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The Influence of DNA Fragmentation Index on Sperm Morphology in Iraqi Men

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ABSTRACT

When evaluating male reproductive potential, it is crucial to evaluate sperm structural abnormalities and sperm DNA damage. Apoptosis and mitochondrial membrane potential disruption are linked to sperm DNA fragmentation (SDF), which is inversely correlated with motility and normal morphology. To enable liquefaction, the semen sample is taken in a sterile collection container and incubated at 37°C for 30 minutes The samples were then split into three groups, P, G, and N, based on the morphological %. To measure chromatin condensation, aniline blue staining (AB), an acidic dye with a strong affinity for non-lysine-rich histones, was employed. The DNA maturity of the spermatozoa was assessed using AB. Sperm concentration, total sperm count, total motility, and total progressively motile sperm all had means that were above the WHO 2010 standard range. The DNA fragmentation index was significantly higher in patients with pattern P than in patients with patterns G and N, and it differed significantly (P=0.003) between the semen morphological patterns.
In conclusion, as DNA fragmentation is an independent test this study showed that a low percentage of normal sperm morphology might indicate DNA integrity status.

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KEYWORD

DNA fragmentation index, Sperm Morphology , SDF

1. *Introduction*

About 15% of couples in the world who are of reproductive age experience infertility, a reproductive health issue; in about 30% of cases, male factors are the independent cause **(Agarwal A. et al.,2015[1]).** Pre-testicular, testicular, or post-testicular abnormalities can lead to male factor infertility. Sometimes, though, there is no apparent cause for the decreased sperm quality **(Agarwal A. et al.,2019[2]).** Conventional semen analysis, which is routinely performed in compliance with the World Health Organization's (WHO) standard standards, is the initial stage in evaluating male fertility. **(World Health Organization, 2010 [3]).** Apoptosis and mitochondrial membrane potential dysfunction are linked to sperm DNA fragmentation (SDF), which is inversely correlated with motility and normal morphology **(Sharbatoghli, M. et., 2012[4]).** Despite the stringent requirements set by the WHO manual of 2010, the seminal examination through the evaluation of the traditional seminal parameters, such as concentration, motility, and morphology, can provide conclusive indications for male infertility when it is derived from azoospermia or globozoospermia **(Dam, A.H.D.M., et al.,2007[5])**. Research generally indicates that male factors, such as immune factors, Genetic abnormalities, urinary tract abnormalities, hormonal issues, and genitourinary infections, are the causes of infertility **(Zeqiraj A. et al.,2018 [6])**. The initial semen sample may not be able to distinguish between fertile men and those who are not, despite its requirement, which highlights the importance of cellular and nuclear components in male infertility **(Denomme, M.M., and Mann,**

M.R., 2013 [7])

Many aberrant sperm, especially those with structural details that are not normal, may be the result of defective mechanisms associated to spermatogenesis and/or sperm maturation. Aberrant sperm morphology is associated with decreased routine semen parameters, lower levels of sperm damage indicators (such as DNA fragmentation levels), and higher levels of reactive oxygen species (ROS) (**Karabulut, A. and Tekin, A.,2013 [8]) (Agarwal, A. et al., 2014 [9])**.

Abnormal chromatin maturity and sperm morphological abnormalities can be used to assess male fertility **(Dariš B. et al., 2010 [10]) (Sunanda P. et al., 2018 [11].** Therefore, sperm structural abnormalities and sperm DNA damage are important factors to consider when assessing male reproductive potential. Nevertheless, the pathophysiology of these illnesses and their connections are still not fully understood because of the distinct and intricate structure of sperm morphogenesis, which includes significant compaction of spermatid chromatin **(Holstein, A.F. et al., 2003 [12]) (Gold, H.B et al.,2018 [13])**

These procedures result in the development of particular sperm structures that are necessary for the fertilization of oocytes. In addition to sperm morphological flaws and reduced maturity, morphogenesis

failure can cause sperm nuclear DNA fragmentation (SDF) because it impairs the repair of nuclear DNA strand breaks in the early spermatid stage. Moreover, sperm with reduced maturity are known to produce more reactive oxygen species and break their DNA more frequently. Last but not least, oxidative stress, teratozoospermia, and "abortive" apoptosis of differentiated germinal.

