International Journal of Science and Research (IJSR)
ISSN: 2319-7064
SJIF (2022): 7.942

Correlation between Lifestyle Factors and Sperm DNA Fragmentation in Infertile Men

Gurbani Kaur Arora[1], Kundavi Shankar[2], Yamini Asokan[3], Geetha V.[4], Rashmi G. V[5], Nithya Naaram[6], Hema Niveda KR[7]

[1]Institute of Reproductive Medicine, Madras Medical Mission, Chennai, India
Corresponding author email: *gurbanik36[at]gmail.com*
Mobile no: +918288981910

[2, 3, 4, 5, 6, 7]Institute of Reproductive Medicine, Madras Medical Mission, Chennai, India

Abstract: ***Background: The sperm DNA fragmentation index (DFI) is regarded as an important tool but its role in evaluating male infertility still remains controversial. The aim of this study is to evaluate the correlation between sperm DNA fragmentation (SDF) with semen parameters and lifestyle patterns. Methods: In our retrospective study which was conducted at the Institute of Reproductive medicine and women health, Madras medical mission, Chennai from 2019-2023, 120 males with subfertility undergoing treatment were included. DFI was measured using the sperm chromatin dispersion assay (SCD) and based on the results they were divided into 3 groups: Low-DFI ≤15%, medium DFI-15%< DFI < 30% and high DFI ≥30%. The correlation between DFI and semen parameters were analysed using Spearman's rank correlation coefficient. Results: In the correlation analysis, DFI showed significant positive correlation with age (p=0.001) and negative correlation with semen parameters like semen volume (p=.0.010), rapid progressive motility (p=0.002) and total motile sperm count (p=0.004). Lifestyle characteristics like body mass index, smoking, consumption of alcohol, diabetes and hypertension showed no significant correlation with DFI. Conclusions: The results indicate a significant relationship between SDF and routine semen parameters, suggesting the inclusion of DNA fragmentation index assessments in male infertility evaluations.***

Keywords: DNA fragmentation, infertility, semen analysis, chromatin

1. Introduction

The success of pregnancy is dependent on factors from both partners. Approximately one-third of infertility cases can be attributed to male factors, either independently or in conjunction with female factors.1Male infertility which is determined by the quality of the spermatozoa is assessed by routine semen analysis in terms of sperm concentration, motility, and morphology and is standard diagnostic tool to assess sperm quality.2 Approximately 15% of men with normozoospermia are diagnosed as infertile thus suggesting that traditional semen analysis alone provides limited information and it does not fully reflect the fertilization potential of the spermatozoa.3 In the era of Assisted Reproductive Technology, standard semen analysis fails to meet the needs of reproductive medicine practice, thus requiring better clinical indicators for evaluating male infertility. The sperm DNA fragmentation index expressed as DFI reflects the integrity of the DNA of the sperm. According to recent studies, sperm DFI is used to predict male infertility and has greater diagnostic and prognostic value than semen analysis.4 For male fertility there is defined DNA fragmentation status as > 30% "significant lack of", 15-30% 'reasonable' and < 15% DNA 'high' fertility status.5 Studies have reported the role of DFI assessment as a valuable tool in assessing male infertility, but its clinical significance in predicting the prognostic outcomes of ART is still unknown.6 There have been various techniques for evaluation of sperm chromatin structure, with the primary being sperm chromatin dispersion (SCD), terminal deoxyuridine nick end labelling (TUNEL), acridine orange test (AOT), sperm chromatin structure assay (SCSA), and aniline blue (AB) staining.7, 8 The SCD test is based on the principle that sperms with fragmented DNA fail to produce the characteristic central core and peripheral halo caused by release of DNA loops. The SCD test is a rapid and accurate procedure to determine SDF whereas other tests require advanced equipment and are expensive.9 Various factors like age, body mass index, stress, consumption of alcohol & caffeine, smoking affect DFI.10

Routine semen analysis, which includes assessments of sperm concentration, motility, and morphology, does not guarantee normal sperm DNA. The purpose of this study was to assess the correlation between DFI and lifestyle factors such as age, body mass index (BMI), diabetes mellitus and hypertension and semen parameters such as days of abstinence, semen volume, concentration, morphology and total motility and to enhance the understanding and evaluation of male infertility.

