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Original Research Article

Comparative analysis of sperm DNA fragmentation index with sperm chromatin dispersion in varied infertility types: a retrospective study

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ABSTRACT

Background: Infertility is a major modern concern amongst couples due to the change in their lifestyle and being carrier-oriented leading to late marriage/s. Out of all the couples suffering from infertility, approximately 30-40% cases are contributed to the male factor. As the age advances, the reduced sperm count magnifies the problem. However, in addition to that, the qualitative change along with quantity is of much more importance. Aims and objectives were to find the prevalence of sperm DNA fragmentation with sperm chromatin dispersion (SCD) test and its comparison with types of infertility

Methods: The present study was retrospective that collected data from the semen sample given for routine check-up during the period of August 2022 to August 2023. A total of 138 semen analysis and sperm DNA Fragmentation Index (DFI) were carried out during this period. All 138 couples were further divided into 2 groups- 62 couples having oligozoospermia as cases and 76 couples with normozoospermia as controls and the data was compared.

Results: Among 138 subjects, (62 cases and 76 control) a significant difference in the age was found. Smoking as a risk factor was found to be statistically significant. While the sperm count was not significantly different in cases and controls, a statistically significant difference was found in DFI (p=0.001) in both the groups. The highest value for DFI was 12.78 % in controls and 24.98% in cases. DFI and sperm count showed negative correlation both in DFI and sperm count with a Karl Pearson's correlation coefficient being 0.213 (p value <0.01) and 0.754 (p value <0.005), respectively. A significant difference was observed in the median value of sperm DFI. When DFI was compared to semen analysis, it yielded 87% sensitivity and 83% specificity. Surprisingly, 13 controls out of 76 i.e. around 17.1% had poor DFI inspite of normal semen analysis parameters.

Conclusions: On comparing, significant difference was observed in the median value of sperm DFI. SCD method is simple, easiest and standard tool to assess DFI.

Keywords: Male infertility, Semen analysis, Sperm DNA fragmentation index

INTRODUCTION

Approximately 15% of all couples trying to conceive are affected by Infertility and recent studies indicate an increase in male infertility rates.[1] Out of them, approximately 50% of the cases are attributed to male factor i.e. around every sixth men is infertile.[2] The problem becomes more, as many men may not accept infertility. In around 30-40% of the cases there are no identifiable factors and are deemed idiopathic.[3] The routinely used test to assess male infertility is semen analysis i.e. sperm concentration, motility and morphology; however, these parameters have been known to be limited as surrogate markers of male fertility. Nearly around 15% of patients with male infertility have a normal semen analysis.

Sperm count is an important test to diagnose male infertility. But it is not a sufficient test as quality required

for positive outcome is not measured. Once sperm count is reported normal, a man feels happy that he has no problem. But the cause of poor pregnancy outcomes (being it miscarriage or failure to implant multiple times i.e. recurrent implantation failure) in couples with otherwise unexplained infertility maybe due to quality of the sperm i.e. due to defective sperm DNA integrity.[4] The biological structure of sperm cannot be determined with routine semen analysis. For its determination, a specialized test has to be carried out including sperm DNA fragmentation index (DFI).[5]

The damage to sperm DNA may originate from the testis and/ or during transit through the reproductive duct. Physiologically, a small amount of reactive oxygen species (ROS) is produced by the spermatozoa for the capacitation and fertilization. But, whenever the concentration increases, it may affect the sperm quality and lead to oxidative stress induced sperm DNA damage.[6] To test the quality, it is worthwhile to check for Sperm DFI that reflects integrity of genetic material of the gamete. DFI can be affected by many factors including age, smoking, alcoholism, high local temperature, varicocele and many others out of which some are modifiable.[7]

Sperm DFI assess the quality of the sperm by evaluating DNA package. The tests are, therefore, distinct and more significant than the conventional semen parameters.[8] Many techniques evaluate sperm chromatin quality in the form of sperm DFI such as sperm chromatin structure assay (SCSA), terminal deoxyuridine nick end labelling (TUNEL) assay, COMET assay (single gel electrophoresis), acridine orange-staining technique (AOT). The newer and economic technique is sperm chromatin dispersion (SCD) test.

