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Original Research Article

Correlation between sperm DNA fragmentation index (DFI) with demographic characteristics, sexual history, social habits, chronic illness, BMI: A cross-sectional study

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ABSTRACT

Background: During an evaluation of infertile men when all standard semen parameters are normal, a significant proportion of infertile men are found to have increased levels of DNA damage that may adversely affect fertility.
Objective: To evaluate the correlation between sperm DNA Fragmentation Index (DFI) with demographic characteristics, sexual history, social habits, chronic illness, BMI, physical characteristics, and abstinence period.
Materials and Methods: The current study was carried out among male patients visiting an infertility clinic at SDM College of Medical Sciences and Hospital, Dharwad.
Results: The present has shown no statistically significant association between DFI and socio-demographic characteristics like age, married life, contraceptive usage, sexual factors, personal habits, chronic illness, BMI, and physical characteristics of semen analysis like liquefaction and viscosity of the study participants.
Conclusions: DFI categories and semen traits including normal forms, head defects, tail defects, amorphous forms, droplet forms, and viable sperms had different means, however these differences (p=0.4378) were not statistically significant.

1. Introduction

Standard semen analysis may miss modest sperm flaws such DNA damage, which might reduce male fertility. In addition to semen analysis, anomalies in sperm chromatin have received a great deal of attention in recent years as a potential cause of male infertility.[1] It is important to know whether the amount of DNA damage exceeds the DNA repair capacity of oocytes. Because of growing worries that assisted reproductive technologies (ART), particularly intracytoplasmic sperm injection (ICSI), may transmit damaged DNA, more attention is being placed on the male gamete's DNA integrity.

Similarly, significant levels of DNA fragmentation are frequently seen in males with aberrant semen parameters, but the opposite is also true for men with normal semen parameters.[2] Semen characteristics such as volume, sperm motility, and the percentage of morphologically normal sperm decline gradually with age but not sperm concentration.[3] Increased paternal age is also linked to an increase in point mutation frequency, increased DNA fragmentation, and numerical and structural chromosomal

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abnormalities.[4]

The assessment of sperm chromatin is difficult for a number of reasons, including the difficulty in establishing a connection between the results of chromatin integrity and understood physiological mechanisms, the ongoing debate over the value of sperm chromatin structure assessment in clinical practice, particularly in ART, and the lack of a standardized method and cutoff for determining sperm chromatin integrity. On the other hand, assessing DNA damage is challenging due to the complex sperm chromatin structure and variability in the sperm population. The majority of DNA contains non-coding sections or introns, and oocytes can repair sperm DNA damage, therefore it is generally known that not all DNA damage is fatal. But it is undeniable that sperm chromatin analysis offers effective diagnostic and predictive capacities for fertility/infertility.

Only the sperm chromatin structure assay (SCSA) and the terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick end labeling (TUNEL) research have so far proven clinical thresholds for sperm chromatin assessment. The documented biological variability of sperm DNA damage within them over time should be taken into account when evaluating infertile men, even if it is more stable than typical semen characteristics.[5] Numerous studies have revealed a sizable difference in the amount of sperm DNA damage between fertile and infertile men.[6]Additionally, infertile patients' spermatozoa are typically more vulnerable to the effects of DNA-damaging substances like radiation and hydrogen peroxide.[7]If the proportion of sperm cells with DNA damage exceeds 30% as detected by the SCSA or 20% as detected by TUNEL, then the probability of fertilization in vivo becomes close to zero.[8] Thus, sperm DNA integrity can be considered as an objective marker of sperm function and it serves as a significant prognostic factor of male infertility.[9] SCSA-defined DNA damage has been found to significantly increase in infertile males with normal sperm parameters. This suggests that infertile men who have been given the diagnosis of idiopathic infertility based on seemingly normal standard semen characteristics may really have a concealed sperm defect when sperm DNA damage is analyzed.[10,11]

