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Original Research Article

CORRELATION BETWEEN SPERM DNA FRAGMENTATION INDEX (DFI)WITH VARIOUS SPERM PARAMETERS IN MEN ATTENDING INFERTILITY CLINIC

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Abstract

Background: Semen analysis is being used routinely to evaluate infertile men. A significant overlap in sperm concentration, motility and morphology between fertile and infertile men has been noted. To study the correlation between sperm DNA fragmentation index with various sperm parameters in a apparently healthy men between the age group of 21 to 45years with infertility. **Materials and Methods:** DFI is calculated by using TUNNEL ASSAY, SEMEN ANALYSIS interpreted by using WHO 2010 criteria. All data was entered and analyzed using SPSS software. Descriptive analysis of data and various factors was compared by using Chi–square test. For statistical analysis of study outcome, we have divided participants into good fertility (DFI <20) and poor fertility (DFI >20).Correlations between variables were analyzed using Spearman's rank correlation coefficient. P< 0.05 was considered statistically significant. **Result:** Statistically significant (p=0.0269) negative correlation has been observed between DFI with midpiece defects of sperms. It could be a spurious association. To explain this correlation further studies are needed with large sample size. In our study we have found a negative correlation between semen volume, sperms with cytoplasmic droplets, tail defects, head defects with DFI, but it is statistically not significant. A positive correlation between semen parameters like sperm concentration, sperm count, progressive motility, normal forms, sperms with tail defects and viable sperms with DFI of study participants is noticed, but it is statistically not significant. **Conclusion:** Even though a large number of tests are available to assess different aspects of sperm DNA integrity, but there are no standard recommendations for clinical cut-off level, and which assay is best.

INTRODUCTION

Infertility is prevalent in 9% of couples of reproductive age and is defined as the inability to establish pregnancy within 12 consecutive months of unprotected intercourse. Amongst infertile couples, 20% is due to male factors alone.[1] Continuous decline in the male fertility over time, which cannot be attributed to any specific cause, results in idiopathic infertility.[2] In many couples seeking andrology clinic the cause for male infertility will not be able to find out, which reflects our still very poor understanding of the mechanisms that govern testicular function.

The causes of male infertility can be divided into four major categories:

- Hypothalamic-pituitary disorders (1–2%), which may be congenital, be acquired, or result from systemic illness.
- Idiopathic (40–50%).
- Primary gonadal disorders (30–40%), both congenital and acquired.
- Disorders of sperm transport (10–20%).

All the above mentioned causes for male infertility can influence sperm DNA fragmentation (SDF). It has been observed that after 35years semen quality decreases and after 40 years SDF increases.[3] While offering advanced reproductive technologies (ART) we employ only a cursory evaluation of the male, which rarely extends beyond semen analysis and anti sperm antibody detection. During semen analysis a significant overlap in sperm

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concentration, motility, and morphology between fertile and infertile men has been demonstrated.[4]

There are concerns regarding sperm chromosomal abnormalities, congenital malformations, and developmental abnormalities in ICSI-born progeny.[5] Currentevidence suggests that a negative relationship exists between sperm DNA damage and the fertility potential of spermatozoa, whether in vivo or in vitro.[6]Abnormalitiesin sperm DNA may be indicative of male sub fertility, regardless of normal semen parameters.[7] Sperm DNA structure evaluation is an independent measure of sperm quality which provides good diagnostic and prognostic capabilities. Therefore, assessment of DNA damage is considered to be a reliable predictor of a couple's inability to become pregnant.[8]

Sperm DNA integrity is directly correlated with pregnancy outcome following in vitro fertilization.[8] Increased sperm DNA fragmentation can compromise embryo quality and it results in pregnancy loss.[9] In addition, high sperm DNA fragmentation can also compromise the progression of pregnancy and result in spontaneous miscarriage following ART. High sperm DNA fragmentation can affect embryo post implantation development in ICSI procedures.[9] Therefore; along with semen analysis sperm DNA fragmentation analysis should be included in the evaluation of the infertile male.[10] Many techniques have been described for the evaluation of sperm chromatin status. In this study we have used TUNEL assay for the evaluation of sperm chromatin status.

