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Sperm Protamination Status in Association with Oxidative Stress-Induced Sperm DNA Damage

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ABSTRACT: Male infertility is a major global health concern, contributing significantly to reproductive challenges among couples worldwide. Oxidative stress is recognized as a key underlying mechanism that compromises sperm quality, causing base modifications and chromatin anomalies. Among various biological fluid markers, 8-hydroxydeoxyguanosine (8-OHdG) serves as a sensitive indicator of oxidative DNA damage, yet its simultaneous expression in systemic and localized reproductive compartments remains controversial.

To assess the concentrations of 8-OHdG in blood and seminal plasma of infertile men and to evaluate their correlations with chromatin maturity

This cross-sectional study enrolled 88 infertile men evaluated at the fertility center in Al-Sader Teaching Hospital and an andrology private clinic from November 2025 to January 2026. Sociodemographic and clinical histories were documented via a structured questionnaire. Systemic and local oxidative markers were quantified by measuring serum and seminal plasma 8-OHdG levels using enzyme-linked immunosorbent assay (ELISA). Sperm chromatin maturity were evaluated utilizing cytochemical Aniline Blue (AB) staining.

Measuring oxidative biomarkers provides complementary information regarding sperm integrity. Further research is required to standardize reference values and explore targeted antioxidant strategies to optimize fertility outcomes in this population.

1. INTRODUCTION

The imbalance between reactive oxygen species (ROS) production and antioxidant defenses leads to what is known as oxidative stress. (1) Low levels of ROS play an important role in normal sperm functions, including capacitation and acrosomal reaction, while excessive production of ROS can lead to lipid peroxidation, protein damage, and DNA fragmentation. (2)

8-hydroxydeoxyguanosine (8-OHdG) is one of the most reliable biomarkers of oxidative DNA damage; it is formed by guanine base oxidation in DNA.(3)

8-OHdG can be detected in various biological fluids such as blood, urine, and seminal plasma, reflecting systemic and local oxidative stress levels. (4)

This intricate, hierarchical packaging system creates a flexible platform for adjusting DNA accessibility, which is essential for other biological processes like gene transcription and DNA replication.(5)

Spermatogenesis preserves male fertility throughout life, ensuring the transfer of genetic information to the following generation. Especially, spermiogenesis entails major modification of chromatin and the cytoskeleton and to accomplish a large compaction of the sperm head. (6)

The substitution of protamines for histones is a significant chromatin remodeling event during spermiogenesis.(7) Where, Protamines are simple, positively charged, arginine-rich proteins that bind to the phosphate backbone of negatively charged DNA. (8)

The compaction of the paternal genome is essential for optimal sperm function. A highly condensed sperm nucleus enhances sperm hydrodynamics, enabling faster movement and conferring an advantage during oocyte fertilization. Additionally, chromatin compaction shields paternal DNA from internal and external threats, such as nucleases and free radicals, which can damage genetic material. DNA damage has been linked to impacts on sperm morphology, fertilization success, and embryo development across various species. Therefore, maintaining sperm DNA integrity is crucial and serves as a vital indicator of sperm quality.(9)

The exact relationship between systemic oxidative markers in blood and local markers in seminal plasma remains unclear in spite of growing evidence on how oxidative stress impacts male infertility, especially the correlation between 8-OHdG levels in these two compartments and their association with sperm parameters and chromatin maturity requires further investigation.

Understanding these relationships may clarify the underlying role of oxidative damage and contribute to identify a reliable biomarkers for diagnosing and managing male infertility.

2. MATERIALS AND METHODS

The study has employed a cross-sectional methodology. It included 88 participants who were chosen from the fertility center at Al-Sader Teaching Hospital throughout the period from November 2025 to January 2026.

All the male participants were informed that their samples would be subjected to additional analysis for scholarly research and that all patient data would be kept private and confidential.

Verbal consent was obtained, and a questionnaire was completed for every patient. Research Ethics Committee approval was obtained (MEC-66)

A male infertile aged 18 – 45 years who complains of either primary infertility—the inability of a pair to conceive after 12 months or more of consistent, unprotected sexual activity—or secondary infertility—the difficulty of a couple to conceive after at least one pregnancy in the past

The couples had either "unexplained infertility" due to an unknown reason or male factor (less than the reference value of sperm concentration, progressive motility, normal morphology, or any combination).(10)

Chronic illness: DM, HTN, CKD, cancer, chemotherapy, Azoospermia, Cryptozoospermia, Severe oligozoospermia; Patients on fertility treatment like antioxidants for more than 3 months and Recent Varicocele surgery in less than 3 months were all excluded

The ELISA kit, Elabscience® 8-OHdG (8-Hydroxydeoxyguanosine) uses the Competitive-ELISA principle. It detects 8-OHdG serum, plasma, urine and other biological fluids. This kit include micro ELISA plate pre-coated with 8-OHdG.