Before beginning chemotherapy, radiation therapy, or surgery, patients with a known case of cancer are frequently transferred to sperm banks for cryopreservation of spermatozoa. Although live births and conceptions utilizing cryo-preserved sperm from cancer patients have been documented, these semen samples frequently have lower fertility potential due to an increased proportion of DNA damage. Only the sperm chromatin structure assay (SCSA) and the terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick end labeling (TUNEL) research have so far proven clinical thresholds for sperm chromatin assessment. The documented biological variability of sperm DNA damage within them over time should be taken into account when evaluating infertile men, even if it is more stable than typical semen characteristics.[5] Numerous studies have revealed a sizable difference in the amount of sperm DNA damage between fertile and infertile men.[6] Additionally, infertile patients' spermatozoa are typically more vulnerable to the effects of DNA-damaging substances like radiation and hydrogen peroxide.[7]

2. Materials and Methods

Infertility clinic patients at SDM Medical College and Hospital in Dharwad between the ages of 21 and 45 participated in the current cross-sectional study. The study sample consisted of 100 men in total. The study was carried out for a period of one year from December 2018 to November 2019 with IEC approval SDMIEC/PG/0176/2018 dated: 12.11.2018.

2.1. Inclusion criteria

Men between the ages of 21 and 45 who attended the SDM HOSPITAL infertility clinic and had at least one year of unprotected sexual contact were apparently healthy.

2.2. Exclusion criteria

Azoospermia, men who have had varicocele repair, an orchidopexy, or a vasectomy. hypergonadotropic hypogonadic males. Men who have been diagnosed with testicular cancer or who have had chemotherapy or radiotherapy for the disease males with congenital testicular or Vas deferens abnormalities.

2.3. Data collection

For the study, a random sample of patients from the SDM Medical College's infertility clinic was chosen. It was obtained with written and full consent. The patient's age, length of marriage, recent use of contraception, sexual dysfunction, and anosmia history were documented. It was noted that the patient's medical history included episodes of bronchiectasis, mumps, orchitis after puberty, diabetes milletus, recurrent chest infections, hypertension, hypothyroidism, and hyperthyroidism. Such individuals were excluded if they had undergone herniorrhaphy, hydrocele drainage, testicular torsion, varicocelectomy, orchidopexy, or correction of hypospadiasis and epispadiasis.

Coitus was observed frequently, along with erectile dysfunction, premature ejaculation, and dyspareunia. The patient's social habits, including smoking, drinking, and drug usage, were elicited. The patient underwent a complete physical examination, including a genital exam, to identify any local diseases. The patient's semen sample was taken, and it was examined using WHO (2010) criteria. Prior to the semen analysis, patients were instructed to abstain for at

least 2-3 days. Masturbation was used to collect the semen sample, which was then promptly delivered to the lab for analysis.

2.4. DNA fragmentation index (DFI)

DNA fragmentation index of the samples carried out according to manufacture protocol using TUNEL assay (ApoTM alert kit)- Clontech.[12]

2.5. Statistical analysis

SPSS software was used to enter and evaluate all data. Using the Chi-square test, descriptive analysis of the data and other parameters were compared. Participants were split into groups with good fertility (DFI 20) and low fertility (DFI>20) for the purposes of statistical analysis of the study results. Spearman's rank correlation coefficient was used to assess the relationships between the variables. Statistics were judged significant at P 0.05.

3. Results

Amongst study participants 23 (63.89%) and 13 (36.11%) participants with good fertility and poor fertility respectively belong to 31-35 years of age. Majority of the participants with good fertility 33 (70.12%) had a married life of 1-5 years and 61 (66.30%) were not using contraceptives. However, there is no statistically significant association between DFI and socio-demographic characters like age, married life and contraceptive usage as mentioned in the Table 1.

In the present, majority of the participants with good fertility had no problems pertaining to coitus like erectile dysfunction 66 (66%), Premature ejaculation 66 (66%), and Dyspareunia 64 (66.67%). Majority of the participants with poor fertility had their frequency of coitus 2-3 times per week. There is no statistical significance associated between DFI and sexual factors of the participants as showed in the Table 2.

