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Research Article

Evaluating the Effects of Life styles and History of Exposure to Radiation on Levels of Significance and Severity of Sperm DNA Damage among Males with Infertility Using 8-Hydroxydeoxyguanosine (8-OHDG) as a Marker

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Abstract

Background: Sperm DNA damage has been established as a known cause of male infertility and severe sperm DNA damage has been linked to failure of some assisted forms of reproductive techniques. While there are different sources of DNA damage, Reactive oxygen species (ROS) are the most clinically relevant sources and this can be quantified by seminal 8-OHDG assay.

This study evaluated the effects of lifestyles and exposure to radiation on sperm DNA damage.

Methods: This was a descriptive cross sectional study of 120 males with at least one SFA parameter abnormalities as test subjects and 120 males with normal SFA results as controls. Seminal 8-OHDG was assayed as a marker of sperm DNA damage using ELISA method.

Results: Significant sperm DNA damage, taken as seminal 8-OHDG levels ≥20.45ng/ml was statistically more associated with smoking, P = 0.001 vs 0.913, Alcohol consumption, P= 0.001 vs 0.759, Exposure to radiation, including mobile phones, P = 0.05 vs 0.699, among test subjects than controls respectively, but not associated with herbal concoction consumption, P=0.201 vs 0.456. Severe sperm DNA damage, taken as seminal 8-OHDG ≥ 26.6ng/ml corresponding to DNA fragmentation index > 30%, was associated with alcohol consumption (P = 0.010), cigarette smoking (P = 0.001), STI (P = 0.027) while exposure to radiation especially from mobile phones was not associated with severe sperm DNA damage (P = 0.797) among test subjects.

Conclusion: Cigarette smoking, alcohol consumption, STI and keeping mobile phones in the pockets were associated with significant and all except histories of exposure to radiation were.

Keywords: *Sperm DNA Damage; Male Infertility; 8-Hydroxyl Deoxyguanosine; Lifestyle*

Abbreviations

ANOVA: Analysis of Variants; ARTU: Artificial Reproductive Technology Unit; ATP: Adenosine Triphosphat; CLSI: Clinical and Laboratory Standards Institute; CT: Computed Tomography; DFI: DNA Fragmentation Index; DNA: Deoxyribonucleic Acid; ELISA: Enzyme Linked Immunosorbent assay; GOPD: General Outpatient Department; ICSI: Intracytoplasmic Sperm Injection; IUI: Intrauterine Insemination; IVF: In vitro- Fertilization;

MRI: Magnetic Resonance Imaging; ROS: Reactive Oxygen Species; SFA: Seminal Fluid Analysis; SPSS: Statistical Package for the Social Sciences; STI: Sexually Transmitted Infection; UITH: University of Ilorin Teaching Hospital; WHO: World Health Organisation; 8-OHDG: 8-Hydoxydeoxyguanosine

Introduction

The ability of cells to replicate and function normally is dependent on the integrity of the deoxyribonucleic acid (DNA). DNA is responsible for the transfer of genetic information from parent cells to their progenies. However, when the DNA integrity is compromised, cell death (apoptosis) or mutations with loss of functions results [1].

In the spermatozoa, DNA damage may transfer mutations during fertilizations [2-4]. This may lead to infertility or early termination of pregnancy, depending on the degree of sperm DNA damage [5].

Sperm DNA integrity is ensured normally by compact organisation of the nuclear chromatin into a tightly packaged dense and insoluble protamine-bound structure, stable enough to withstand the rigours of all the stages between deposition of semen in female genital tract, post-copulation and immediate post-fertilization stage i.e. sperm transportation, capacitation, fertilization and acrosomal reaction [1]. The delicate intricate arrangement of the chromatin makes DNA within it susceptible to damage.

Spermatozoa DNA damage results from exposure to excessive free radicals/reactive oxygen species, which are mostly generated from effects of urbanization and industrialization. Activities such as exposure to radiations, petrochemicals, herbicides, fertilizers, alcohol consumption and cigarette smoking etc. increase reactive oxygen species (ROS) in the semen, causing peroxidation of the lipid-rich spermatozoa and most importantly damage to the sperm DNA, a major cause of male infertility [6-8].