2. Materials & Methods

This retrospective study was conducted on medical records of 120 men from subfertile couples who had undergone non donor IVF-ICSI cycles in the Institute of Reproductive medicine and women health, Madras medical mission, Chennai from the year 2019-2023 and was approved by the Ethics committee. The inclusion criteria for the study were subfertile males between 21-55 years of age. Exclusion criteria included men with sperm count under 2 million/ml, men with azoospermia, men suffering from retrograde ejaculation and the use of testicular sperm.

General characteristics like age, BMI, occupation, type of infertility, smoking, alcohol consumption, history of diabetes and hypertension were recorded. After an

abstinence period of 2-5days, semen sample collected by masturbation was analysed according to the WHO Laboratory Manual for Human semen examination and Processing, 5th & 6th edition. Microscopic examination assessing the sperm motility, concentration and sperm morphology was done. Sperm morphology assessment was done using Kruger's strict criteria. All semen samples were evaluated for fragmented DNA by Sperm chroma kit which is based on the sperm chromatin dispersion (SCD) technique. This method is based on the principle that intact DNA loops expand following denaturation, however when DNA is fragmented minimal or no expansion is seen. This method uses light microscopy method to evaluate the susceptibility of sperm DNA to acid denaturation. Normal spermatic DNA is represented by a central core and peripheral halo and fragmented DNA shows no halos or small halos.

After counting 200 spermatozoa, the DNA fragmentation index (DFI) was calculated as indicated below

$$\frac{\text{Number of sperms with fragmented DNA}}{\text{Total number of sperms}} \times 100\,\%$$

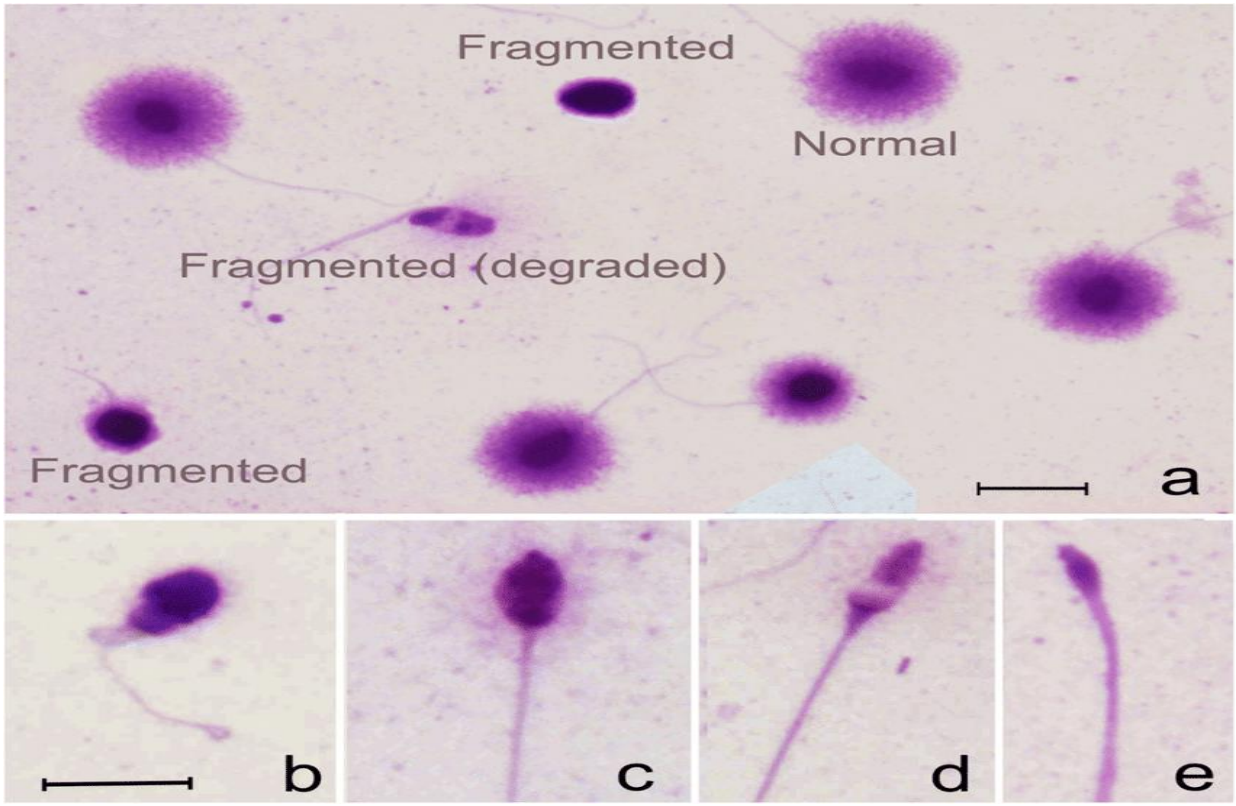

Figure 1: Classification of sperm DNA fragmentation a: Big halo without fragmentation, b: Medium halo without fragmentation, c: Small halo with fragmentation, d: Without halo with fragmentation, e, Degraded: no halo and weakly stained.

