IRAQI JOURNAL OF EMBRYOS AND INFERTILITY RESEARCHES (IJEIR)
Narges, et al., Vol. 12, Issue 2, Pp. 1-11 (2022) Original Research
DOI: http://doi.org/10.28969/IJEIR.v12.i2.r1.22 E-ISSN: 2616-6984 P-ISSN: 2218-0265

The Correlation Between Sperm DNA Integrity and Conventional Semen Parameters

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ABSTRACT

The integrity of sperm deoxyribonucleic acid (DNA) and chromatin is very important for genetic material transmission into offspring. Therefore, the aim of the present study was to investigate the correlation between sperm deoxyribonucleic acid integrity and sperm parameters. The current study was conducted on 96 semen samples. This study was done at the High Institute for Infertility Diagnosis and Assisted Reproductive Technologies, Al-Nahrain University, for the period between November 2020 and May 2021. The samples were collected, and seminal fluid analysis was performed according to World Health Organization guidelines. Sperm Chromatin Dispersion test was performed. The results showed that the deoxyribonucleic acid fragmentation of sperms in infertile men in this study was higher than reported in fertile men in previous studies, indicating that deoxyribonucleic acid fragmentation (DNA) is actively contributing at least partially to male infertility. In terms of enrolled men, sperm deoxyribonucleic acid fragmentation (SDF) showed a significant positive correlation with round cell count.

Received: 30-Sep-2021
Accepted: 08-Dec-2021
Published: 17-Aug-2022

How to cite:

Khalaf NM; Mohammed AA; Rahim AI; The Correlation Between Sperm DNA Integrity and Conventional Semen Parameters; Iraqi Journal of Embryos and Infertility Researches (IJEIR), (2022); 12 (2): 1-11.
Doi: http://doi.org/10.28969/IJEIR.v 12.i2.r1.22

KEYWORDS

Infertility, Sperm DNA fragmentation, Sperm Chromatin Dispersion Test

1. Introduction

Infertility is a global reproductive health issue that affects 15% of couples, with male factor infertility accounting for almost half of those affected patients (**Lee and Foo, 2014 [1]**). Despite the fact that the analysis of semen is an important diagnostic method in the assessment of infertile couples' male partners (**Kale and Bhoyar, 2020 [2]**), Semen analysis is normal in about 15% of patients with male factor infertility (**Agarwal and Allamaneni, 2005 [3]**). However, basic semen analysis frequently fails to provide a precise diagnosis of male fertility (**Naji, Issa, and Alhalabi, 2016 [4]**). The genomic quality of the oocyte and sperm, the embryo's ability to implant, and the endometrial receptivity are all factors that influence whether or not a pregnancy occurs (**Kale and Bhoyar, 2020 [2]**). The integrity of sperm DNA and chromatin is obviously critical for the correct transfer of genetic material to future generations. Accumulating evidence shows that the damage of sperm DNA or sperm chromatin abnormalities may have an impact on human male fertility (**Fernández et al., 2009 [5]**). Human ejaculates contain several sperm cells with substantial DNA breakage, but many of infertile men have a higher percentage of sperm cells with DNA fragmentation than fertile controls (**Fernández et al., 2009 [5]**). A number of tests are currently available for the measurement of sperm DNA fragmentation. These include the TUNEL assay, the comet assay, the chromonychia A3 test, the DNA Breakage Detection-Fluorescence In Situ Hybridization (DBD-FISH) test, and the SCSA test. (**Fernández et al., 2003 [6]**). The diagnostic test used today (SCD) is a lightweight and fast test based on sperm chromatin dispersion (**Fernández et al., 2005 [7]**). Normal sperm creates DNA halo zones (**Yılmaz et al., 2010 [8]**). The aim of this study is to investigate the correlation between sperm DNA integrity and sperm parameters.

2. Materials and Methods

The current study was conducted on 96 semen samples, and the study was done at the High Institute for Infertility Diagnosis and Assisted Reproductive Technologies, Al-Nahrain University, for the period between November 2020 and May 2021. The study was approved By the Ethical Approval Committee. High Institute for Infertility Diagnosis and Assisted Reproductive Technologies/Al-Nahrain University. The samples were collected. Collected in sterile containers from patients by masturbation method with 2-7 days abstinence, and seminal fluid analysis was performed according to World Health Organization guidelines 1999. Sperm Concentration, Motility, Morphology, Round Cells, and Agglutination were evaluated microscopically. According to seminal

fluid characteristics, the patients were sub-classified into normozoospermia, oligozoospermia, asthenozoospermia, teratozoospermia,oligoasthenozoospermia,oligoteratozoo-spermia, oligoasthenoteratozoospermia, asthenoteratozoospermia. After that, each semen sample was mixed with agarose gel, which is melted before in a water bath as a preparation step for the Sperm Chromatin Dispersion test. The sperm chromatin Dispersion test kit was supplied by Avicenna Research Institute, Iran.