Sperm DNA damage is a common cause of high failure rate in In-vitro fertilization and Intra-uterine Insemination [9] leading to huge financial losses and emotional trauma in infertile couples and the attending physicians. Therefore, quantitation of the degree of sperm DNA damage, to determine the levels of significance and severity of damage, before expensive procedures such as Artificial Reproductive Techniques (ARTs), are embarked upon, will save cost and improve results, especially now that it has been found that patients with severe sperm DNA will benefit from Intra-cytoplasmic Sperm Injection (ICSI) rather than IVF and IUI.

The human body has enzymatic and non-enzymatic anti-oxidant system to keep ROS within the acceptable limits. The enzymatic anti-oxidants include superoxide dismutase, glutathione peroxidase and catalase while non-enzymatic anti-oxidants includes vitamins C and E. Exposure to radiations such as X-ray, computed tomography (CT) scan, Magnetic Resonance Imaging (MRI) and close body contacts with phones, cigarette smoking, alcohol consumptions, sexually transmitted infections (STIs) generates ROS that may overwhelm the body's total antioxidant capacity, predisposing it to deleterious effects of its highly oxidative effects.

This study aimed to evaluate the effects of life style, relevant medico-surgical histories and exposure to radiations, particularly investigative radiological modalities such as x-ray, CT scan and MRI, and mobile phones on levels of significance and severity of sperm DNA damage among infertile males.

Materials and Methods

The study was conducted at the Department of Chemical Pathology and Immunology, University of Ilorin Teaching Hospital (UITH), Kwara State, Nigeia. The hospital receives referrals of infertile male patients from Kwara State and other neighbouring States. The Hospital has a General Outpatient Department (GOPD) which serves as a referral point for infertile couples and Specialist Gynaecological and Urology clinics attending to female and male infertility cases respectively, as well as an Artificial Reproductive Technology Unit (ARTU). The Department of Microbiology and ARTU receives request for semen fluid analysis (SFA) of about 400/year from Gynaecology, Urology and GOPD Clinics in the hospital as part of evaluation of infertile couples.

The study population included male partners of infertile couples who were requested to carry out seminal fluid analysis (SFA) at the Microbiology Laboratory UITH and at the ARTU.

This was a descriptive cross-sectional study of consecutive infertile male partners of infertile couples.

Study was conducted using the consecutive sampling method.

The minimum sample size required for the study was estimated using the Fisher formula [10]

Given as: $n = \frac{Z^2pq}{d^2}$

n = The desired minimum sample size

z = The standard normal deviation usually set at 1.96 which corresponds to 95% confidence interval.

p = The prevalence of male infertility in the target population from previous study. This was estimated to be 8.45% [11]

q = The proportion in the target population who do not have a particular characteristic, i.e.

q = 1 – p = 1 – 0.0845 = 0.9155,

d = Tolerable margin of error, an observed difference of 5% was taken as being significant.

Therefore, n= $\frac{1.96^2 \times 0.0845 \times 0.901}{0.05^2}$ =119

The sample size was 120

120 Participants with normal semen parameters according to WHO guideline served as control.

Study participants were consenting male patients who were referred from Gynaecology, Urology and General Out-Patient Department (GOPD) clinics to the Medical Microbiology laboratory or ARTU for SFA. Patients found to have at least one defective semen parameter were recruited as subjects while patients with normal semen parameters were recruited as controls.

Recruitment into the study was preceded by obtaining an informed consent. The process was repeated for consecutive patients until the required sample size was achieved.

Included in this study were all consenting male partners of infertile couples. Those with at least one defective semen parameter after seminal fluid analysis (SFA) were recruited as subjects while those with normal semen fluid analysis parameter were regarded as controls.

- Patients with abnormalities like cryptorchidism (undescended testes) or atrophic testes.
- Patients with Azoospermia (absence of spermatozoa in the semen).
- Patients with Aspermia who are unable to produce semen.

Written consent for inclusion into the study was obtained after explanation of the study and the procedure before samples were taken from the patients. Written permission were sought and obtained from the Heads of Chemical Pathology and immunology and Medical Microbiology of UITH. Clearance was obtained from the Ethical Committee of UITH before commencing the study.

Semen samples were collected by the patients from their respective homes using masturbation into a sterile, wide mouthed container, after at least 72 hours (3 – 4 days) of sexual abstinence. The samples were transported from their respective homes to the laboratory within 1hour of collection, to preserve the viability of the spermatozoa. During transportation to the laboratory samples were kept as much as possible close to the body temperature, this is best achieved by placing the container inside a plastic or polythene bag and transporting it in the front pocket of the person's clothing.