Based on the results from sperm DFI assessment, the cases were divided into the following three groups: low DFI (DFI ≤15%), medium DFI (15%< DFI < 30%) and high DFI (≥30%).

All statistical analyses were performed using SPSS software (version 22.0, SPSS Inc). All numeric data are presented as the mean value ± standard deviation. Frequencies were expressed as percentages by comparison with mean values among three groups using an analysis of variance test. The association of the standard parameters and the DFI was measured by Spearman's rho correlation coefficient (r). Comparison of quantitative variables between the study groups was done using Kruskal-Wallis test. For comparing categorical data, chi square test was performed. Differences between the values were considered statistically significant when $p< 0.05$.

3. Results

A total of 120 men of sub fertile couples were enrolled in the study. There are 22 patients with High DFI (18.3%), 55 patients with medium DFI (45.8%) and 43 patients with low DFI (35.8%) with the maximum DFI of 44% and minimum DFI of 2%.

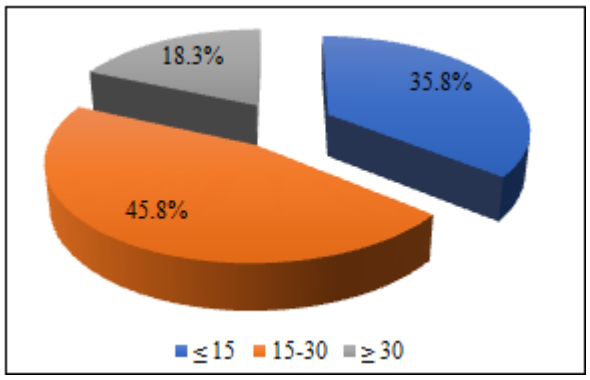

Figure 2: DFI distribution

The age of the patients in our study ranged from 21 to 53 years with a mean of 37.59±7.37 years. Most of the patients belonged to the age group less than 40 years (n= 66) with a mean DFI of 13.47±6.31 %**.** The mean DFI in patients above 40 years was27.5±7.79 % and this difference was statistically significant (p=0.001).55 % of the study population belonged to overweight group (n=66) according to their BMI with a mean DFI of 19.58±10.64. Hypertensive men (n=13) showed higher DFI (22.08±13.13) whereas normotensive men showed a DFI of 19.50±9.47. Men with history of smoking (n=14) and consumption of alcohol (n=42) showed a mean DFI of 15.43±8.19 and 19.83±9.71 respectively.

DFI showed no statistically significant relationship to male characteristics such as body mass index (BMI), type of infertility, smoking and consumption of alcohol. The prevalence of diabetes and hypertension in the study group also showed no significant association with DFI with p value of 0.367 and 0.378 respectively.

Table 1: General characteristics of study population and DNA fragmentation index

	Total (n=120)	Mean±SD	DFI % Median (IQR)	p value
AGE				
<40 Years	66	13.47±6.31	13 (8-19)	**0.001**
≥40 Years	54	27.5±7.79	27 (22.75-32.25)	
INFERTILITY TYPE				
Primary	51	21.61±10.23	21 (12-29)	0.082
Secondary	69	18.43±9.49	17 (12.50-25)	
BMI KG/M2				
<22.9	10	19.70±8.72	19.5 (13-24.25)	
23-24.9	35	20.09±9.40	18 (13-27)	0.994
25-29.9	66	19.58±10.64	18 (11.75-26.25)	
>30	9	20.22±8.63	19 (11.50-29.50)	
Diabetes				
YES	19	17.89±9.13	16 (12-23)	0.367
NO	101	20.14±10.03	19 (13-27)	
Hypertension				
YES	13	22.08±13.13	24 (11.50-32.50)	0.378
NO	107	19.50±9.47	18 (13-26)	
Smoking				
YES	14	15.43±8.19	13 (10.75-18.25)	0.080
NO	106	20.36±9.99	19 (13-27.25)	
Alcohol				
YES	42	19.83±9.71	17.5 (12.75-27)	0.968
NO	78	19.76±10.35	18 (12-26)	