Aims and objectives

To assess sperm DFI with sperm chromatin dispersion (SCD) test and find its prevalence. To compare sperm DFI with types of infertility.

METHODS

It was a retrospective study conducted in one of the reputed IVF centres of western India wherein sperm DFI was routinely assessed in all the patients. Data was obtained from andrology laboratory regarding the tests done during 1 August 2022 to 31 August 2023 of all the subjects who were subjected to sperm DFI tests. During the specified time period, total 138 semen analysis along with sperm DFI were carried out. Type of infertility was decided using the semen parameters using the WHO guidelines.[9] Based on the total sperm count, the couples were divided in two subgroups; 76 couples who were normozoospermic (>15 million/ml) were labelled as controls and 62 couples who were oligozoopermic (<15 million/ml) were labelled as cases. Then the sperm DFI in both the groups were compared and analysed.

Period of study was from August 2022 to August 2023.

A standard pre-evaluated proforma was designed to obtain information from the available records. It consisted of sociodemographic data, personal history (addiction), family history, other co-existing medical history.

The information was recorded by the investigator directly in google forms and the data was exported to the excel sheet. Both the groups were compared using unpaired t-test while for comparing the addictive behaviour between the groups, χ^2 test was used. Further, correlation carried out between the cases and controls in respect of DFI and sperm count. Also, both the groups were compared to find out the sensitivity and specificity. All the analysis was carried out using SPSS version 26 and MS-excel.

Laboratory methods

Patient was asked to given semen sample usually after 3 days of abstinence and the sample was divided into 2 parts. One part of the sample was used to evaluate basic sperm parameters including sperm count. While the second part was used to assess sperm DNA fragmentation.

The sperm count was carried out by examining the semen under a microscope to observe how many sperm appear within squares on a grid pattern. The reference values were according to the World Health Organization (WHO) reference values for human semen characteristics- semen volume, 1.5 ml (1.4-1.7); total sperm number, 39 million per ejaculate (33-46); sperm concentration, 15 million per ml (12-16); vitality, 58% live (55-63); progressive motility, 32% (31-34); total (progressive + non-progressive) motility, 40% (38-42); morphologically normal forms, 4.0% (3.0-4.0).[9]

DFI was carried out using sperm chromatin dispersion, done by CANfrag DNA fragmentation kit, Candore Biosciences- Belgium. The sample was mixed with 1% low melting agarose and was allowed to solidify under the slide and then treated with reagent I (lysis solution) for seven minutes followed by reagent II (neutralizing and lysis solution 2) and washed. The slide was then dehydrated, stained and examined. On each slide, 500 sperms were evaluated for halo size and dispersion pattern as described by Fernandez et al.[10]

DFI were calculated as percentage of number of spermatozoa with fragmented DNA out of total number of spermatozoa evaluated. The subjects were further divided according to DFI with cut-off values 25%, 15%-25% and <15%.[11] As per the guidelines, if DFI is ≤25%, it is within acceptable limits and considered good whereas if >25%, it is considered poor for the reproductive outcomes.

RESULTS

The clinical characteristics of 138 couples in this study showed that the cohort had a mean age of 31 years and a

mean BMI of 24.1 kg/m^2. The mean Infertility duration of the cases as well as controls was around 4 years. Based on the established diagnostic criteria, 62 (44.9%) subjects were labelled as group 1- cases having oligozoospermia whereas 76 (55.1%) couples were labelled as group 2- controls having normozoospermia. Both the groups were compared.

Table 1: Comparison of various parameters between two groups: case and control.