Samples were allowed to liquefy at room temperature (25 - 30ºc) monitored by thermometer fitted on the wall inside the laboratory, for at least 45 minutes. After liquefaction, samples were analysed for volume, pH, sperm count, motility, morphology and viability (where necessary). The basic laboratory procedures for seminal fluid analysis (SFA) was according to WHO guideline [12].

Samples were centrifuged for 20 minutes at 1000×g (validated by a timed stop watch). The sediments were collected and divided into aliquots and stored at -80°C inside a freezer (Thermofisher, UK, located in the Rotavirus Research Lab.) monitored by 12 hourly temperature chart of automatic factory fitted thermometer, for not more than 6months to avoid the loss of bioactivity and contamination. Semen samples that are found to be normospermic, according to WHO criteria, were taken as controls.

Laboratory procedures were in two phases, which include: 1. Sperm DNA isolation and 2. Sperm DNA damage quantification.

Protocol for sperm DNA extraction using the zymo kit by bio techniques [13]

Add 200 ul Trizol reagent to 200 ul of sperm cells in an Eppendorf tube.

Add 20 ul of Proteinase K to the mixture.

Incubate the mixture to 60°c and vortex the mixture intermittently to disrupt and solubilize the material for an hour.

Add 500 ul of ethanol (95 - 100%) to the homogenized mixture and vortex for 30 seconds.

Transfer the mixture into a Zymo Spin™ IICR Column in a Collection Tube and centrifuge at 8000rpm for 2 minutes.

Transfer the column into a new collection tube and discard the flow-through and reassemble the column into the collection tube.

Add 700 ul Direct-zol™ DNA Wash 1 to the column and centrifuge for 2 minutes. Discard the flow-through and reassemble the column into the collection tube.

Add 700 ul Direct-zol™ DNA Wash 1 to the column and centrifuge for 2 minutes. Discard the flow-through and reassemble the column into the collection tube.

To ensure complete removal of the wash buffer centrifuge the reassembled tubes for 3 minutes.

Carefully, transfer the column into a nuclease-free tube.

To elute DNA, 50ul of Direct zol™ Elution Buffer was added directly to the column matrix and centrifuged for 1 minute.

Sperm dna damage quantification

The OxiSelect™ Oxidative DNA Damage ELISA kit (produced by Cell Biolabs, Incorporated, 7758 Arjons Drive San Diego, CA 92126) was used [14]. It is a competitive enzyme immunoassay developed for rapid detection and quantitation of 8-hydroxydeoxyguanosine (8-OHdG) in urine, serum, or other cell or tissue DNA samples. Among numerous types of oxidative DNA damage, the formation of 8-hydroxydeoxyguanosine (8-OHdG) is a ubiquitous marker of oxidative stress. The quantity of 8-OHdG in unknown sample is determined by comparing its absorbance with that of a known 8-OHdG standard curve. The kit has an 8-OHdG detection sensitivity range of 100 pg/mL to100ng/mL. Each kit provides sufficient reagents to perform up to 96 assays, including standard curve and unknown samples.

Sperm DNA samples preparation

- DNA was extracted from Sperm cell samples as described above.
- The extracted DNA was then dissolved in water at 1 - 5 mg/mL.
- The DNA samples were then converted to single-stranded DNA by incubating the samples at 95ºC for 5 minutes and rapidly chilling on ice.
- The DNA samples were then digested to nucleosides by incubating the denatured DNA with 5 - 20 units of nuclease P1 (previously reconstituted in the manufacturer's recommended buffer) for 2 hrs at 37ºC in a final concentration of 20 mM Sodium Acetate, pH 5.2.
- Then 5 - 10 units of alkaline phosphatase (previously reconstituted in the manufacturer's recommended buffer) was added, plus sufficient Tris buffer to a final concentration of 100mM Tris, pH 7.5, and incubated for 1 hr at 37ºC.
- The reaction mixture was then centrifuged for 5 minutes at 6000 x g and collected the supernatant for use in the ELISA.

Assay principle

The OxiSelect™ Oxidative DNA Damage ELISA kit is a competitive ELISA method for the quantitative measurement of 8-OHdG. The unknown 8-OHdG samples or 8-OHdG standards were first added to an 8-OHdG/BSA conjugate preabsorbed microplate. After a brief incubation, an anti-8-OHdG monoclonal antibody is added, followed by an HRP conjugated secondary antibody. The 8-OHdG content in unknown samples was determined by comparison with predetermined 8-OHdG standard curve.

