia respectively in a related study in Benin, Edo State while Green & Nwachuku [21] reported a prevalence of 20.1% as asthenozoospermia in their study done in Port Harcourt. The prevalence of teratozoospermia of 13.5%, asthenoteratozoospermia of 16.6% and oligoasthenoteratozoospermia of 11.4% observed in our study relates closely to the findings of Anaezichukwuolu (2016) who also reported 11.5% and 13.8% for teratozoospermia and oligoasthenoteratozoospermia respectively. Likewise, Green & Nwachuku [21], also gave similar report of 18.3% prevalence of teratozoospermia among infertile males in Port Harcourt.

The prevalence observed in our study indicates increase in the number of male affected with infertility issues in 2022 compared against studies done in 2016 – 2018 in the Niger Delta. These increases could also be environmental related since Rivers State/Port Harcourt is the hub of oil and gas exploration in the Niger Delta and Nigeria at large. There have been reported cases of oil spills, gas flaring, and contamination of seafoods and farmlands which in turn could exposure these subjects to contaminants that are mostly toxic and disruptors of endocrine function.

The result of significantly lower values observed in TSC, normal morphology, and active motility in oligospermic infertile males compared to normospermic fertile males are similar to the work done in Port Harcourt by Green & Nwachuku [21]. Similarly, significantly lower values of testosterone, TAC, and GPX and significantly higher values of MDA in seminal plasma of the azoospermic and oligozoospermic infertile males compared to normospermic fertile males concur with the findings of Waheed et al. [22], Parrish [23], and Fraczek et al. [24]. They all reported lower values of GPX and TAC in azoospermic and oligospermic subjects in their studies. The significantly higher values of HSP*70*, PKA and significantly lower values of OPN in azoospermic and oligospermic infertile male subjects are in accordance with the finding of Waheed et al. [22], Cao et al. [25], and Agarwal et al. [26]. Cao et al. [25] and Agarwal et al. [26], documented higher values of PKA and HSP*70* in oligospermic infertile males compared against fertile male while Waheed et al. [22], reported lower values of OPN in infertile subjects.

Also, the pattern of results observed in azoospermic and oligospermic infertile males are similar to those observed in AZS, OAS, TZS, ATS, and OAT infertile males. Cologar et al. [27], reported significant reduction in sperm volume of ATS infertile males but not in OAT subjects. The findings of Cologar et al., [27], contradict our findings, which indicated no significant difference in the ejaculatory sperm volume compared against normospermic fertile subjects. Cologar et al. [27], further documented significantly reduced sperm count, motility, and sperm morphology only in ATS and OAT subjects which again contradict our findings. In our study, significantly lower values of sperm count, motility, and active motility

were not only seen in ATS and OAT subjects but in AZS, OAS, TZS, ATS, and OAT infertile subjects. In addition, the significantly higher values of abnormal sperm cells morphology in TZS, ATS, and OAT subjects (table 4) are in accordance with the findings of Cologar [27].

The significantly lower and higher values of TAC and MDA respectively in our findings (table 4, table 5) are in line with the reports of Nabil et al. [28] and Khosrowbeygi et al. [29]. They reported significantly lower and higher values of TAC and MDA respectively in infertile azoospermic and oligospermic subjects as well as in AZS, ATS and OAT subjects. Our results (table 5) further indicated highest and lowest values of MDA and TAC in OAT subjects. In addition, the significantly higher values of HSP*70*, PKA and significantly lower values of OPN and testosterone in infertile males with sperm cells deformities observed in our study are in accordance with the findings of Moghadam et al. [30], Blommaert et al. [31], and Waheed et al. [22] respectively. Waheed et al. [22], reported significantly higher levels of OPN in males with higher fertility compared against sub-fertility male subjects.

PSA results showed no significant differences except in OAT subjects (table 5) were significantly higher values were seen. Our PSA result is in agreement with the findings of Shang et al. [32], who documented that inflammatory reaction of the prostate are usually observed in oxidative stress cases. They further documented reduced sperm viability and progressive sperm motility in infertile males compared against control subjects. PSA is a protein used in the diagnosing the enlargement/hyperplasia of the prostate. Therefore, significant increase in PSA is an indication of possible prostate hyperplasia. The poor sperm viability, motility and deformities observed in the OAT subjects could also be due to the prostate malfunction. The prostate contributes majorly to the seminal plasma and if the environment is under stress, it could affect the quality of sperm cells produced resulting in deformed sperm cells.