Parameters	Cases	Controls	P value	
Age (years)	31.71±4.01	30.33±4.09	0.0487	S
Infertility duration (years)	3.96±0.78	3.82±0.98	0.3628	NS
BMI (kg/m^2)	24.25±4.44	23.96±3.98	0.6867	NS
Count (millions/ml)	10.25±2.98	61.75±5.87	0.1415	NS
Sperm DNA fragmentation index (DFI) (%)	24.98±10.63	12.78±7.06	0.0011	S

To obtain p value, unpaired t-test was used; S: Significance, NS: non significance

Table 2: Comparison of addictive behaviour between two groups: case and control.

Parameters		Cases	Controls	Total (%)	χ^2	P value	
Smoking	Yes	44	34	78 (56.5)	9.56	0.001	S
	No	18	42	60 (43.5)			
Alcohol	Yes	26	30	56 (40.5)	0.0086	0.76	NS
	No	36	46	82 (59.5)			

To obtain p value, χ^2 test was used; S: Significance, NS: non significance

Both the groups were compared using the unpaired t test and p value was obtained to find out any significant difference in both of them. Accordingly, statistically significant difference was found between the 2 groups with Age and DFI with p value <0.05 indicating the difference between the groups. However, no statistically significant difference was found when counts of both the groups was compared indicating the necessity of qualitative sperm assays including sperm DFI along with the regular semen analysis.

When the addictive behaviour of both the groups was compared using the chi-square test, it was found that smoking proved to be a significant factor affecting the sperm count as well as DFI that correlated well with the existing literature.

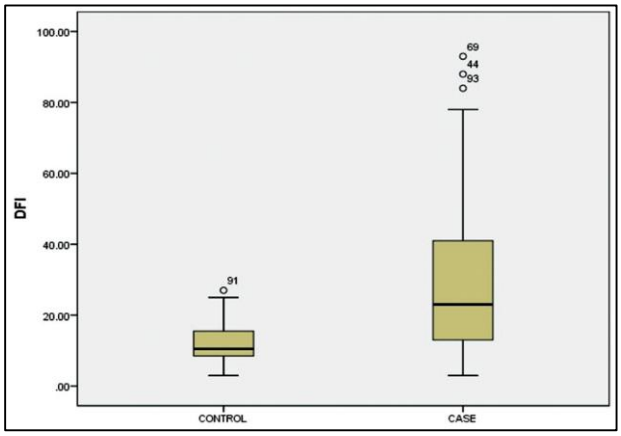

Figure 1: Box plot comparing DNA fragmentation index for cases and controls.

When a DFI was plotted on a box plot, it yielded the following graph that showed the distribution of different values of DFI between the two groups and a huge difference between them suggesting a role of DFI with male factor infertility.

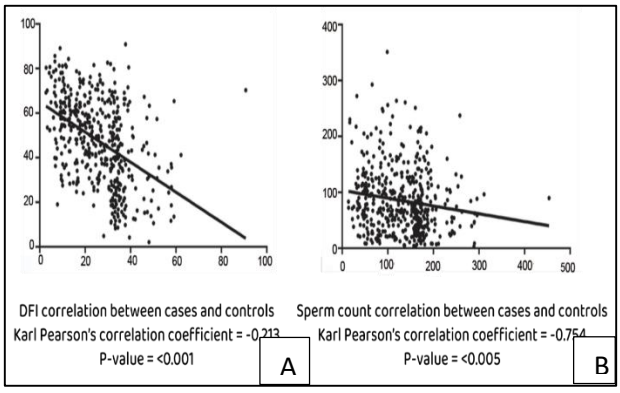

Figure 2 (A and B): Correlation between the cases and controls in respect of DFI and sperm count.

When correlation was carried out between the cases and controls in terms of DFI as well as sperm count, both of them yielded a negative correlation. It suggested that whether it was sperm count or DFI, both tend to move in opposite directions in cases as well as controls.