Assay procedure

- All reagents were prepared and mixed thoroughly before use. High content 8-OHdG samples were diluted at least 10 - 20 folds in Assay Diluent.
- 50 µL of unknown samples or 8-OHdG standard to the wells of the 8-OHdG Conjugate coated plate and incubated at room temperature for 10 minutes on an orbital shaker.
- Then 50 µL of the diluted anti-8-OHdG antibody was added to each well and incubated at room temperature for 1 hour on an orbital shaker.

- The microwell strips were thereafter washed 3 times with 250 µL 1X Wash Buffer per well with thorough aspiration between each wash. After the last wash, the wells were emptied and the microwell strips were tapped on absorbent pad or paper towel to remove excess 1X Wash Buffer.
- Then 100 µL of the diluted Secondary Antibody-Enzyme Conjugate were added to all wells.
- And then incubated at room temperature for 1 hour on an orbital shaker.
- The microwell strips were then washed 3 times according to step 4 above and proceed immediately to the next step.
- The substrate solution was warmed to room temperature and added 100 µL of Substrate Solution to each well, including the blank wells and incubated at room temperature on an orbital shaker. Actual incubation time may vary from 2 - 30 minutes.
- The enzyme reaction ended by adding 100 µL of Stop Solution into each well, including the blank wells and the results were read immediately (colour will fade over time).
- The absorbance of each microwell was read on a spectrophotometer using 450 nm as the primary wave length.

Quality control

The performance of the equipment, reagents, analytical method and analyst was validated by running control with each batch of samples. This was done using mean, standard deviation and coefficient of variation. The control samples were obtained from the same reagent kit's manufacturer.

Estimation of severity of sperm DNA damage

A previous study established a relationship between sperm DNA damage estimation using sperm DNA fragmentation index (DFI%) and seminal 8-OHDG concentration (in ng/ml), where sperm DFI of 21.73 ± 9.49% was equivalent to seminal 8-OHDG of 19.27 ± 5.01 ng/ml [15]. Sperm DFI of > 30% is accepted as cut-off point for severe sperm DNA damage. Seminal 8-OHDG in ng/ml equivalent to Sperm DFI of 30% was estimated using the above stated relationship (Sperm DFI 21.73% ≈ 19.27 ng/ml seminal 8-OHDG).

Statistical analysis

Statistical analysis was done with the Statistical Package for Scientific Solutions (SPSS) version 20.0 (SPSS-IBM, USA). Normally distributed data was expressed as mean ± SD, while non-gaussian distributed data was expressed as median and interquartile range. Non-parametric statistical methods (median, interquartile range, Mann-Whitney U test) were used to analyse non-gaussian distributed data. ANOVA was used for multiple comparisons of mean. P-value ≤ 0.05 was considered significant.

Results and Discussion

A total of 120 males who had abnormal SFA results, comprising low sperm counts, low motility or both were recruited for this study as test subjects and 120 males with no sperm count or motility abnormalities were recruited as controls. The mean age of the test subjects was 36.22 ± 7.56 while the mean age of control was 35.84 ± 6.27 years as shown in table 1. The test subjects and controls were mainly civil servants, constituting 56% and 50%, respectively and were educated to tertiary level. The socio-demographic distribution is as shown in table 1 below.

Variables	Subjects	Controls
	N (%)	N (%)
Age Group		
21 – 30	22 (18.3)	25 (20.8)
31 - 40	70 (58.3)	78 (65.0)
41 – 50	22 (18.3)	12 (10.0)
51 – 60	6 (5.0)	5 (4.2)
Mean ± SD	36.22 ± 7.56	35.84 ± 6.27
Range	23 – 58	23 – 55
Place of Domicile		
Urban	120 (100.0)	118 (98.3)
Rural	0 (0.0)	2 (1.7)
Occupation		
Unemployed	4 (3.3)	9 (7.5)
Petrochemicals	3 (2.5)	2 (1.7)
Agriculture	14 (11.7)	2 (1.7)
Mechanic Technician	11 (9.2)	7 (5.8)
Car Battery Technician	1 (1.7)	1 (0.8)
Printing	0 (0.0)	1 (0.8)
Civil Servants	56 (46.7)	50 (41.7)
Others	30 (25.0)	48 (40.0)
Education		
None	12 (10.0)	8 (6.7)
Primary	10 (8.3)	6 (5.0)
Secondary	8 (6.7)	12 (10.0)
Tertiary	90 (75.0)	94 (78.3)

Table 1: *Distribution of the Socio-Demographic Characteristics of Participants.*

NB: All Participants are Male.

