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

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Authors' contributions

This work was carried out in collaboration among all authors. Author EASB designed the study and wrote the protocol. Author IE performed the statistical analysis. Author AMA wrote the first draft of the manuscript and managed the literature searches. Authors HB and ABC managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

Aim: Infertility is a global problem affecting both males and females. It is a condition with psychological, economic, and medical implications resulting in trauma and stress, particularly in a society where there is a strong emphasis on childbearing. The study therefore aims to investigate the pattern of some sperm proteins, anti-oxidants, and prostate-specific antigen in the seminal plasma of infertile males with sperm cell deformities.
Study Design: The study is a case-control study designed to investigate semen parameters and sperm proteins in infertile males with sperm cell 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 between the periods of September 2019 and Feb. 2022. However, some of the Laboratory investigations were done in the Chemical Pathology Unit and Medical Microbiology Unit of the University of Port Harcourt Teaching Hospital, Port Harcourt. The Study was between the periods of September 2019 and Feb. 2022.

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Methodology: A total of 193 male subjects participated in the study. These subjects were grouped into normospermic males (100), azoospermic (40) and oligospermic males (53). Based on their sperm cell morphology and active motility, they were further classified into asthenozoospermic (40), oligoasthenozoospermic (48), teratozoospermic (26), asthenoteratozoospermic (32), and oligoasthenoteratozoospermic (22). HSP70, PKA, MDA, TAC, and GPX (in seminal plasma), PSA, and testosterone (in serum) were analyzed using ELISA while TAC was done using spectrophotometric methods. Results obtained were statistically analyzed using GraphPad Prism and SPSS.
Results: Sperm motility, TSC, and normal sperm morphology were poor in oligospermic subjects but were very severe in OAT subjects. HSP70 and PKA were significantly increased while OPN, TAC, and GPX were significantly decreased in the seminal plasma of infertile males with abnormal sperm cells. Testosterone and PSA in blood plasma were significantly lower and higher respectively.
Conclusion: Oxidative stress played a 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.

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.

1. INTRODUCTION

Infertility is a global problem affecting both males and females. It is a condition with psychological, economic, and medical implications resulting in trauma and stress, particularly in a society where there is a strong emphasis on childbearing [1]. Infertility is a disease of the reproductive system defined by the failure to achieve a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse [1]. However, due to cultural, socio-economical, and religious influence, male infertility 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 not put in place, male infertility might cause over 70% of all infertility cases in Nigeria with a rapid decrease in normospermia (14.1%), increasing cases of severe oligospermia (44.1%), and azoospermia (74.7%).

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

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

2. MATERIALS AND METHODS

2.1 Sample Size Determination

The sample size of infertile subjects to be recruited was calculated as 93 using the equation 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 by 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 a 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 the 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 a 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 with a total 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 lifestyles like smoking, and alcohol consumption. Individuals with known infertility case like varicocoele, obstruction of seminal ducts, and cryptorchidism were also not included in this study. In addition, participants with a 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 193 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 into azoospermic and oligospermic groups depending on their total sperm count based and were re-grouped into asthenozoospermia (AZS), oligoasthenozoospermia (OAS), teratoz-oospermia (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 a sperm concentration of ≤ 19 x 10^6/ml were grouped as oligozoospermia, and 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%, and progressively motile sperms of ≥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 a 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 classification is based on WHO [10] criteria.

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 liquefaction before analyses. The liquefied semen samples were centrifuged at 4500rpm for 15 minutes as described by Conquer et al. [11] to obtain seminal plasma. The seminal plasma specimens were aliquoted into plain tubes and kept frozen at -20^{0}C before 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 a Neubauer hemocytometer was used for sperm quality analysis, while an Auto Elisa P microplate reader (Labtech) was used for the quantitative analysis of sperm proteins. A Digital Olympus microscope (Olympus, Japan) was also used. Other equipment used includes a mermert oven (at 37^{0}C), universal bucket centrifuge model 320, Omron digital blood pressure kit (Omron healthcare co., Ltd, Japan), pipettes, microscopic glass slides and coverslips. Commercially available Osteopontin (OSP), Heat shock protein *70* (HSP70), 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 anti-oxidant 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) and chemical of analytical grade (AR).

2.8 Laboratory Analysis of Sperm Parameters

The semen volume was determined using graduated glass Pasteur pipettes while the pH was determined using combi-9 strips. Determination of viscosity was done as described by Vasan [12]. Sperm viability was determined as described by WHO [10,13] 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 the Neubauer cell counter as described by WHO [13] and Ochei & Kolhatkar [15]. The estimate and quantitation of pus cells, epithelial cells, and motility of the sperm cells were done as described by WHO [13]. The morphology examination of the sperm cells was done using the methylene blue-eosin staining technique after incubation at 25^{0}C with trypsin for 10minutes described by WHO [13].

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 which 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 the ELISA method as described by Moron et al. [17]. More so, Total Anti-oxidant Capacity (TAC) was done using the Colorimetric Method as Described by Mencci et al. [18].

2.10 Statistical Analysis

Statistical packages 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 a prevalence of 20.8%, 25.0%, 13.5%, 16.6%, and 11.4% respectively (Table 1).

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 significantly higher values in normospermic subjects compared to oligospermic subjects. On the other hand, the results of abnormal sperm morphology indicated significantly higher values in oligospermic subjects compared to normospermic subjects at *P*=.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 to other groups with sperm cell 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 to other forms of sperm cell deformities at *P*=.05 (Table 3).