8- OHdG within samples or standard competes with a fixed amount of pre-coated 8-OHdG for binding sites on the Biotinylated Detection Ab specific to 8-OHdG.

Sperm chromatin packaging and maturity are evaluated by aniline blue (AB) staining technique to detect residual histone proteins according to WHO criteria. (10)

The staining procedure includes:

1. Smear Preparation: Centrifugation of semen samples at 1000 × g for 20 minutes at 2–8°C is used to separate seminal plasma and sperm cells. After diluting the pellet with 0.5 ml of phosphate buffer saline, 10 microliters of the diluted pellet were aspirated using a micropipette.
2. Aliquots ARE USED to clean glass slides to make thin, uniform smears which are left to air-dry at room temperature.
3. Fixation: The smears then immersed in the glutaraldehyde fixative solution for 30 minutes at room temperature.
4. Rinsing: Slides are gently rinsed with distilled water to remove excess fixative and allowed to air-dry.
5. Staining: The fixed smears were flooded with the aniline blue staining solution and incubated for 5 minutes at room temperature.
6. Final Wash: The slides are rinsed under a gentle stream of distilled water to eliminate unbound dye, air-dried, and prepared for microscopic examination.

A brightfield light microscope equipped with a 100 times oil immersion objective lens is used to assess chromatin maturity. Random selection of minimal 200 spermatozoa is done for evaluation per slide. Cells were categorized into two distinct profiles based on head coloration:

Mature (AB-Negative): Spermatozoa presenting with pale blue, light pink, or unstained heads, indicating successful protamination and normal chromatin condensation

Immature (AB-Positive): Spermatozoa presenting with dark blue or purple-blue heads, indicating persistent histone retention and incomplete chromatin condensation.

The final data are expressed as the percentage of abnormal aniline blue-positive spermatozoa relative to the total number of evaluated cells.

3. RESULTS

Table (3.1) illustrates the comparison of 8-OHdG levels in serum and semen across different categories of DNA integrity (Good, Fair, and Bad). The statistical analysis revealed no significant differences in 8-OHdG levels among the three groups in either serum or semen (P value was 0.750 and 0.926 respectively). Although the "Bad DNA integrity" group recorded numerically higher mean levels of 8-OHdG in both serum and semen (27.90 ± 7.57 vs 15.97 ± 12.58), these findings did not reach statistical significance. Furthermore, Pearson correlation analysis demonstrated a weak positive correlation between 8-OHdG levels and chromatin maturity, with correlation coefficients of 0.307 for serum and 0.185 for semen. However, these correlations were statistically non-significant, as the P-values (0.145 and 0.386, respectively) exceeded the standard significance threshold of 0.05.

Table (3.1): Comparison of 8-OHdG levels in serum and semen across different categories of DNA integrity

Sperm Chromatin Maturity	No.	Serum 8-OHdG		Semen 8-OHdG	
		Mean ± St. deviation	Pearson Correlation (r)	Mean ± St. deviation	Pearson Correlation (r)
Good DNA integrity	54	24.04 ± 13.90	0.307	14.81 ± 9.56	0.185
Fair DNA integrity	31	22.09 ± 14.84		14.15 ± 7.75	
Bad DNA integrity	2	27.90 ± 7.57		15.97 ± 12.58	
P value		0.750	0.145	0.926	0.386

The relationship between sperm chromatin maturity (DNA integrity) and serum 8-OHdG levels is presented in Table 3.2. Among participants with normal serum 8-OHdG levels (≤ 4.2 ng/ml) (11), the sample was equally distributed between good and fair DNA integrity (50% each), with a significantly lower mean value observed in the good DNA integrity group (8.38 ± 1.80 vs. 18.63 ± 2.66; P < 0.001).

Similarly, within the cohort presenting with high serum 8-OHdG levels (> 4.2 ng/ml), a highly significant statistical difference was observed (P < 0.001). In this group, the majority of cases exhibited good DNA integrity (63.8%, mean: 9.82 ± 2.99),

followed by fair DNA integrity (33.7%, mean: 18.54 ± 3.14). Notably, bad DNA integrity was uniquely reported in this high-serum group (2.5%), showing the highest overall mean concentration of 35.00 ± 2.83.

Table (3.2): Distribution and comparison of sperm chromatin maturity across serum 8-OHdG levels

Sperm Chromatin Maturity	Normal Serum 8-OHdG (≤ 4.2 ng/ml)			High Serum 8-OHdG (> 4.2 ng/ml)		
	N (%)	Mean ± St. Deviation	P Value	N (%)	Mean ± St. Deviation	P Value
Good DNA integrity	4 (50%)	8.38 ± 1.80	< 0.001	51 (63.8%)	9.82 ± 2.99	< 0.001
Fair DNA integrity	4 (50%)	18.63 ± 2.66		27 (33.7%)	18.54 ± 3.14	
Bad DNA integrity	0 (0%)	-		2 (2.5%)	35.00 ± 2.83	

The findings illustrated in Table 3.3 outline the association between sperm chromatin maturity (DNA integrity) and semen 8-OHdG concentrations. highly significant statistical difference was observed (P < 0.001) within the normal semen 8-OHdG group (≤ 20 ng/ml) (12), where mean levels increased progressively alongside DNA degradation. The mean values rise from 9.82 ± 3.16 in cases with good DNA integrity to 18.65 ± 3.09 in those with fair integrity, peaking at 37.0 ± 0.0 for the single case categorized with bad DNA integrity.