The mean seminal 8-OHDG level among the 120 fertile controls is 12.45 ± 4.00 ng/ml. Therefore, taking the reference value as mean ± 2S.D based on the clinical and laboratory standard institute (CLSI) recommendation, reference value of seminal 8-OHDG was 4.45 - 20.45 ng/ml [16]. Values of Seminal 8-OHDG more than 20.45 ng/ml, the upper limit of the reference interval, were considered as suggestive of significant sperm DNA damage.

Histories of STIs, alcohol consumption, cigarette smoking and exposure to radiation among participants were compared with the degree of significant sperm DNA damage, using seminal 8-OHDG concentration > 20.45 ng/ml, as explained above, as a threshold of significant sperm DNA damage. Histories of alcohol consumption, cigarette smoking, STIs and exposure to radiation, including mobile phones were associated with significant sperm DNA damage among test subjects compared to controls, as shown in (Tables 2 to 5).

Variables	Significant DNA Damage (8-OHDG >20.45ng/Ml)			X^2	*P* Value
	No	Yes	Total		
	N (%)	N (%)	N		
Past History of STIs					
Yes	22 (62.9)	13 (37.1)	35	31.666[Y]	0.001
No	85 (100.0)	0 (0.0)	85		
Social History					
Smoking					
Yes	12 (52.2)	11 (47.8)	23	35.711[Y]	0.001
No	95 (97.9)	2 (2.1)	97		
Alcohol Ingestion					
Yes	19 (63.3)	11 (36.7)	30	24.184[Y]	0.001
No	88 (97.8)	2 (2.2)	90		
Use of herbal concoction					
Yes	54 (85.7)	9 (14.3)	63	1.637	0.201
No	53 (93.0)	4 (7.0)	57		

Table 2: *Assessment of the Effects of Medico-Social History on Significant Damage to Sperm DNA among Study Subjects.*

Variable	Significant DNA Damage (8-OHDG >20.45ng/Ml)			X^2	*P* Value
	No	Yes	Total		
	N (%)	N (%)	N		
Radiation History					
Yes	26 (78.8)	7 (21.2)	33	3.702[Y]	0.05
No	81 (93.1)	6 (6.9)	87		
If yes, which (n=33)					
X-ray	22 (84.6)	4 (15.4)	26	1.118[Y]	0.115
CT Scan	4 (57.1)	3 (42.9)	7		
How long is phone kept on your body per day					
<1 hour	16 (100.0)	0 (0.0)	16	6.815[Y]	0.009
1 – 3 hours	18 (94.7)	1 (5.3)	19		
>3 hours	73 (85.9)	12 (14.1)	85		

Table 3: *Assessment of the Effects of Radiation History on Significant Damage to Sperm DNA among Subjects.*

NB: Every respondent in the subject keeps phone in pocket.

Variables	Significant DNA Damage (8-OHDG >20.45ng/Ml)			X^2	*P* Value
	No	Yes	Total		
	N (%)	N (%)	N		
Past History of STI					
Yes	13 (100.0)	0 (0.0)	13	0.012[Y]	0.913
Alcohol					
Yes	10 (100.0)	0 (0.0)	10	0.094[Y]	0.759
No	106 (96.4)	4 (3.6)	110		
Smoking					
Yes	6 (100.0)	0 (0.0)	6	0.490[Y]	0.484
No	110 (96.5)	4 (3.5)	114		
Herbal Concoction					
Yes	35 (100.0)	0 (0.0)	35	0.556[Y]	0.456
No	81 (95.3)	4 (4.7)	85		

Table 4: *Assessment of the Effects of Medico-Social History on Significant Damage to Sperm DNA among Study Controls.*

Variables	Significant DNA Damage			X^2	*P* Value
	No	Yes	Total		
	N (%)	N (%)	N		
Exposure to radiation					
Yes	9 (100.0)	0 (0.0)	9	0.149[Y]	0.699
No	107 (96.4)	4 (3.6)	111		
If yes, which (n=9)					
X-ray	7 (100.0)	0 (0.0)	7	0.161[Y]	0.688
CT Scan	2 (100.0)	0 (0.0)	2		
Where phone is kept					
Pocket	115 (96.6)	4 (3.4)	119	5.283[Y]	0.173
None	1 (100.0)	0 (0.0)	1		