Advancing paternal age is associated with an increased percentage of ejaculated spermatozoa with DNA damage.[11] A number of studies have observed that advanced paternal age is associated with an increase in the prevalence of birth defects (e.g., neural tube defects, cardiac defects, and limb defects); congenital diseases (e.g., Wilms tumor); increase in new autosomal dominant mutations (e.g., achondroplasia and Alpert, Waardenburg, Crouzon, Pfeiffer, and Marfan syndromes);increased incidence of X-linked diseases (hemophilia A and Duchenne muscular dystrophy); increased risk for schizophrenia in offspring.[12] Similarly, increasing paternal age isassociated with increase in the risk of spontaneous abortion and autism in children, which could be because ofde novo mutations or errors in genetic imprinting.[13] Overall, the incidence of anomalies is increased two to threefold in children whose fathers are over age 45 years, possibly due toemerging mutations during spermatogenesis.[14] The above association between paternal age and incidence of anomalies in the offspring could be because of increasedDNA damage, hence sperm DNA damage assessment should beincluded in the evaluation of infertile men.

It has been shown that the proportion of sperm with DNA damage is significantly higher in men from couples with recurrent pregnancy loss than in the general population or fertile donors.[15] It has also been reported that 39% of miscarriages could be predicted using a combination of selected cut-off values for percentage spermatozoa with denaturated (likely fragmented) DNA and/or abnormal chromatin packaging as assessed by SCSA.[8] An increased trend of spontaneous abortions following IVF/ICSI was also demonstrated when sperm from men with a large amount of damaged DNA were used.[16] Thus, it is possible that the assessment of sperm DNA damage could be a good predictor of possible miscarriage. In this study we are evaluating the correlation between DNA FRAGMENTATION INDEX (DFI) and with various semen parameters.

MATERIALS AND METHODS

Present Study was conducted among Men attending infertility clinic at SDM COLLEGE OF MEDICAL SCIENCES AND HOSPITAL, DHARWAD. Informed consents were obtained from subjects involved and participation-information sheet was filled out. Based on the inclusion and exclusion criteria, the subjects were selected.

Inclusion Criteria

- Apparently healthy men between the age group of 21- 45 years, attending infertility clinic at SDM HOSPITAL, who had at least one year of unprotected sexual intercourse.

Exclusion Criteria

- Azoospermia
- Men who have undergone vasectomy, orchidopexy, varicocele repair.
- Men with hypergonadotrophic hypogonadism.
- Men who are diagnosed with testicular malignancy or those who have received chemotherapy or radiotherapy for testicular malignancy
- Men with congenital anomalies of testes or Vas deferens

Data Collection

Patients attending infertility clinic at SDM medical college were randomly selected for the study. Witten and informed consent was taken. Patient's age, duration of marriage, contraception used in the recent past, sexual dysfunction and history of anosmia were noted. Medical history of the patient like history of mumps orchitis after puberty, history of diabetes milletus, recurrent chest infections, hypertension, hypothyroidism, hyperthyroidism, hyperprolactinemia and bronchiectasis was recorded. If the patient has undergone herniorrhaphy, hydrocele drainage, detorsion of the testes, varicocelectomy, orchidopexy, correction of hypospadiasis and epispadiasis such patients were excluded.

Frequency of the coitus, erectile dysfunction, premature ejacualation, dyspareunia was noted. Social habits of the patient like smoking, alcohol consumption and substance abuse were elicited. A thorough physical examination of the patient including genital examination was done to note down the local pathologies. Semen sample of the

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patient was collected and analysed by applying WHO (2010) standards. Patients were instructed to remain abstinent for atleast 2-3 days prior to semen analysis. Semen sample was collected by masturbation in a clean wide mouthed dry glass jar and sent to the laboratory immediately for analysis.

DNA Fragmentation index (DFI)

DNA fragmentation index is done in our hospital by using TUNEL assay.(ApoTM alert kit)

Principle

- The TUNEL is a direct assay, which quantifies the incorporation of dUTP at double strand DNA breaks in a reaction catalyzed by the template independent enzyme terminal deoxynucleotidyl transferase (TdT).
- This enzyme (TdT) incorporates biotinylated deoxyuridine to 3′-OH of DNA to create a signal, which increases with the number of DNA breaks.
- Sperm with normal DNA therefore have only background staining/fluorescence, whereas those with fragmented DNA (multiple chromatin3′-OH ends) stain/fluoresce brightly.17