2.1. Sperm Chromatin Dispersion Test

The SCD test is based on the principle that sperm with fragmented DNA fail to produce the characteristic halo of dispersed DNA loops that is observed in sperm with non-fragmented DNA, following acid denaturation and removal of nuclear proteins. **(Fernández et al., 2003 [6]).** Under the light microscope, the slide was examined, and counting at least 300 sperm. Apply this equation:

SDF = fragmented + degraded / total sperm counted × 100

The Spermatozoa with a big or medium halo is considered normal, while Spermatozoa with a small or does not have halo or Sperm cells with degradation is considered fragmented DNA spermatozoa. Data were collected, summarized, analyzed, and presented using Statistical Package for Social Sciences (SPSS) version 23 and Microsoft Office Excel 2010.

3. Results

The sociodemographic factors of infertile men enrolled in the present study are shown in **Table 1**. The current study included 96 Infertile men. The mean age of all enrolled infertile men was 33.23 ±7.15 years, with an age range of 20 to 54 years. Most of the enrolled men were between 20 to 39 years as they, accounted for 77 (80.2%). The mean body mass index (BMI) of infertile men was 28.11 ±5.06 kg/m^{2,} and it ranged from 17.3-40.12 kg/m^2. According to BMI, there were 27 (28.1 %) men with normal weight, 40 (41.7 %) mean with overweight, 17 (17.7 %) men with class I obesity, 10 (10.4 %) with class II obesity, and a single man with class III obesity. The mean infertility duration was 5.07 ±3.99 years, and it ranged from 1 to 20 years. According to infertility duration, there were 62 (64.6 %) men with 1-5 years, 26 (27.1 %) men with 6 to Ten years, 4 (4.2 %) men with 11 to 15 years and 4 (4.2 %) men with 16 to 20 years. Active smoking was seen in 43 (44.8 %), passive smoking was seen in 9 (9.4 %) and 44 (45.8 %) men were not smokers. The baseline seminal fluid characteristics of infertile men enrolled in the current study are shown in **Table 2**. The

sperm DNA fragmentation (SDF) in infertile men enrolled in the current study is shown in **Table 3**. The correlations of Sperm DNA fragmentation (SDF) to seminal fluid characteristics in all enrolled infertile men are shown in **Table 4**. The mean SDF was 38.98 ±16.73 %, and it ranged from 9 -70 %. The correlations of sperm DNA fragmentation (SDF) to seminal fluid characteristics in all enrolled infertile men are shown in **Table 5**. SDF showed a significant positive correlation with round cell count. The mean highest level was observed in Oligoteratozoo spermia, and the lowest level was reported in Oligoasthenozoospermia, 24.96 versus 19.91
µmole / L, respectively.

4. Discussion

In the current study, the mean SDF was 38.98
±16.73 %, and it ranged from 9 -70 %. In compare to our study outcomes, the study carried out by Zeqiraj et al. in 2018, the DNA fragmentation mean in the infertile men group was 34.53 ± 4.68 %, whereas that of the fertile group was 14.91 ± 4.02%, so the DNA fragmentation mean in the current study in both infertile and fertile males, was higher than that reported by Zeqiraj et al. in 2018 which Included oligozoospermia and asthenozoospermia men. While the current research encompassed a wide range of anomalies, this could explain the discrepancy between the current study's results and those of Zeqiraj et al. in addition of DNA fragmentation. While another study found that infertile men have a much higher percentage of DNA fragmentation (**Sergerie et al., 2005 [9]**). Based on the findings of this study and previous findings, it is suggested that the use of DNA fragmentation index in the diagnostic workup of men presumed of infertility, a suggestion previously adopted by other authors, be considered (**Diallo et al., 2015 [10]**), because it appears clearly that, DNA fragmentation could be considered as an independent factor affecting men fertility status. While other researchers have concluded that sperm DNA fragmentation mean was considerably high among infertile males 24.80

Table (1): Sociodemographic factors of infertile men enrolled in the present study.