A parallel highly significant trend (P < 0.001) was detected within the high semen 8-OHdG group (> 20 ng/ml). The majority of these subjects exhibited good DNA integrity (73.9%), showing a mean concentration of 9.48 ± 2.42, while 21.7% fell into the fair integrity category (mean: 18.0 ± 3.06). Similar to the normal group, the highest mean value was documented in the bad DNA integrity subgroup (33.0 ± 0.0), further highlighting the consistent increase in 8-OHdG levels as chromatin maturity deteriorates.

Table (3.3): Distribution and comparison of sperm chromatin maturity across semen 8-OHdG levels

Sperm Chromatin Maturity	Normal Semen 8-OHdG (≤ 20 ng/ml)			High Semen 8-OHdG (> 20 ng/ml)		
	N (%)	Mean ± St. Deviation	P Value	N (%)	Mean ± St. Deviation	P Value
Good DNA integrity	38 (58.5%)	9.82 ± 3.16	< 0.001	17 (73.9%)	9.48 ± 2.42	< 0.001
Fair DNA integrity	26 (50%)	18.65 ± 3.09		5 (21.7%)	18.0 ± 3.06	
Bad DNA integrity	1 (1.5%)	37.0 ± 0.0		1 (4.4%)	33.0 ± 0.0	

4. DISCUSSION

8-OHdG is generated through the oxidation of guanine residues by reactive oxygen species (ROS) and is released during DNA repair processes; therefore, its concentration reflects the extent of oxidative DNA injury occurring in the body. (13) 8-OHdG is one of the most widely recognized biomarkers of oxidative DNA damage.

This study tests for chromatin maturation which is an important indicator of sperm DNA integrity and reflects the degree of chromatin condensation achieved by replacement of histones with protamines. Using aniline blue stain that binds to lysine-rich histones, a higher proportion of aniline blue-positive sperm indicates defective chromatin remodeling and reduced chromatin maturity.

Comparison of serum and seminal plasma 8-OHdG levels across the three DNA integrity categories (Good, Fair, and Bad) demonstrated no statistically significant differences (serum: P = 0.750; semen: P = 0.926). Although participants with poor DNA integrity exhibited numerically higher mean concentrations of 8-OHdG in both serum (27.90 ± 7.57 ng/mL) and seminal plasma (15.97 ± 12.58 ng/mL), these increases were not statistically significant. Similarly, Pearson correlation analysis revealed weak positive correlations between 8-OHdG levels and chromatin maturity for both serum (r = 0.307, P = 0.145) and seminal plasma (r = 0.185, P = 0.386). Overall, these findings indicate that neither serum nor seminal plasma 8-OHdG levels were significantly associated with sperm DNA integrity or chromatin maturity in the studied population.

These results support recent research (14) (15) that also did not find a significant relationship between these variables.

Another finding of the present study demonstrated that although men with poor sperm DNA integrity exhibited numerically higher concentrations of 8-hydroxy-2'-deoxyguanosine (8-OHdG) in both serum and seminal plasma, these differences were not statistically significant. Similarly, the weak positive correlations observed between 8-OHdG levels and sperm chromatin maturity failed to reach statistical significance. These results suggest that, within the present study population, oxidative DNA damage measured by 8-OHdG was not significantly associated with sperm DNA integrity or chromatin maturity. Supporting the results of (14).

5. CONCLUSIONS

This study evaluate how 8-OHdG level in both serum and seminal plasma relate to conventional semen parameters, and sperm chromatin maturity in infertile men. This study provide several important insights:

1. Serum 8-OHdG acts as an independent biochemical indicator that reflects generalized systemic oxidative load. It is considered a complementary test rather than a replacement for conventional diagnostic evaluations in male infertility.
2. The normal baseline measurement of 8-OHdG does not exclude the active presence of other non-mutagenic reactive oxygen species (ROS) within the seminal microenvironment, meaning that local oxidative stress may still persist through alternate pathological pathways.
3. As 8-OHdG is considered a valuable biomarker of oxidative DNA damage, its current clinical application is limited by methodological variability between laboratories, lack of standardized universal reference values, and inconsistent correlations with classic semen characteristics.
4. Recent international reviews and updates emphasize that extensive testing for sperm DNA fragmentation and genomic integrity is no longer recommended or considered a beneficial routine diagnostic test due to these wide procedural uncertainties, as results are controversial and indicate that there are unknown and undiscovered multifactorial underlying causes of male factor infertility that require further investigation.

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