In our study**,** majority of the participants with good fertility indicators did not have personal habits like smoking 60(65.22%), Alcohol consumption 59 (67.05%) and substance abuse 59 (64.13%). The statistical significance is no found between DFI and personal habits of the study participants as represented in Table 3.

Majority of the patients with good fertility had no diabetes mellitus 64(65.98%), hypertension 66(66.67%), bronchiectasis 66(66%), and anosmia 66(66%). None of the patients had undergone herniorrhaphy 66(66%). There is no statistical significance associated between DFI and chronic illness of the participants as mentioned Table 4.

Majority of the study participants with good fertility 39(70.91) had their BMI within normal limits. Most of the participants with poor fertility 16(29.09) had their BMI within normal limits. However, there is no statistically significant association between DFI and BMI of the study participants as noted in Table 5.

Majority of the participants with good fertility 36(66.67%) were remaining abstinent for a period of 2-3 days prior to semen analysis. Normal liquefaction time 53(62.35%), and normal viscosity 50(65.79%) was observed in majority of the patients with good fertility. The study participants with poor fertility had their abstinence period of 2-3 days 18(33.33%), normal liquefaction time 32(37.65%), normal viscosity 26(34.21%). There is no statistical significance associated between DFI and physical characteristics of semen analysis like liquefaction and viscosity. (p = >0.05) as represented in Table 6.

4. Discussion

We found no statistically significant correlation between DFI and sociodemographic characteristics including age, marital status, or recent use of contraception in the current study. According to a study by Marij et al., no other demographic factors significantly affect DFI in infertile patients; only an increase in paternal age is linked to an increase in sperm DNA damage.[13] Belloc et al. and Das et al. have found a link between a high level of DFI and paternal age that is advanced (>40 years).[14] DFI and sexual variables do not statistically significantly correlate.

Studies conducted by Sun et al., and Potts et al., have shown that paternal smoking increases sperm DNA damage as measured by TUNEL technique and it is associated with an increased incidence of childhood cancer[15] Sepania et al., and Viloria et al., in their studies, have found an association between smoking which increases reactive oxygen species (ROS) and sperm DNA damage in infertile men.[16] Social habits like smoking and alcohol are associated with high DFI rate (Sepaniak et al., and Sharma et al.)[14]

There is no statistically significant link between participants' DFI and chronic illness. (p= 1.000). DFI and BMI of the study subjects did not show any statistically significant correlation (p= 0.3070). Cabler et al. and McPherson et al. have discovered a link between high DFI and obesity-related impaired spermatogenesis.[14]

The physical properties of the semen analysis, such as liquefaction (p=0.9210), viscosity (p=0.1440), and abstinence period (p=0.3620), are not significantly associated with DFI. Cohn-Bacrie et al.'s prospective analysis revealed a positive connection between DFI and length of abstinence (p 0.006).[17]

We discovered a statistically significant difference (p=0.0217) between fertility categories and research participants' height. It will take more research with a big sample size to understand this. The marital status and BMI of the study participants' t-values are negative in our study, indicating a reversal in the direction of the impact, although this has no bearing on the significance of the difference between the groups.

Table 1: Association between DFI and demographic characteristics

Profile	Good fertility (DFI< 20)	%	Poor fertility (DFI>20)	%	Total	Chi-square	p-value
Age groups							
<=30yrs	8	66.67	4	33.33	12		
31-35yrs	23	63.89	13	36.11	36	0.6130	0.8940
36-40yrs	19	63.33	11	36.67	30		
41-45yrs	16	72.73	6	27.27	22		
Married life							
1-5yrs	33	70.21	14	29.79	47		
6-10yrs	26	65.00	14	35.00	40	1.2450	0.5370
>=11yrs	7	53.85	6	46.15	13		
Contraception							
Not used	61	66.30	31	33.70	92		
Used	5	62.50	3	37.50	8	0.04700	0.8280
Tota	66	66.00	34	34.00	100		