Men with High DFI (≥ 30 %) showed lower semen volume (1.80 ± 0.50), sperm concentration (41.32 ±23.31), total motility (43.41± 12.40), rapid progressive motility (5 ± 5.02) and total motile sperm count (21.48± 21.29). Sperm morphology was comparable in all three groups of low (2.09 ± 1.27), medium (2.69 ± 2.69) and high DFI (2.14 ±1.98). Men with normal DFI had a total motile sperm count (TMSC) of 41.27 ± 35.01 which was higher when compared with mean TMSC (33.20 ± 29.96), whereas men with HIGH DFI (≥ 30 %) showed a lower TMSC of 21.48± 21 and this difference was statistically significant (p value-0.035). For all the other semen parameters tested, no significant difference was seen in the three groups in terms of days of abstinence, volume, concentration, total motility, progressive motility and morphology.

Table 2: Semen analysis parameters and sperm DNA Fragmentation Index

Semen Parameters	Total Mean ± SD	DFI ≤ 15% (n=43)	DFI 15-30% (n=55)	DFI ≥ 30% (n=22)	p value
Abstinence	1.08± 0.32	1.07 ± 0.26	1.04 ± 0.27	1.18 ± 0.50	0.2
Volume (ml)	2.05 ± 0.59	2.16 ± 0.57	2.07 ± 0.63	1.80 ± 0.50	0.067
Concentration (mil/ml)	47.38 ±26.12	51.53 ± 26.47	46.55 ± 26.78	41.32 ±23.31	0.315
Total Motility (%)	47.43 ±14.69	48.88± 13.77	47.91 ± 16.12	43.41± 12.40	0.348
RPM (%)	7.18 ± 5.54	8.37 ± 4.93	7.13 ±5.99	5 ± 5.02	0.066
TMSC	33.20 ± 29.96	41.27 ± 35.01	31.59 ±27.17	21.48± 21.29	0.035
Morphology	2.38 ± 2.15	2.09 ± 1.27	2.69 ± 2.69	2.14 ±1.98	0.336

In the correlation analysis, statistically significant the negative correlations of semen volume (p=0.010), rapid progressive motility (p=0.002) and total motile sperm count (0.004) with DFI were observed in our study. Additionally, a significant positive correlation was found between age (p=0.001) and DNA fragmentation index. Other parameters like BMI, days of abstinence, sperm concentration, total motility and total morphology showed no correlation with DFI.

Table 3: Correlation between age, BMI, standard semen parameters and sperm DNA Fragmentation Indices

Factors	DNA Fragmentation Index	
	Correlation coefficient	p value
AGE	0.682	**0.001**
BMI	0.057	0.537
Abstinence	0.057	0.537
Volume	-0.234	**0.01**
Concentration	-0.17	0.063
Total motility	-0.161	0.079
RPM	-0.277	**0.002**
TMSC	-0.262	**0.004**
Morphology	-0.053	0.565

4. Discussion

Sperm DNA damage has a negative impact on reproductive outcomes in couples with infertility. The potential role of assessing sperm DNA fragmentation index in reproductive medicine has been recommended by various studies. DFI directly reflects the degree of sperm DNA destruction. Sperm DNA integrity is essential in transmitting genetic materials to the offspring. The major factors known to cause sperm DNA damage are oxidative stress, abnormal apoptosis of sperms and aberrant sperm chromatin assembly.[1]During the process of sperm maturation, protamination occurs where histones are gradually replaced by positively charged protamine to form tight junction which reduces the ability of sperm DNA to repair itself and thus prevents sperm DNA damage. Some DNA damage or breaks that occur during this process can be repaired by oocytes. However, it leads to infertility when the DNA damage is beyond the threshold of oocyte repair. Poor lifestyle habits including obesity, smoking, caffeine & alcohol consumption, exposure to radiation, systemic diseases and environmental pollution can all lead to raised sperm DNA fragmentation. Inflammatory processes in the genital tract and varicocele produce reactive oxygen species in the sperm and increases the risks of SDF.[12]This study's significance lies in its potential to influence clinical practices by integrating sperm DNA fragmentation assessments into routine infertility evaluations, thereby offering a more comprehensive understanding of male infertility factors.