Table 5: *Assessment of the Effects of Radiation History on Significant Damage to Sperm DNA among Controls.*

A previous study established a relationship between sperm DNA damage estimation using sperm DNA fragmentation index (DFI%) and seminal 8-OHDG concentration (in ng/ml), where sperm DFI of 21.73 ± 9.49% was equivalent to seminal 8-OHDG of 19.27 ± 5.01 ng/ml [15]. Sperm DFI of > 30% which is will be equivalent to seminal 8-OHDG concentration of > 26.6 ng/ml in this study is established as severe sperm DNA damage. 3.3% of subjects in this study had severe sperm DNA damage compared to 0% among controls.

Severity of sperm DNA damage was significantly associated with histories of STI, alcohol and cigarette smoking among research subjects while histories of exposure to radiation including keeping phones in the front pockets of trousers and consumption of herbal concoction were not significantly associated with severe sperm DNA damage (Tables 6 and 7).

Variables	Severity				X^2	*P* Value
	Mild	Moderate	Severe	Total		
	N (%)	N (%)	N (%)	N		
Past History of STI						
Yes	30 (85.7)	1 (2.9)	4 (11.4)	35	7.188[Y]	0.027
No	85 (100.0)	0 (0.0)	0 (0.0)	85		
Exposure to radiation						
Yes	31 (93.9)	0 (0.0)	2 (6.1)	33	0.455[Y]	0.797
No	84 (96.6)	1 (1.1)	2 (2.3)	87		
If yes, which (n=33)						
X-ray	26 (100.0)	0 (0.0)	0 (0.0)	26	3.686[Y]	0.158
CT Scan	5 (71.4)	0 (0.0)	2 (28.6)	7		
Where phone is kept						
Pocket	115 (95.8)	1 (0.8)	4 (3.3)	120		

Table 6: *Assessment of the Effects of Medico-Social History on Severity of Sperm DNA Damage to Sperm DNA among Study Subjects.*

Variables	Severity				X^2	*P* Value
	Mild	Moderate	Severe	Total		
	N (%)	N (%)	N (%)	N		
Alcohol						
Yes	25 (83.3)	1 (3.3)	4 (13.3)	30	9.157[Y]	0.010
No	90 (100.0)	0 (0.0)	0 (0.0)	90		
Smoking						
Yes	18 (78.3)	1 (4.3)	4 (17.4)	23	13.373[Y]	0.001
No	97 (100.0)	0 (0.0)	0 (0.0)	97		
Herbal concoction						
Yes	59 (93.7)	1 (1.6)	3 (4.8)	63	0.190[Y]	0.909
No	56 (98.2)	0 (0.0)	1 (1.8)	57		

Table 7: *Assessment of the Effects History of Exposure to Radiation on Severity of Sperm DNA Damage to Sperm DNA among Study Subjects.*

Discussion

The mean seminal 8-OHDG level of 12.45 ± 4.0 ng/ml obtained among the 120 controls was used to estimate the reference values for seminal 8-OHDG, using mean ± 2SD in line with the CLSI recommendation for a normally distributed measurement [15], since there was no known established reference value for seminal 8-OHDG level. The reference values of 4.45 - 20.45 ng/ml was obtained for seminal 8-OHDG and values greater than 20.45 ng/ml, the upper limit of the reference interval, were taken as suggestive of significant sperm DNA damage. This is equivalent to 23.06% sperm DNA damage by DFI using 21.73% DFI equivalent to 19.27 ng/ml of seminal 8-OHDG, as obtained in a previous study [16].

Based on this, the prevalence of sperm DNA damage was 10.8%, which was statistically significantly more than 3.3% among controls. This is similar to findings of 16.8% and 19.25% obtained among infertile men in previous studies [17,18]. The higher values obtained in those studies maybe due to differences in their method of DNA damage evaluation. Their evaluations were based on sperm DNA fragmentation (SDF) which would have detected sperm DNA damages from other causes aside ROS while the method in this study detected sperm DNA damage from ROS only.