Table 2: Association between DFI and sexual history

Sexual history	Good fertility (DFI <20%)	%	Poor fertility (DFI >20%)	%	Total	Chi-square	p-value
Frequency of coitus /week							
1—2	2	50.00	2	50.00	4		
2—3	44	69.84	19	30.16	63	1.2980	0.5220
4—5	20	60.61	13	39.39	33		
Erectile dysfunction							
No	66	66.00	34	34.00	100	0.0000	1.0000
Yes	0	0.00	0	0.00	0		
Premature ejaculation							
No	66	66.00	34	34.00	100	0.0000	1.0000
Yes	0	0.00	0	0.00	0		
Dyspareunia							
No	64	66.67	32	33.33	96		
Yes	2	50.00	2	50.00	4	0.0230	0.8800
Total	66	66.00	34	34.00	100		

Table 3: Association between DFI and social habits

Social habits	Good fertility (DFI<20%)	%	Poor fertility (DFI>20%)	%	Total	Yates Chi-square	p-value
Smoking							
No	60	65.22	32	34.78	92	0.0290	0.8640
Yes	6	75.00	2	25.00	8		
Alcohol consumption							
No	59	67.05	29	32.95	88	0.074	0.7850
Yes	7	58.33	5	41.67	12		
Substance abuse							
No	59	64.13	33	35.87	92		
Yes	7	87.50	1	12.50	8	0.9010	0.3420
Total	66	66.00	34	34.00	100		

Table 4: Association between DFI and chronic illness

Chronic illness	Good fertility (DFI <20%)	%	Poor fertility (DFI>20%)	%	Total	Yates Chi-square	p-value
Diabetes Mellitus							
No	64	65.98	33	34.02	97	0.0000	1.0000
Yes	2	66.67	1	33.33	3		
Hypertension							
No	66	66.67	33	33.33	99	0.0000	1.0000
Yes	0	0.00	1	100.00	1		
Bronchiectasis							
No	66	66.00	34	34.00	100	0.0000	1.0000
Yes	0	0.00	0	0.00	0		
Anosmia							
No	66	66.00	34	34.00	100	0.0000	1.0000
Yes	0	0.00	0	0.00	0		

Table 5: Association between DFI and BMI

BMI	Good fertility (DFI<20%)	%	Poor fertility (DFI>20%)	%	Total	Chi-square	p-value
Normal	39	70.91	16	29.09	55	2.3640	0.3070
Over weight	19	55.88	15	44.12	34		
Obese	8	72.73	3	27.27	11		
Total	66	66.00	34	34.00	100		

Table 6: Association between DFI and physical characteristics and abstinence period

Semen Analysis	Good fertility (DFI<20%)	%	Poor fertility (DFI>20%)	%	Total	Chi-square	p-value
Abstinence							
2-3days	36	66.67	18	33.33	54	4.3430	0.3620
4-5days	25	64.10	14	35.90	39		
>=6days	5	71.43	2	28.57	7		
Liquefaction time							
<30min	53	62.35	32	37.65	85	0.1650	0.9210
31-44min	5	83.33	1	16.67	6		
>44min	8	88.89	1	11.11	9		
Viscosity							
Grade 1	50	65.79	26	34.21	76	5.4150	0.1440
Grade 2	6	46.15	7	53.85	13		
Grade 3	8	88.89	1	11.11	9		
Grade 4	2	100	0	0.00	2		
Total	66	66.00	34	34.00	100		

5. Conclusion

There is no statistically significant association between DFI and socio-demographic characters like age, married life, and contraceptive usage, sexual factors, personal habits, chronic illness, BMI, or physical characteristics of semen analysis like liquefaction and viscosity of the study participants.

6. Source of Funding

Nil.

7. Conflict of Interest

Nil.

8. Ethical Approval

Ethical approval was obtained from Shri Dharmasthala Manjunatheshwara college of Medical Science and Hospital. SDMIEC/PG/0176/2018 dated: 12.11.2018.

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