Sperm DNA integrity is a pre-requisite for proper embryo development, implantation, and pregnancy and routine semen analysis fails to identify sperm DNA defects. According to WHO guidelines, SDF testing could represent an important addition in the evaluation of male infertility.2 At present there are several techniques to measure DNA fragmentation index but the SCD test (Halosperm test) seems to be a simple, cost effective and popular test.[13] It has also been reported that SCD has shown significant correlation with semen parameters when compared with other tests such as TUNEL, acridine orange, or SCSA.[14] The different mechanisms underlying these methods results in discrepancies and inconsistencies thus drawing different conclusions. Various researches have reported that infertile men group have higher sperm DNA fragmentation and the additional step in testing male infertility should be measuring sperm DFI.

Ageing in the male is associated with altered semen parameters, infertility and sperm DNA damage. There are many potential mechanisms cause these changes with age which include impaired DNA repair and cell division. Additionally, reduced antioxidant capacity, increasing apoptosis in testes, increase ROS, accumulated damage from infection, tobacco and other toxins also increase with age and affect the integrity of sperm.[15]The interplay of these factors gives rise to oxidative stress and contributes to the effects of age on sperm DNA.[16]Das et al. reported a higher DFI in men with subfertility having with greater paternal age.[17] In addition, Belloc et al. demonstrated that men with normal semen parameters, increasing paternal age was a significant predictor of greater DNA sperm fragmentation.[18] Campos et al reported that age was a significant predictor of DFI in patients with altered semen parameters when compared with men with normozoospermia (39.50 ± 6.87 vs 37.26 ± 6.76, respectively).[19]In our study population, 55% of the men were in the age group of less than 40 years with a mean DFI of 13.47±6.31 % and remaining 45 % belonged to the age group more than 40 years with a mean DFI of 27.5±7.79 %. In the group with age more than 40 years, 32 patients had medium DFI (15-30 %), 21 patients had high DFI (≥ 30 %) and only 1 patient had low DFI (≤ 15%). Age showed a statistically significant correlation with DFI (p value=0.001) as shown in Figure 3. Similar findings were reported by studies conducted by Kaarouch et al.[20] and Alshahrani et al. [21] that men more than the age of 40 years had higher DFI than younger men.

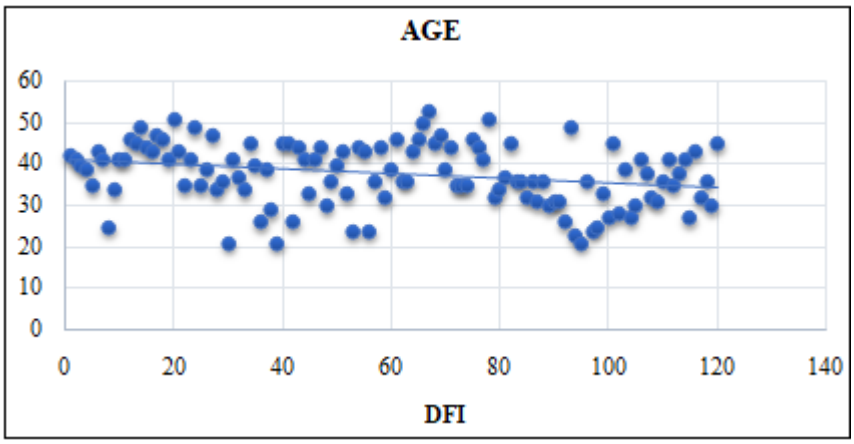

Figure 3: Correlation between age and DFI.
Individual data points and the regression line are shown.
Spearman´s correlation coefficient= 0.682 p=0.001.