Cells can occur **(Sakkas D. et al., 2003[14]) (Grunewald S. et al., 2017 [15]).** Sperm's DNA is more concentrated than somatic cells' to better withstand physical and chemical denaturation, yet sperm fragmentation can result from DNA damage caused by heat, radiation, oxidative stress, and aberrant apoptosis **(Agarwal, A. et al. 2020 [16])**

There is growing evidence that male infertility, which is strongly linked to low conception rates, high miscarriage rates, and poor embryo quality, is directly impacted by high sperm DNA damage **(McQueen, D.B. et al., 2019 [17]) (Zhu, C et al., 2020 [18]).**

Massive and abnormal fragmentation can happen during the intra-testicular spermatogenesis or post-testicular phase, for instance, along with the transmission in the epididymis, as a result of apoptosis brought on by too many oxygens free. Radicals, or as a result of unhealthy lifestyle choices like drug use, smoking, and poor working conditions that raise scrotal temperature. It can also happen as a result of clinical conditions like varicocele or exposure to certain environmental **pollutants (Cissen M. et al., 2016 [19]) (Mostafa R.M. et al., 2018 [20]) (Alamo, A., et al., 2020 [21]) (Bosco, L. et al., 2018 [22]) (Ruvolo, G., et al., 2013 [23])**

From the ≥80.5% stated in the first edition of the WHO manual **(World Health Organization, 1987 [24]) (Menkveld, R., 2010 [25])** to ≥14% in the fourth edition **(World Health Organisation, 1999 [26])** and even down to ≥4% in the fifth and most recent edition, the reference values for normal sperm morphology have undergone significant revisions. This research aims to find the correlation between laboratory assessments of sperm.

Morphology and sperm immaturity index as a type of Sperm DNA fragmentation assays.

2. Patients and Methods

A group of 120 infertile patients aged 30 to 50, were contributed in this study. The samples were normozoospermic, asthenozoospermic, teratooligozoospermic, or teratozoospermic. Based on the number of visitors, a 95 percent confidence interval and a power of 0.8 were used to calculate the sample size. Budgetary and schedule limitations, along with the fact that a sample size of 120 was found to be statistically adequate, led to this study being self-funded. Al Nahrain University has assigned the ethical clearance number IEC/2020/222. Following two to seven days without sexual activity, samples were taken by masturbating and subjected to macroscopical and microscopical analysis in accordance with WHO 2010 .

Following WHO (2010) guidelines, the semen samples were collected in a sterile, dry, and well-defined container and then allowed to liquefy by incubating at 37°C for half an hour. Considering the results of the morphology, the samples were divided into three groups :

1 -**Group P** includes sperm with a percentage of normal forms lesser than 4 were

2 -**Group G** includes sperm with a (4-14) percentage of normal form.

3 -**Group N** includes samples with a percentage of normal forms greater than 14.

Aniline blue staining (AB), an acidic dye that has a strong affinity for non-lysine-rich histones, was employed to measure chromatin condensation **(Shimal, R.A.A. et al.,2023 [27]).** AB was utilized to gauge the spermatozoa's DNA maturity.

4. Results

1) Seminal fluid analysis

These semen analysis parameters were found in 120 infertile men in this study based on the 2010 WHO normal reference values for semen analysis: sperm volume, sperm concentration, total sperm count, motility type (immotile, progressive, and non-progressive), total motile sperm, total progressive sperm, sperm morphology, and round cell counts (Table 1). Overall, The following were the results of the seminal fluid examination: the average of total motility, total sperm count, total sperm concentration, and total progressive motile sperm were 18.80, 47.41, 44.10, 15.60 respectively, which is more than the reference range of WHO 2010. While the percentage of progressive motile sperm was (28.40), which is considered less than the normal range in WHO 2010.

Table (1): **Seminal fluid analysis according to the World Health Organization 2010.**

Parameters	Mean ± SD	Reference range
Days of Abstinence	3.50 ± 0.60	2-7 days
Semen Volume (ml/ejaculate)	2.40 ± 0.80	>1.5 ml
Sperm Concentration (ml)	18.80 ± 14.20	≥15*106 /ml
Total Sperm Count	47.41 ± 39.91	≥39*106/ejaculate
Total Motility	44.10 ± 19.60	≥42%
Progressive Motility	28.40 ± 16.10	30%
Non-progressive Motility	15.51± 7.90	1%
Immotile	55.95 ± 19.55	≤20
Total Progressive Sperm (million)	15.60 ± 18.51	≥7.2*106/ejaculate
Morphology	8.10 ± 7.25	>4
Round Cells Count	8.32 ± 6.50	≤5/HPF

(2) Comparison by morphology pattern of semen

When comparing the morphological patterns of semen, it was evident that the mean DNA fragmentation index varied significantly (P=0.003), and it was significantly greater among patients in group P than those in the G and N groups (38.54 vs. 31.59 and 18.78, P=0.001), respectively (Table 2).