Clinical Significance

- The TUNEL assay is being widely used in male infertility research related to sperm chromatin abnormalities. Many studies have shown a negative correlation between the percentage of DNA-fragmented sperm and the motility, morphology, and concentration in the ejaculate.
- DFI assessment also serves as a good predictor for IUI pregnancy rate, IVF embryo cleavage rate, and ICSI fertilization rate.
- In addition, DFI assessment provides an explanation for recurrent pregnancy loss in infertile couples.18
- Various studies have recommended a predictive threshold DFI for in vivo fertility between fertile and infertile men (20% of TUNEL-positive cells),[19] although it differs from that demonstrated for IUI (12%),[20] and other ART procedures.

RESULTS

The mean age of the participants with good and poor fertility was 36.05±5.10 and35.91±4.94years respectively. deference is not statistically significant.

The mean height and weight of the participants with good and poor fertility were 166.42±7.73 cms, 69.52±10.17 kg and 162.50±8.42 cms, 66±6.71 kg respectively. We have found statistically significant difference between fertility categories with height of the study participants. In this table t- value is negative for married life and BMI of the study participants which indicates a reversal in the directionality of the effect, which has no effect on the significance of the difference between groups. [Table 1]

Table 1: Comparison of groups of DFI with age, married life and BMI by independent t test

Variables	Good fertility (DFI<20%)		Poor fertility (DFI>20%)		t-value	p-value
	Mean	SD	Mean	SD		
Age	36.05	5.10	35.91	4.94	0.1254	0.9004
Married life	6.52	3.28	7.06	3.92	-0.7334	0.4650
Height	166.42	7.73	162.50	8.42	2.3321	0.0217*
Weight	69.52	10.17	66.00	6.71	1.8190	0.0720
BMI	24.88	3.75	25.14	3.34	-0.3428	0.7324

*p<0.05

The mean semen volume in participants with good and poor fertility is 2.3+1.32 and 2.01+ 0.94 respectively.The mean sperm concentration in participants with good and poor fertility outcome is 67.97+52.53 and 78.18+45.75 respectively.The mean sperm count among the participants with good and poor fertility outcome is 145.10+124.83 and 146.82+91.54 respectively.The difference in semen volume, sperm concentration, sperm count between good and poor fertility groups of the participants is not statistically significant.

Table 2: Comparison of groups of DFI with other parameters by independent t test

Parameters	Good fertility(DFI<20%)		Poor fertility(DFI>20%)		t-value	p-value
	Mean	SD	Mean	SD		
Volume (ml)	2.33	1.32	2.01	0.94	1.2521	0.2135
Sperm concentration (mill/ml)	67.97	52.53	78.18	45.75	-0.9600	0.3394
Total count(mill/ejaculate	145.10	124.83	146.82	91.54	-0.0713	0.9433

The mean progressive motility, non progressive motility of the sperms of participants with good and poor fertility were 46.88+16.35, 49.24+15.41 and 16.17+9.01 , 17.21+ 9.02 respectively. Total motility (PR+NP) of sperms with good and poor fertility is 63.05+13.72 and 66.29+13.83 respectively. The above differences between DFI and progressive motility (PR) is not statistically significant.

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Table 3: Comparison of groups of DFI with other parameters by independenttests.

	Good fertility(DFI <20)		Poor fertility(DFI > 20)		t-value	p-value
	Mean	SD	Mean	SD		
PR %	46.88	16.35	49.24	15.41	-0.6960	0.4881
NP %	16.17	9.01	17.21	9.02	-0.5462	0.5862
PR+NP%	63.05	13.72	66.29	13.83	-1.1184	0.2661

The mean of normal froms, head defects of sperms with good and poor were 11.92+10.89, 9.91+4.89 and 46.17+ 14.88, 41.06+14.49 respectively. The mean of tail defects, mid piece defects of sperms with good and poor fertility were 21.39+ 15.57, 25.79+ 15.54 and 19.30+ 10.99, 16.53+18.19 respectively. The mean of amorphous forms and pin head forms of sprms with good and poor fertility were 4.15+4.10, 3.75+3.70 and 3.54+ 2.03, 2.00 +0.71 respectively.