As the cases and controls were defined using the semen parameters, the sensitivity and specificity of DFI was compared to semen analysis. It yielded 87% sensitivity i.e. in 87% patients, infertility described by semen analysis that is quantitative was validated by DFI that measures the

quality required for the reproductive outcomes. Specificity was around 83% when compared to traditional semen analysis. Surprisingly, 13 controls out of 76 i.e. around 17.1% had poor DFI in spite of normal semen analysis parameters that would suggest the regular use of DFI for prediction of better reproductive outcomes in such patients who would else not be treated considering the normal semen parameters and would give the adverse reproductive outcomes.

Table 3: Comparison of semen analysis with DFI.

Parameters		Cases (n=62)	Controls (n=76)	
DFI	POOR (>25%)	54 (TP)	13 (FP)	PPV=80.50%
	GOOD (≤25%)	8 (FN)	63 (TN)	NPV=88.73%
		Sensitivity =87.09%	Specificity =82.89%	

DISCUSSION

There are periodic amendments in the semen analysis techniques and their cut-offs from WHO. However, in spite of that upto 30% men with normal semen parameters remain infertile.[12] Many authors have in past recommended the testing of sperm DFI before going for any treatments. However, due to the monetary constraints, analysis should be done regarding whom to offer this test. Studies advocate doing sperm DFI before going for IVF as it has strong association with IVF outcomes. Bungun et al carried out DFI using sperm chromatin structure assay (SCSA) whereas Garolla et al had done it with high power microscopy.[13,14] Our study too showed that the DFI was negatively correlated in both the groups and 13 controls out of 76 i.e. around 17.1% had poor DFI in spite of normal semen analysis parameters.

In present study, there was no significant difference in the Infertility duration, BMI and Sperm count of two groups (Table 1). The decreased sperm counts maybe due to ageing which decreases the function of all organs. Frattareli et al, have found that there is an age-related decrease in ability of sperms to fertilize ova.[15] Many other studies have reported that age has no effect on fertilization rate and while others have shown negative correlation with paternal age for ICSI.[16]

In our study, DFI was significantly higher in men from cases as compared to men from control group which suggests the association of high DFI with infertility. Wiweko and Utami in their study found significant difference in DFI in healthy fertile men and infertile men.[17]

Sperm proteins play a major role in compacting the sperm DNA and plays a vital role in its integrity. In current study, there was a negative correlation between the cases as well as controls in relation to sperm DFI and count. It means that there is high probability of oligospermic cases to have high DFI as compared to normospermic cases. However, Fernandez et al, found no statistically significant differences in sperm DFI values from infertility patients with normal or abnormal semen parameters.[10]

TUNEL and SCD are widely used tests to carry out DFI. Our setup uses SCD. When SCD and TUNEL were compared by Ragosta et al they found out that SCD mainly detects DFI in unviable spermatozoa and that SCD was less reliable than TUNEL for diagnostic purposes.[18] Whereas, Kaiyal et al found out that there was limited predictive power for pregnancy and live birth outcomes with SCD.[19]

Limitations of present study were: a very small sample size was enrolled in the study; participants were selected from one Institute and therefore may not be representative of all patients in general central Indian population. Many other environmental factors may contribute to sperm DNA damage in different parts of India.

CONCLUSION

In current study, DFI was significantly higher in cases as compared to control which suggests that high DNA fragmentation is associated with infertility. Also, 17.1% of patients having normal semen analysis parameters had poor DFI according to SCD. It suggests semen analysis alone can only provide limited information for the assessment of male fertility, and it does not fully reflect the fertilization potential of the sperm. With the development of assisted reproductive technology (ART), traditional semen analysis has failed to meet the needs of reproductive clinical practice. DFI reflects integrity and damage to the DNA, the genetic material of sperm, thereby detecting potential sperm damage and is considered a crucial indicator in evaluating semen quality.

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Ethical approval: The study was approved by the Institutional Ethics Committee

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