Seminal 8-OHDG > 26.6 ng/ml equivalent to sperm DFI of > 30% was taken as threshold of severe sperm DNA damage and 3.3% of the subjects in this study had severe sperm DNA damage. Only few studies have been done on assessment of severity of sperm DNA damage, however, the severity in this study is lower than 8.4% severity of sperm DNA among infertile males obtained from a similar study, though subjects selected for the study were men with unexplained infertility. Infertile males with > 30% sperm DNA damage, classified as severe sperm DNA damage will benefit from ICSI as a means of assisted reproductive technique rather than the conventional IVF. Sperm DNA damage evaluation therefore becomes very important for infertile males selected for ART before choosing the specific ART method, to minimize failure rate which is very common with such procedures.

Mean 8-OHDG was estimated and compared between research participants with histories of some conditions that have been implicated in generation of excessive ROS such as cigarette smoking, alcohol consumption, STI, exposure to radiations and herbal consumption. The median (IQR) seminal 8-OHDG levels were significantly higher among subjects who smoked 19.90 (12.40 - 22.50) vs 12.90 (11.90 - 16.80), $p < 0.001$), those who consumed alcohol, 16.60 (12.30 - 21.20) vs 13.30 (11.88 - 16.90), $p < 0.05$) and those with past history of STI, 18.60 (12.30 - 20.80) vs 12.80 (11.80 - 16.60, $p < 0.001$) than subjects without similar histories. Median (IQR) seminal 8-OHDG levels were significantly higher among test subjects who kept phone in the pockets of their trousers than controls with similar history. Mean seminal 8-OHDG levels were not significantly higher among test subjects who consumed herbal concoction.

Similar histories where compared among participants as a whole, subjects and controls separately with significant sperm DNA damage (seminal 8-OHDG levels > 20.45 ng/ml) and those without significant sperm DNA damage. Significant sperm DNA were higher among research participants as a whole and among subjects with histories of STI (< 0.001), smoking (< 0.001) and alcohol consumption (< 0.001). Significant sperm DNA was associated with history of exposure to radiation (x-ray, CT scan and keeping phones in the trouser pocket) among all research participants and among test subjects but not controls separately. Herbal concoction was also not associated with significant sperm DNA damage.

Severity of sperm DNA damage were also found to be associated with cigarette smoking ($p < 0.001$), alcohol consumption, STI and exposure to radiation through keeping phone in trouser pockets among all research participants combined and among subjects only compared with controls, where only exposure to phone radiation alone was found to be significantly associated with sperm DNA damage.

Cigarette smoking has been associated with disruption of sperm structural integrity including sperm DNA and sperm plasma membrane damage by many similar studies [20,21]. Cigarette is known to contain substances like alkaloids, nitrosamines and nicotine which can generates ROS [22]. Through excessive ROS generation, cigarette smoking causes sperm DNA damage by affecting the DNA protamination process and suppression of miRNA, thereby disrupting spermatogenesis and sperm chromatin formation [23].

Alcohol consumption is associated with sperm DNA damage through the generation of excessive ROS. Alcohol consumption compromise sperm DNA integrity including chromatin breakage, as demonstrated in many other studies [24,26]. STIs have also been implicated in previous other studies as a cause of male infertility, by compromising sperm DNA integrity including chromatin fragmentation [27].

Cell phones have also been associated with sperm DNA damage in several other studies. The proposed mechanism is through the radiofrequency electromagnetic field (RF-EMF), non-ionizing radiation, that negatively impact sperm quality, including increase sperm DNA fragmentation [28,29].

Conclusion

Cigarette smoking, alcohol consumption, STI and keeping mobile phones in the pockets, all established sources of reactive oxygen species, were associated with significant sperm DNA damage and all except histories of exposure to radiation were associated with severe sperm DNA damage, using 8-OHDG as biomarker of DNA damage. Since cigarette smoking, alcohol consumption, STI and exposure to radiation, especially mobile phones, are all modifiable factors, adjustments or prevention of these associated lifestyles may reduce or prevent sperm DNA damage.

Conflict of Interest

None.

Bibliography

1. Agarwal A and Said TM. ''Role of sperm chromatin abnormalities and DNA damage in male infertility''. *Human Reproduction Update* 9 (2003): 331-345.

2. Schulte RT., *et al*. ''Sperm DNA damage in male infertility: etiologies, assays , and outcomes''. *Journal of Assisted Reproduction and Genetics* 27 (2010): 3-12.

3. Wright C. ''Sperm DNA damage caused by oxidative stress : modifiable clinical , lifestyle and nutritional factors in male infertility''. *Reproductive Biomedicine Online* 28 (2014): 684-703.