Table (2): **Comparison of DNA fragmentation index by morphology patterns of semen**

Morphology Pattern	DFI Levels	Morphology Pattern
Group P	38.54 ± 15.65	0.001
Group G	31.59 ± 17.07	
Group N	18.78 ± 16.0	

5. Discussion

The results showed that the DNA fragmentation index levels for the three varied significantly in their values. Moreover, the patterns of morphology indicated that the P group (normal form <4%) achieved significant superiority, with the highest value in the DFI level reaching 38.54, while the group that recorded the lowest value of 18.78, while the G model (normal form >4> 14) recorded 31.59. A study has shown that DNA fragmentation is a large and strong indicator of the possibility of fertility in men, higher than traditional semen indicators. It is known that in men who have high levels of DNA fragmentation, the probability of pregnancy is naturally very low or by intrauterine insemination methods **(Santi D. et al., 2018 [28])**

Muratori et al. (2019) explained that the process of DNA fragmentation occurs due to several factors, including lifestyle habits, medications, infections, aging, and exposure to various pollutants at the cellular level, and that all of these factors work to stimulate the breakage of sperm DNA through three main mechanisms, namely programmed cell death and poor sperm purification sperm chromatin and oxidative stress **(Muratori, M. et al., 2019 [29]).**

While Hamilton et al. (2020) confirmed that problems with the protamine process, apoptosis, and ROS are thought to be some of the most significant causes of DNA fragmentation and that sperm DNA.

Fragmentation is one of the primary causes of male infertility **(dos Santos Hamilton, T.R. and Assumpção, 2020 [3])**. Despite its importance as a predictor of pregnancy outcomes after intrauterine insemination, Yang et al. (2019) found that sperm DFI has proven to be highly beneficial in evaluating male fertility **(Yang, H. et al., 2019 [31])**. Tamburrino et al. (2012) found that the most common of the several DNA abnormalities that can arise in male gametes, especially in infertile guys, is fragmented DNA **(Tamburrino, L. et al., 2012 [32])**

There is also strong proof that sperm with broken DNA can nonetheless be living and moving. It has a normal morphology. And capable of fertilizing the egg. It was also found that fragmentation of sperm DNA may be the most common reason for the transmission of DNA abnormalities from parents to offspring, as there is a high percentage of sperm in infertile men, as well as heavy smokers elderly. Men and people exposed to toxic substances or who receive chemoradiation therapy **(Brahem, S.et al., 2011 [33])**

Brahem et al. (2011) concluded that men with a history of recurrent pregnancy loss have higher rates of DNA damage and impaired sperm motility compared to the control group, and this explains the pregnancy loss in these patients **(Liu, K et al., 2023 [34])** Liu et al., (2023) analyzed the sperm DNA fragmentation index was found to correlate with semen routine and appearance. Based on the DFI levels, 468 cases were in the first group (DFI greater than or equal to 15%), 518 cases were in the second group (DFI between 15 and 30%), and 476 cases were in the third group (DFI greater than 30%). **Kleshchev et al., 2021[35])** noted that a high level of DNA fragmentation is associated with a defect in sperm formation as a result of genetic mutations and the influence of environmental factors.

William et al. (2019) indicated that male physiological conditions not only affect the phenotypic characteristics of sperm but also affect their genetic and epigenetic signatures, which affects the fitness of the resulting offspring **(Silva, W.T et al., 2019 [36])**

Campos et al. (2021) found that high levels of DNA fragmentation were accompanied by significant declines in all essential parameters **(Campos, L.G.A, et al., 2021 [37]).**

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Author Contribution

Sahar Alhalafi performed the study.

Conflict of Interest

The authors declare no conflict of interest .

Ethical Clearance

The study was approved by the Ethical Approval Committee.

Financial Disclosure

There is no financial disclosure.

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