Characteristic	Results
Age (years)	
Mean ±SD	33.23±7.15
Range	20 -54
20-39, *n* (%)	77 (80.2 %)
40-54, *n* (%)	19 (19.8%)
BMI (Kg/m^2)	
Mean ±SD	28.11±5.06
Range	17.3 -40.12
Normal, *n* (%)	27 (28.1%)
Overweight, *n* (%)	40 (41.7%)
Class I obesity, *n* (%)	17 (17.7%)
Class II obesity, *n* (%)	10 (10.4%)
Class III obesity, *n* (%)	1 (1.0 %)
Infertility Period (years)	
Mean ±SD	5.07±3.99
Range	1 -20
1-5 y, *n* (%)	62 (64.6%)
6-10 y, *n* (%)	26 (27.1%)
11-15 y, *n* (%)	4 (4.2%)
16-20 y, *n* (%)	4 (4.2%)
Type of infertility	
Primary, *n* (%)	62 (64.6%)
Secondary, *n* (%)	34 (35.4%)
Smoking	
Active smoker, *n* (%)	43 (44.8 %)
Not smoker, *n* (%)	44 (45.8 %)
Passive smoker, *n* (%)	9 (9.4 %)

n: number of cases; **SD**: standard deviation

Table (2): Baseline seminal fluid characteristics of infertile men enrolled in the current study

Characteristic	Results	Characteristic	Results
Duration of abstinence (days)		**Progressive motility (%)**	
Mean ±SD	3.38±0.80	**Mean ±SD**	33.17±18.45
Range	2 -7	**Range**	0-80
Volume (ml)		**Nonprogressive motility (%)**	
Mean ±SD	2.96±1.13	**Mean ±SD**	33.25±13.99
Range	1 -6	**Range**	5 -72
Liquefaction time (minutes)		**Immotile sperm (%)**	
Mean ±SD	34.04±11.87	**Mean ±SD**	33.58±17.44
Range	20 -60	**Range**	10 -95
pH		**Normal sperm morphology (%)**	
Mean ±SD	7.97±0.21	**Mean ±SD**	32.79±20.22
Range	6 -8	**Range**	0 -80
Sperm concentration (million/ml)		**Round cells (million/ml)**	
Mean ±SD	43.43±26.15	**Mean ±SD**	8.83±7.20
Range	3 -150	**Range**	0 -35
Total count million /ejaculate		**Viscosity**	
Mean ±SD	129.19±95.66	**Normal, *n* (%)**	93 (96.9 %)
Range	12 -510	**Viscous, *n* (%)**	3 (3.1%)

n: number of cases; **IQR**: inter-quartile range; **M**: Mann Whitney U test; **NS**: not significant at $p > 0.05$

Table (3): Sperm DNA fragmentation (SDF) in infertile men enrolled in the current study.

Characteristic	Results
Sperm DNA fragmentation (SDF) (%)	
Mean ±SD	38.98±16.73
Range	9 -70

Table (4): Sperm DNA fragmentation (SDF) according to seminal fluid analysis

Group	*N*	Mean	SD
Normozoospermia	19	40.68	20.09
Oligozoospermia	1	25.00	---
Asthenozoospermia	47	38.06	16.34
Teratozoospermia	1	28.00	---
Oligoasthenozoospermia	7	35.71	12.61
Oligoteratozoo spermia	1	37.00	---
Oligoasthenoteratozoospermia	8	31.50	10.43
Asthenoteratozoospermia	12	49.00	17.02

Table (5): Correlations of Sperm DNA fragmentation (SDF) to seminal fluid characteristics in all enrolled infertile men.

Characteristic	Sperm DNA fragmentation SDF (%)	
	r	*p*
Duration of abstinence (days)	0.005	0.959
Viscosity	-0.047	0.653
Volume (ml)	-0.197	0.055
Liquefaction time (minutes)	0.052	0.617
pH	0.058	0.577
Sperm concentration (million/ml)	0.114	0.268
Total count (million /ejaculate)	-0.035	0.736
Progressive motility (%)	-0.010	0.927
Nonprogressive motility (%)	0.010	0.922
Immotile sperm (%)	0.002	0.985
Normal sperm morphology (%)	-0.115	0.265
Round cells (million/ml)	0.243	**0.017 ***

± 13.1 % (**Atshan et al., 2020 [11]**), supporting the existence of significantly higher levels of DNA fragmentation in men with infertility regardless to the sperm parameters status and this against the study done by Virro in 2004, which has been established that if sperm DNA fragmentation exceeds 30%, sperm quality, and man fertility are significantly reduced (**Virro et al., 2004 [12]**). Based on the percentage of DNA fragmentation, it is possible to choose the right technique in assisted medical care clinics (**Zeqiraj et al., 2018 [13]**).

5. Conclusions

Sperm DNA fragmentation shows a positive correlation with round cell count and a negative correlation with other sperm parameters, so it can conclude that sperm DNA fragmentation could be consider as an independent factor affecting men's fertility status.

Acknowledgment

We would like to acknowledge Al Nahrain University, Baghdad, Iraq.

Funding

This work received no funding.

Author Contribution

Khalaf, NM, performed the study, examined and reviewed results, and manuscript writing with the help and supervision of Mohammed, AA, and Rahim, AI.

Conflict of Interest

The authors declare no conflict of interest.

Ethical Clearance

The study was approved by the Ethical Approval Committee.

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