4. Zabludovsky N., *et al*. ''Relationship between human sperm lipid peroxidation, comprehensive quality parameters and IVF outcome''. *Andrologia* 31 (1999): 91-98.

5. Agarwal A and Allamaneni SSR. ''Sperm DNA damage assessment : a test whose time has come''. *Middle East Fertility Society Journal* 84 (2005)'': 5-8.

6. De kretser DM. ''Male infertility''. *Lancet* 349 (1997): 787-790.

7. Sikka SC. ''Oxidative stress and role of antioxidants in normal and abnormal sperm function''. *Frontiers in Bioscience* 1 (1996): 78-86.

8. Gharagozloo P and Aitken RJ. ''The role of sperm oxidative stress in male infertility and the significance of oral antioxidant therapy''. *Human Reproduction* 26 (2011): 1628-1640.

9. González-marín C. ''Types , Causes , Detection and Repair of DNA Fragmentation in Animal and Human Sperm Cells''. *International Journal of Molecular Sciences* (2012): 14026-14052.

10. Belle G Van. ''Biostatistics: A Methodology for the Health Sciences''. *Second* (2004).

11. Uhuonrenren OB., *et al* ''The Prevalence and Patterns of Endocrinopathies Amongs Azoospermic Male Partners at a Fertility Clinic in Benin City''. *Endocrinology and Metabolism International Journal* 1 (2014): 1-6.

12. Chessbrough M. ''District Laboratory Practise in Tropical Countries''. (2006).

13. Khaldoun S. ''Sperm DNA fragmentation testing: To do or not to do?'' *Middle East Fertility Society Journal* 18 (2013): 78-83.

14. WHO. World Health Organization reference values for human semen Human Reproduction (2009).

15. Wang Q., *et al*. ''Increased Oxidative DNA Damage in Seminal Plasma of Infertile Men with Varicocele''. *Andrology* 3 (2014): 1-6.

16. Wayne P. ''Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline''. *Clinical Laboratory Standard Institute* (2008) C28-A3.

17. Sharma R., *et al*. ''Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay using bench top flow cytometer for evaluation of sperm DNA fragmentation in fertility laboratories: protocol, reference values, and quality control''. *Journal of Assisted Reproduction and Genetics* 33 (2016): 291-300.

18. Sharma R., *et al*. ''TUNEL as a test for sperm DNA damage in the evaluation of male infertility''. *Urology* 76 (2010): 1380-1386.

19. Oleszczuk K., *et al*. ''Prevalence of high DNA fragmentation index in male partners of unexplained infertile couples''. *Andrology* 1(2013): 357-360.

20. Arabi M and Moshtaghi H. ''Influence of cigarette smoking on spermatozoa via seminal plasma''. *Andrologia* 37 (2005): 119-124.

21. Potts R. ''Sperm chromatin damage associated with male smoking''. *Mutation Research* 423 (1999): 103-111.

22. Eftekhar M., *et al*. ''Relation of Second Hand Smoker and Effect on Pregnancy Outcome and Newborns Parameters''. *Gynecology and Women's Health Research* 6 (2016): 2.

23. Pembrey M., *et al*. ''Sex-specific, maleline transgenerational responses in humans''. *European Journal of Human Genetics* 14(2006): 159-166.

24. Aboulmaouahib S., *et al*. ''Impact of alcohol and cigarette smoking consumption in male fertility potential: Looks at lipid peroxidation, enzymatic antioxidant activities and sperm DNA damage''. *Andrologia* 50 (2017): 3.

25. Komiya A., *et al*. ''Clinical Factors Associated with Sperm DNA Fragmentation in Male Patients with Infertility''. *The Scientific World Journal* 2014 (2014): 868303.

26. Wdowiak A., *et al*. ''Relationship Between Alcohol Consumption and Sperm Nuclear DNA Fragmentation and Pregnancy''. *Advance Andrology Online* 3 (2016): 14-21.

27. Agarwal A., *et al*. ''Role of oxidative stress, infection and inflammation in male infertility''. *Andrologia* 11 (2018): 50.

28. Gallegos G., *et al*. ''Sperm DNA fragmentation in infertile men with genitourinary infection by Chlamydia trachomatis and Mycoplasma''. *Fertility and Sterility* 2 (2008) 90: 328-334.

29. Zalata A., *et al*. ''In vitro effect of cell phone radiation on motility, DNA fragmentation and clusterin gene expression in human sperm''. *International Journal of Fertility and Sterility* 9 (2015): 129-136.

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