Table 1. Prevalence of morphological abnormal sperm cells in infertile males

Classification	No	Prev.	% Prev.	X^2	Pvalue
1. 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

Table 2. Results of Sperm parameters of Azoospermic, oligospermic, Normosper-mic subjects

Parameters	Azoospermia	Oligospermia	Normospermia	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: Norm. Sperm Morph = Normal Sperm Morphology, Ab. Sperm Morph= Abnormal Sperm Morphology, TSC=Total Sperm Count. Tukey's Post Hoc: Within the 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 to azoospermic and normospermic subjects. The normospermic subjects also had higher values of HSP70 compared to azoospermic subjects. In addition, significantly higher values of OSP and MDA were observed in azoospermic subjects compared to 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 to oligospermic and normospermic subjects. In addition, significantly lower values were seen again in oligospermic subjects compared to normospermic subjects. Finally, the results of PSA in azoospermic, oligospermic, and normospermic subjects were not significantly different.

When some sperm proteins in seminal plasma were considered in subjects with sperm cell 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 the MDA of AZS, OAS, and TZS subjects compared to ATS and OAT subjects. In addition, a significantly higher value was observed in PSA of OAT subjects when compared to normospermic subjects as well as AZS, OAS, TZS, and ATS subjects. However, significantly lower values were seen in OSP, GPX, TAC, and testosterone of AZS, OAS, TZS, and ATS subjects when compared to normospermic subjects at $p<0.05$ (Table 5).

4. DISCUSSION

The results indicated 20.8% and 27.1% as prevalence of azoospermic and oligozoospermic males respectively summating as 47.1% of male infertilities. Our findings are in support of the report of Uadia & Emokpe [2], who reported a prevalence of 42.6% as male factor infertility in Southern Nigeria. In addition, our findings regarding oligospermic status are similar to the reports of Anaezichukwuolu [19] and Odunvbun et al. [20], who reported prevalences of 22.8% and 25% 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 AZS of 20.8% and OAS 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 prevalences of 7.3% and 2% for AZS and OAS respectively in a related study in Benin, Edo State while Green & Nwachuku [21] reported a prevalence of 20.1% as AZS in

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 the same row, values with different superscripts differ significantly when various groups were compared. ‡Parameters were assayed in serum. S=Significant, NS=Not Significant at P=.05

Table 4. Results of one-way ANOVA of age, sperm parameters of infertile males with sperm cell deformities

Parameters	Normospermic	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 the same row, values with different superscripts differ significantly at P=.05. 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 cell deformities

Parameters	Normospermic	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 the same row, values with different superscripts differ significantly at P=.05. AZS =Asthenozoospermic, OAS = Oligoasthenozoospermic, TZS = Teratozoospermic, ATS = Asthenoteratozoospermic, OAT = Oligoasthenoteratozoospermic. ‡Parameters were assayed in serum.S=Significant at P=.05

their study done in Port Harcourt. The prevalence of TZS of 13.5%, ATS of 16.6%, and OAT of 11.4% observed in our study relate closely to the findings of Anaezichukwuolu (2016) who also reported 11.5% and 13.8% for TZS and OAT respectively. Likewise, Green & Nwachuku [21], also gave a similar report of an 18.3% prevalence of teratozoospermia among infertile males in Port Harcourt.

The prevalence observed in our study indicates an increase in the number of males affected with infertility in 2022 compared to 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 seafood and farmlands which in turn could exposure these subjects to mostly toxic contaminants 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 support of 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 to fertile males 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 is similar to those observed in AZS, OAS, TZS, ATS, and OAT infertile males. Cologar et al. [27], reported a 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 to normospermic fertile subjects. The discrepancy between our result and that of Cologar et al. [27] regarding the seminal volume could be that our study did not include infertile males with known infertility cases like varicocoele, obstruction of seminal ducts, and cryptorchidism which was not very clear if the subjects they selected probably have one or any of the aforementioned. 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 cell morphology in TZS, ATS, and OAT subjects (Table 4) support 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 higher and lower 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 cell deformities observed in our study support 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 to infertility male subjects.

PSA results showed no significant differences except in OAT subjects (table 5) where significantly higher values were seen. Our PSA result is in agreement with the findings of Shang et al. [32], who documented that inflammatory reactions of the prostate are usually observed in oxidative stress cases. They further documented reduced sperm viability and progressive sperm motility in infertile males compared to control subjects. PSA is a protein used in diagnosing the enlargement/hyperplasia of the prostate. Therefore, the 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 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 forms 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 parameters suggest severe oxidative stress (OS) in the sperm cells. In addition, the significantly 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 damage are most severe in OAT subjects compared to other forms of sperm cell 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 on the optimal activities of the leydig and sertoli cells of the testes. OS has been reported to infer with androgen metabolism by hindering steriodogenesis and therefore affecting spermatogenesis.

The significant increase seen in HSP70 of the oligospermic subjects compared to 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 documentation 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 toward preventing sperm cell 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 is involved in the regulation of spermatozoa motility and interacts with 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 signals in the production of ATPs in the flagella motion of the spermatozoa. Therefore, higher values of PKA seen in the oligopermic subjects could be due to 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 the semen of males with sperm cell deformities might affect motility.

5. CONCLUSION

Sperm proteins were altered in individuals with abnormal sperm cell morphologies. HSP70 and PKA were significantly increased while OPN, TAC, and GPX were significantly 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 a significant role in sperm cell deformities and fertility.

DISCLAIMER

The products used for this research are commonly and predominantly used products in

our area of research and country. There is no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by the personal efforts of the authors.

CONSENT AND ETHICAL APPROVAL

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 following the ethical standards laid down in the 1964 Declaration of Helsinki.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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