Stress and lifestyle factors like obesity can affect sperm DNA and raise the fragmentation index. In clinical studies, the association between smoking and consumption of alcohol with DFI has been proved.2[2]The impact of metabolic syndrome encompassing elevated blood pressure, impaired glycaemic control, dyslipidaemia and obesity on semen quality, particularly on sperm DNA integrity, is supported by limited data.2[3] Obesity and increased weight are linked with hypogonadism, impaired spermatogenesis, elevated scrotal temperatures and an increased likelihood of sperm DNA damage.2[4] Kort et al. evaluated 520 male partners of infertile couples and found a positive correlation between BMI and sperm DNA fragmentation with the mean SDF rising from 19.9% in men with a normal BMI to 27.0% in obese men.2[5] The mean BMI of our study population was 26.08 ± 2.61kg/m2 and 55% (n=66) of them had a BMI more than 25 kg/m2 with a mean DFI of 19.58±10.64%. Systemicillness like diabetes mellitus and hypertension also increase the oxidation stress and raise sperm DNA fragmentation. Our results showed that the mean DFI (22.08±13.13) was higher in the semen samples from hypertensive men (n = 13) whereas DFI (17.89±9.13) was lower in men suffering from diabetes mellitus (n=19) and both showed no statistical significant relationship with DFI. In our study, 11.7 % (n=14) were smokers with a mean DFI of 15.43±8.19 and showed no significant correlation with sperm DNA fragmentation (p=0.058). In clinical studies, the association between smoking and DFI has been controversial. Researches have revealed that consumption of alcohol and caffeine cause significant morphological changes in sperm, leading to head and tail defects.2[2] In our study population, 35% (n=42) consumed alcohol with a mean DFI of 19.83±9.71 and it showed no significant correlation with sperm DNA damage (p=0.988).

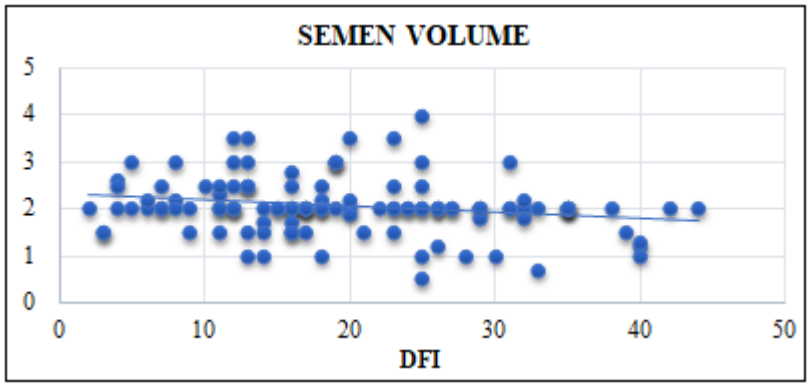

Figure 4: Correlation between semen volume and DFI.
Individual data points and the regression line are shown
Spearman´s correlation coefficient=-0.234 p=0.010.

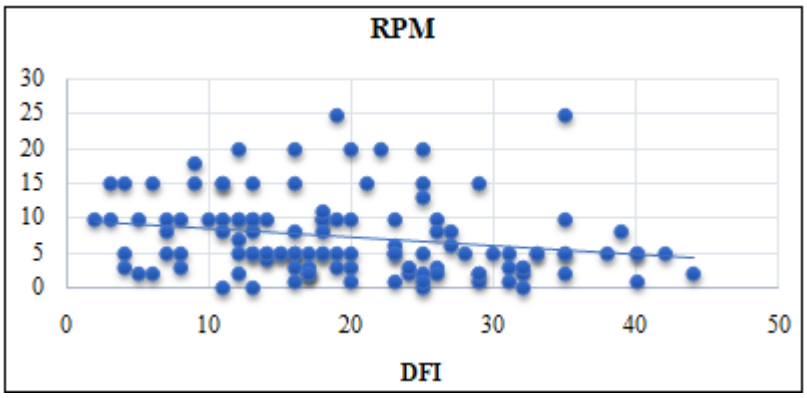

Figure 5: Correlation between RPM and DFI
Individual data points and the regression line are shown.
Spearman´s correlation coefficient=-0.277 p=0.0002.

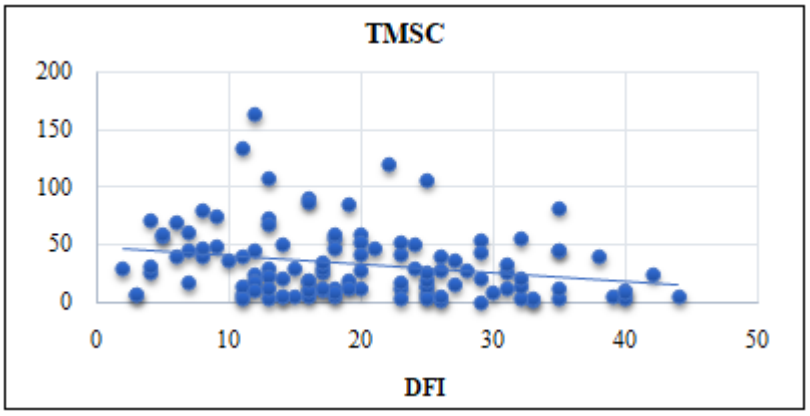

Figure 6: Correlation between TMSC and DFI.
Individual data points and the regression line are shown.
Spearman´s correlation coefficient=-0.262 p=0.0004.