The mean of droplet forms and viable sperms with good and poor fertility were 3,29+1.20, 1.50+ 0.71 and 60.06 +13.10, 62.29+14.48 respectively. The above differences in mean between DFI and semen parameters like normal forms, head defects, tail defects, amorphous forms, droplet forms and viable sperms were not statistically significant.

Table 4: Comparison of groups of DFI with other parameters by independent t test

Parameters	Good fertility (DFI <20%)		Poor fertility(DFI >20%)		t-value	p-value
	Mean	SD	Mean	SD		
Normal forms %	11.92	10.89	9.91	4.89	1.0242	0.3083
Head defect %	46.17	14.88	41.06	14.49	1.6372	0.1048
Tail defect %	21.39	15.57	25.79	15.54	-1.3273	0.1876
Midpiece defect %	19.30	10.99	16.53	18.19	0.9386	0.3503
Amorphous forms %	4.15	4.10	3.75	3.70	0.2880	0.7750
Pin head %	3.54	2.03	2.00	0.71	1.6338	0.1218
Cytoplasmic droplet %	3.29	1.20	1.50	0.71	2.0090	0.0642
Vitality %	60.06	13.10	62.29	14.48	-0.7791	0.4378

Majority of the patients with good fertility DFI are not yet conceived 30(66.67%), however this is not statistically significant (p> 0.05). Among the study participants with good fertility, two (66.67%) are conceived spontaneously and three(75.00%) conceived by IVF.

Table 5: DFI and Pregnancy Status

Pregnancy Status	Good fertility (DFI<20%)	%	Poor fertility (DFI>20%)	%	Total	Chi-square	p-value
IUI Failed	22	62.86	13	37.14	35	0.3680	0.9850
Conceived spontaneously	2	66.67	1	33.33	3		
Delivered (IVF Conception)	3	75.00	1	25.00	4		
Gamates cryopreserved	30	66.67	15	33.33	45		
Pregnant (IVF Conception)	9	69.23	4	30.77	13		
Total	66	66.00	34	34.00	100		

This table shows positive correlation between semen parameters like sperm concentration, sperm count, progressive motility, normal forms, sperms with tail defects and viable sperms with DFI of the study participants, but it is statistically not significant.

There is negative correlation between semen volume, sperms with cytoplasmic droplets, tail defects, head defects and DFI of the study participants, but it is statistically not significant.

Statistically significant (p= 0.0269) negative correlation is observed between DFI and midpiece defects. It could be a spurious association and it could be because of small sample size.

Table 6: Correlation between DFI scores with other variables related to Semen Analysis by spearman R correlation coefficient.

Variables	Correlation between DFI scores with		
	Spearman R	t-value	p-level
Volume (ml)	-0.0239	-0.2365	0.8135
Sperm concentration (mill/ml)	0.1454	1.4552	0.1488
Total count(mill/ejaculate	0.1012	1.0069	0.3164
PR %	0.0803	0.7977	0.4270
NP %	0.0115	0.1138	0.9097
PR+NP %	0.1022	1.0174	0.3115
Normal forms %	0.1244	1.2414	0.2174
Head defect %	-0.0878	-0.8678	0.3877
Tail defect %	0.0357	0.3462	0.7300
Mid piece defect %	-0.2236	-2.2480	0.0269*
Amorphous forms %	-0.1043	-0.6376	0.5276
Pin head %	-0.1934	-0.7887	0.4418

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Cytoplasmic droplet %	-0.2065	-0.7897	0.4429
Vitality %	0.0875	0.8700	0.3864

*p<0.05

In this study there is positive correlation between married life and BMI with DFI of the study participants, buti tis statistically insignificant. There is negative correlation between DFI and age of the study participants, however it is not statistically significant