The significantly lower values of GPX and TAC could be an indication of oxidative stress in the seminal plasma of the infertile males. GPX is an enzymatic form of antioxidant component of the sperm protein of spermatozoa as well as seminal plasma of the semen. It has been documented to be one of the most versatile intracellular enzymatic form of anti-oxidants involved in the mitigation or elimination of reacting oxygen (ROS) and reactive nitrogen species (RNS) in seminal plasma. TAC measures the comprehensive anti-oxidant activities of the seminal plasma including enzymatic and non-enzymatic anti-oxidant activities. Therefore, the significant reductions in these anti-oxidants parameter suggest severe oxidative stress (OS) of the sperm cells. In addition, the significant higher values of MDA possibly indicate lipid peroxidation of the spermatozoa in the oligospermic infertile male. Spermatozoa are readily susceptible to oxidative stress due to limited cytoplasmic enzymatic repair mechanism to oxidative stress. Also, owing to the rich polyunsaturated fatty acid (PUFAs) in their cell membrane, spermatozoa are very susceptible to oxidative stress inducing cell damages and hence lipid peroxidation. The lipid peroxidation may induce intracellular energy loss, decreased sperm viability, and increased mid-piece sperm morphological deformities. Therefore, the significantly reduced sperm count and morphological defects observed in the oligospermic infertile males could also be due to dead sperm cells and oxidative induced distortions.

The results of MDA and TAC in OAT subjects further suggest that the oxidative stress induced damages are most severe in OAT subjects compared to other form of sperm cells defects. Oxidative stress has also been responsible for the lower values of testosterone in the infertile subjects as seen in our results. The production of testosterone is dependent of the optimal activities of the leydig and sertoli cells of the testes. OS have been reported to infer with androgen metabolism by hindering steriodogenesis and therefore affecting spermatogenesis.

The significant increases seen in HSP*70* of the oligospermic subjects compare against control subjects (table 4 and table 5) is in line with the findings of Moghadam et al. [30]. They also reported significantly higher values of HSP70 in oligospermic subjects. Likewise, the significantly higher values in PKA in the oligospermic and azoospermic subjects concur with the documentations of Blommaert et al. [31], who also reported significantly higher values of Anchor kinase activated protein (AKAPs) and PKA in oligospermic and azoospermic subjects. The higher values of HSP*70* could be due to their protective function of mitigating oxidative stress or distress associated with cells. HSP*70* exist in the plasma in relatively low concentrations but their concentration could increase exponentially due to oxidative stress beyond the physiological limit. Therefore, their increase could be targeted towards preventing sperm cells deformation or death by free radicals or reactive oxygen species. The HSPs including HSP*70* are known to conserve and protect cells such as spermatozoa that are prone to lipid peroxidation and degradation. On the other hand, PKA are involved in the regulation of spermatozoa motility and interacts with a several other kinase proteins in the fibrous sheath. Anchor kinase activated proteins (AKAPs) alongside PKA are the major fibrous proteins that acts on glycolytic enzymes and several phosphorylation signal in the production of ATPs in flagella motion of the spermatozoa. Therefore, higher values of PKA seen in the oligopermic subjects could be as a result of reduced spermatozoa concentration or poor motility of spermatozoa, which in turn signals the up-regulation of AKAPS genes inducing increased synthesis of the proteins in the plasma and seminal fluid. In previous research as reported by Brown et al.[33], Moss et al. [34], Krisfalusi et al. [35], also indicated in their respective study increased activities of AKAPs and PKA proteins in semen of males with sperm cells deformities that might affect motility.

5. CONCLUSION

Sperm proteins are clearly altered in individuals with abnormal sperm cells morphologies. HSP70, PKA were severely increased while OPN, TAC, and GPX were severely decreased in the seminal plasma of infertile males with abnormal sperm cells. Testosterone and PSA in blood plasma were significantly low and high respectively. These indicate oxidative stress plays significant role in sperm cell deformities and fertility.

ETHICAL APPROVAL AND CONSENT

Ethical approval was granted from the Ethical Review Boards of the Rivers State Government through the ministry of Health with approval no MH/PRS/391/VOL.2/726 and MH/PRS/391/VOL.2/727 for Teaching Hospital and Primary Healthcare Centres respectively. In addition, oral and written informed consents were obtained from all the participants. We therefore, declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

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ABBREVIATIONS

ATS	=	Asthenoteratozoospermia
AZS	=	Asthenozoospermia
GPX	=	Glutathione Peroxidase
MDA	=	Malonaldehyde
Norm	=	Normospermia
OAS	=	Oligoasthenozoospermia
OAT	=	Oligoasthenoterotozoospermia
OS	=	Oxidative stress
PSA	=	Prostate-Specific Antigen
TAC	=	Total Antioxidant capacity
TSC	=	Total Sperm Count
TZS	=	Teratozoospermia