Most of the men with infertility have higher seminal ROS leading to oxidative stress and altered semen parameters. DNA damage induced by oxidative stress accelerates the apoptosis of germ cells, leading to a reduction in sperm count. Studies have reported a significant correlation between the sperm DFI (SCD assay) and semen parameters like sperm motility, morphology and concentration.2[6] Study conducted by Sivanarayana et al reported a negative correlation of sperm concentration, motility and normal morphology which were significantly lower in the High DFI group (DFI ≥ 30%) than in the normal DFI group (DFI < 30%) and used the SCD assay to calculate DFI.2[7] Study conducted by Le MT et al showed that DFI established a significantly positive correlation (p=0.0003) with abnormal head and negative correlation (p=0.0027) with progressive motility.2[8]Our results in men with subfertilty showed a significant negative correlation between DFI and semen volume (p=0.010), rapid progressive motility (p=0.002) and total motile sperm count (p =0.004) as depicted in Figure 4, 5 and 6 respectively. However, other parameters such as days of abstinence, concentration, total motility, and morphology exhibited no significant correlation in our study.

In conclusion, our study demonstrated a strong correlation between sperm DNA fragmentation with routine semen parameters and age. Hence, the evaluation of the sperm DNA fragmentation index should be considered as an additional step in assessing male infertility.

References

[1] Agarwal A, Mulgund A, Hamada A, Chyatte MR. A unique view on male infertility around the globe. ReprodBiol Endocrinol. 2015; 13: 37.
[2] Boitrelle F, Shah R, Saleh R, et al. The Sixth Edition of the WHO Manual for Human Semen Analysis: A Critical Review and SWOT Analysis. Life (Basel, Switzerland). 2021; 11: 1368.
[3] Wang C, Swerdloff RS. Limitations of semen analysis as a test of male fertility and anticipated needs from newer tests. FertilSteril. 2014; 102: 1502-7.
[4] Lewis SE, Aitken JR, Conner SJ, et al. The impact of sperm DNA damage in assisted conception and beyond: recent advances in diagnosis and treatment. Reprod Biomed Online 2013; 27: 325-37.
[5] E. Evgeni, G. Lymberopoulos, S. Touloupidis, B. Asimakopoulos, “Sperm nuclear DNA fragmentation and its association with semen quality in Greek men”, Andrologia, 2015; 47: 1166-74.
[6] Agarwal A, Cho CL, Majzoub A, et al. The Society for Translational Medicine: clinical practice guidelines for sperm DNA fragmentation testing in male infertility. TranslAndrolUrol 2017; 6: 720-33.
[7] Tandara M, Bajić A, Tandara L, Šunj M, Jurišić Z, Jukič M. Correlation between proportions of sperm with DNA fragmentation assessed by Halosperm test and values of standard quality parameters of semen and possible impact on embryo quality.