DISCUSSION

The difference in semen volume, sperm concentration, sperm count between good and poor fertility groups of the participants is not statistically significant. The mean progressive motility, non-progressive motility of the sperms of participants with good and poor fertility were 46.88+16.35, 49.24+15.41 and 16.17+9.01, 17.21+ 9.02 respectively. Total motility (PR+NP) of sperms with good and poor fertility is 63.05+13.72 and 66.29+13.83 respectively. There are no statistically significant differences between DFI categories and progressive motility (p=0.4881) of sperms. A prospective study conducted by Cohn-Bacrie et al. has shown an inverse correlation between DFIwith rapid progressive motility of sperms($p < 0.0001$).[21]
Thedifferences in mean between DFI categories and semen parameters like normal forms, head defects, tail defects, amorphous forms, droplet forms and viable sperms(p=0.4378) were not statistically significant. A prospective study conducted by Cohn-Bacrie et al. has showed a negative correlation between DFIwith viablity of sperms($p < 0.0001$).[21]
There is no statistically significant association between DFI categories and pregnancy outcome parameters. Fortunate A, et al. in their studies have shown a significant inverse relationship between pregnancy outcomes and the DFI detected only by the Halo test.[22] Simon L, et al. in their studies have observed a close inverse relationship between sperm DFI assessed by the Comet assaywith live- birth rates after IVF.[23]Benchaib, et al. in their studies have shown that in ICSI conception DNA fragmentation rate was significantly on lower side. when the DNA fragmentation was more than 20% no pregnancies were reported.[24] The clinical pregnancy rates following IVF but not with ICSI decreases significantly in patients with a high degree of sperm DFI by TUNEL assay (Li et al.).[25] When TUNEL cutoff value of 15% is used no significant difference was seen in pregnancy rates after IVF or ICSI (Benchaib et al).[26] Studies conducted by Sun et al. and Borini et al. have shown a negative correlation between DFI with both fertilization and embryo cleavage rate after IVF, which suggest that sperm with DNA damage will fertilize less efficiently and impair embryonic development.[27] In couples with recurrent pregnancy loss, a significant increase in sperm DFI rate has been noticed (Carrell et al).[28] There is no correlation between the clinical pregnancy rate and pregnancy loss rate in groups with less than 10% or more than 10% DFI has been observed. Similarly, no correlation was noted between sperm DFI and the fertilization rate in ICSI (Borini et al).[27] For IVF or ICSI, semen parameters like sperm concentration, progressive motility and normal sperm morphology do not influence the clinical pregnancy rate and pregnancy loss rate (Borini et al).[27] When natural conception is considered, a higher percentage of DFI by TUNEL in infertile patients than in normozoospermic controls has been observed (Gandini et al).[29]
In our study a positive correlation between married life(p=0.4404) and BMI(p=0.8134)with DFI of the study participants has been observed, but it is statistically insignificant. There is negative correlation between DFI with age (p=0.7622) of the study participants and it is not statistically significant. A prospective studyconducted by Cohn-Bacrie et al. has shown a negative correlation between DFIby TUNEL assay and age of the study participants.[21]
Statistically significant(p= 0.0269) negative correlation is observed between DFI and midpiece defects. It could be a spurious association. To explain this correlatio further study is needed with large sample size.
A study conducted by Fortunato A, et al. has shown that a significant inverse correlationbetween sperm morphology and the pregnanciesobtained with DFI in patients undergoing assisted reproductive techniques.[22]
Various studies have found that the percentages of sperms with amorphous heads and overall head abnormalities were significantly higher in sperm samples with high degree of DFI.[22]
There is a negative correlation between the levels of sperm DFI whether measured by COMET (Irvine et al.)TUNEL (Bench et al and Zini et al. a,b) or SCSA (Chohan et al.)with various semen parameters, such as sperm concentration, motility and morphology.
Reason for negative correlation could be high variability of sperm parameters over time within individuals in contrast to measures of sperm DFI by TUNEL, which is stable over time within an individual(Sergerie et al.a,b).[30]
Gandini et al., have showed that in normozoospermic men DNA damage was associated with a lower percentage of apoptosis and the percentage of abnormal sperm head forms ($p < 0.0006$).151 Irvine et al. have showed that Sperm with low motility carry higher rates of DFI as shown by TUNEL and comet assays.152 Borini et al.in their study have showed that there is a significant negative correlation between sperm DFI with semen parameters like concentration, total sperm count, and morphology before and after preparation of sperms.[27]

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Even though we have been using different tests to assess SDF, still there is a lack of optimisation and clear-cut clinical reference values, which makes the routine use of the SDF assays controversial. In future more comprehensive studies are needed for SDF testing to infertile couples for better management.

CONCLUSION

Even though different tests are available to assess SDF, still they lack optimisation and clear-cut clinical reference values, which makes the routine use of the SDF assays controversial. As this is just the beginning of the use of SDF assays in clinical practice, in future more comprehensive studies may increase the scope of providing SDF testing to infertile couples for better management.

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