ZdravniskiVestnik-slovenian Medical Journal. 2013; 82: 298-307.
[8] Evenson DP. The Sperm Chromatin Structure Assay (SCSA (®)) and other sperm DNA fragmentation tests for evaluation of sperm nuclear DNA integrity as related to fertility. AnimReprod Sci. 2016; 169: 56-75.
[9] Gosalvez J, Caballero P, Lopez-Fernandez C, et al. Can DNA fragmentation of neat or swim-up spermatozoa be used to predict pregnancy following ICSI of fertile oocyte donors? Asian J Androl. 2013; 15: 812-18.
[10] Petersen CG, Mauri AL, Vagnini LD, et al. The effects of male age on sperm DNA damage: an evaluation of 2, 178 semen samples. JBRA Assist Reprod. 2018; 22: 323-30.
[11] Gomes M, Goncalves A, Rocha E, et al. Effect of in vitro exposure to lead chloride on semen quality and sperm DNA fragmentation. Zygote 2015; 23: 384-93.
[12] Ni K, Steger K, Yang H, et al. Sperm protamine mRNA ratio and DNA fragmentation index represent reliable clinical biomarkers for men with varicocele after microsurgical varicocele ligation. J Urol 2014; 192: 170-6.
[13] Feijó CM, Esteves SC. Diagnostic accuracy of sperm chromatin dispersion test to evaluate sperm deoxyribonucleic acid damage in men with unexplained infertility. FertilSteril. 2014; 101: 58-63.
[14] Chohan KR, Griffin JT, Lafromboise M, De Jonge CJ, Carrell DT. Comparison of chromatin assays for DNA fragmentation evaluation in human sperm. J Androl. 2006; 27: 53-9.
[15] Jarak I, Almeida S, et al. Senescence and declining reproductive potential: insight into molecular mechanisms through testicular metabolomics. BiochimBiophys Acta (BBA)-Molecular Basis of Disease. 2018; 1864: 3388-96.
[16] S. Gunes, G. N. Hekim, M. A. Arslan, R. Asci, “Effects of aging on the male reproductive system”, J Assist Reprod Genet. 2016; 33: 441-54.
[17] M. Das, N. Al-Hathal, M. San-Gabriel, S. Phillips, I. J. Kadoch, F. Bissonnette et al., “High prevalence of isolated sperm DNA damage in infertile men with advanced paternal age”, J Assist Reprod Genet, 2013; 30: 843-8.
[18] S. Belloc, M. Benkhalifa, M. Cohen-Bacrie, A. Dalleac, E. Amar, A. Zini, “Sperm deoxyribonucleic acid damage in normozoospermic men is related to age and sperm progressive motility”, FertilSteril, 2014; 101: 1588-93.
[19] L. G. A. Campos, L. C. Requejo, C. A. R. Minano, J. D. Orrego, E. C. Loyaga, L. Gonzales Cornejo, “Correlation between sperm DNA fragmentation index and semen parameters in 418 men seen at a fertility center”, JBRA Assisted Reproduction 2021; 25: 349-57.
[20] I. Kaarouch, N. Bouamoud, A. Madkour, N. Louanjli, B. Saadani, S. Assou et al., “Paternal age: negative impact on sperm genome decays and IVF outcomes after 40 years”, Mol Reprod Dev. 2018; 85: 271-80.
[21] S. Alshahrani, A. Agarwal, M. Assidi, A. M. Abuzenadah, D. Durairajanayagam, A. Ayaz et al., “Infertile men older than 40 years are at higher risk of sperm DNA damage”, ReprodBiol Endocrinol. 2014; 12: 103.
[22] La Vignera S, Condorelli RA, Balercia G, Vicari E, Calogero AE. Does alcohol have any effect on male reproductive function? A review of literature. Asian J Androl. 2013; 15: 221-5.
[23] Pearce KL, Hill A, Tremellen KP. Obesity related metabolic endotoxemia is associated with oxidative stress and impaired sperm DNA integrity. Basic Clin Androl. 2019; 29: 6.
[24] Ventimiglia E, Capogrosso P, Colicchia M, Boeri L, Serino A, Castagna G, et al. Metabolic syndrome in white European men presenting for secondary couple’s infertility: investigation of the clinical and reproductive burden. Int J Androl. 2016; 49: 944-51.
[25] Kort HI, Massey JB, Elsner CW, et al. Impact of body mass index values on sperm quality and quantity. J Androl 2006; 27: 450-2.
[26] Velez de la Calle JF, Muller A, Walschaerts M, et al. Sperm deoxy-ribonucleic acid fragmentation as assessed by the sperm chromatin dispersion test in assisted reproductive technology programs: resultsof a large prospective multicenter study. FertilSteril. 2008; 90: 1792-9.
[27] Sivanarayana T, Ravi Krishna C, Jaya Prakash G, et al. Sperm DNA fragmentation assay by sperm chromatin dispersion (SCD): correlation between DNA fragmentation and outcome of intracytoplasmic sperm injection. Reprod Med Biol. 2014; 13: 87-94.
[28] Le MT, Nguyen TA, Nguyen HT, Nguyen TT, Nguyen VT, Le DD, Nguyen VQ, Cao NT. Does sperm DNA fragmentation correlate with semen parameters?. Reproductive medicine and biology. 2019 Oct; 18: 390-6.