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Clinical Correlation of Sperm DNA Fragmentation Index (DFI) With Seminal Profile in Male Infertility

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Abstract
Background: *Apart from the WHO 2010 guided sperm parameters like sperm count, morphology, motility, vitality the sperm DNA fragmentation becomes a deciding factor of male infertility, recurrent pregnancy loss and lower pregnancy rate. Male infertility is a heterogeneous groups of disorders, out of which 30-40% are of known causes, rest constitute of unknown (idiopathic) causes. Male infertility has been found to be major cause of infertility, involving about 40%. Though in present days there is no guideline from WHO about standardization in value of DFI, or inclusion of this criteria as routine procedure in reproductive practice, it is expected to be important parameter in evaluation of the problem, prognosis and follow ups.*
Objective: *The primary aim of the study was to evaluate the clinical correlation of sperm DNA index with seminal profile in male infertility.*
Materials and Methods: *100 numbers of samples from persons having infertility (presuming the couple as one unit) of more than 2 years are analysed. All the patients received for analysis were only those reported to Swagat ART centre OPD for infertility. The sampling period was from January 2018 to February 2020. Sperm analysis was done according to WHO guidelines 2010. Sperm Chroma Kit test was used to detect sperm DNA damage rate and DFI rate was calculated.*
Results:
1. Even normozoospermia is having significantly high DNA fragmentation rate
2. Effect of medicine is more when it is used for early rejection by DNA fragmentation test than normal semen analysis. So DNA fragmentation has more prognostic value.
3. Both the normal report (WHO guided semen analysis report, DNA fragmentation rate) can determine and assure high pregnancy rate (natural, IUI, IVF), less pregnancy loss.
4. High age group males (> 45 years), patient with Diabetes, Obesity, are having more DFI, may be cause of reduced fertility in elderly.
Conclusion: *It can be concluded that DNA fragmentation evaluation can be one of the sensitive tests in male infertility (MI), when it is combined with normal procedure of semen analysis the accuracy in assessment of MI is more precise. It is expected that DFI can be a routine and compulsory procedure in selection of sperm specially when sperm is selected for recurrent ART failure or even in first time ART.*
Key Words: *WHO, DNA fragmentation, ART, Sperm Chroma Kit test*

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I. Introduction

Sperm DNA fragmentation (SDF) has a major impact on fertility. In the last decade, SDF has become a biomarker of male infertility, since it was discovered that spermatozoa with poor-quality or fragmented genetic material may hinder embryonic growth and development, increasing the risk of miscarriage in early pregnancy, issues involving fetal development.[1] High levels of SDF have been associated with repeated failure of assisted reproductive technology. Various methods to gauge SDF have been assessed for clinical use in studies that sought to identify threshold values for conception and to investigate their significance, sensitivity, and specificity. Now a days five most widely used techniques of measuring DNA fragmentation index (DFI), to identify correlations among them, and to determine their sensitivity, specificity, and cut-off values for predicting male infertility. More specifically, the terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay, the sperm chromatin dispersion (SCD) test, the sperm chromatin structure assay (SCSA), and the comet assay were compared. Of these methods, the SCD test and SCSA characterize SDF on basis of denatured chromatin in spermatozoa. The SCSA utilizes acridine orange staining to label double and single-stranded DNA with green and red fluorescence, respectively, after treatment with an acidic denaturing agent; higher levels of denaturation are associated with lower levels of sperm DNA integrity, and the clinical utility of this technique

has been firmly established. The SCD assay measures the extent to which chromatin in spermatozoa is dispersed based on the appearance of a radiant halo, and it enables non-divided (with a halo) spermatozoa to be distinguished from divided (without a halo) spermatozoa.

Apart from the WHO 2010 guided sperm parameters like sperm count, morphology, motility, vitality the sperm DNA fragmentation becomes a deciding factor of male infertility, recurrent pregnancy loss and lower pregnancy rate. Male infertility is a heterogeneous groups of disorders, out of which 30-40% are of known causes, rest constitute of unknown (idiopathic) causes. Male infertility has been found to be major cause of infertility, involving about 40%. Though in present days there is no guideline from WHO about standardization in value of DFI, or inclusion of this criteria as routine procedure in reproductive practice, it is expected to be important parameter in evaluation of the problem, prognosis and follow ups.

The primary aim of the study was to evaluate the clinical correlation of sperm DNA index with seminal profile in male infertility.

II. Materials And Methods

100 numbers of samples from persons having infertility (presuming the couple as one unit) of more than 2 years are analysed. All the patients received for analysis were only those reported to Swagat ART centre OPD for infertility. The sampling period was from January 2018 to February 2020. Sperm analysis was done according to WHO guidelines 2010. Sperm Chroma Kit test was used to detect sperm DNA damage rate and DFI rate was calculated.

After 3 to 5 days of abstinence, a complete sample of neat ejaculate produced by masturbation in a room in the laboratory was collected into a sterile container. In the first30 min after the collection, sperm concentration was deter-mined by using counting chamber technique, while wet preparation for sperm motility and eosin-stained for vitality determination were prepared. The slides were examined with phase-contrast Ffeoptics at x400 magnification and only morphologically normal spermatozoa were assessed. An air dried, fixed, and Diff quik stained preparation was made for sperm morphology determination by using bright-field optics. The prominent semen analysis parameters in this study were sperm concentration, motility, and morphology by referring to the World Health Organization (WHO) standards 2010. The sperm DFI was determined by Chroma Kit test. Both determinations, semen analysis and sperm DFI, were carried out by the same expert.

Statistical analysis: Statistical analysis was performed using SPSS version 23.Descriptive method was used to determine the distribution of the demographic profiles and the risk factors. Logistic regression was done to assess the smoking habit as the potential confounding factor. Due to the normality test of Kolmogorov-Smirnov showed abnormal data distribution, the Mann Whitney test was used to determine the association of semen parameters (concentration, motility, or morphology) and sperm DFI with male infertility. Categorization of sperm DFI was based on the cut-off point obtained from the Receiver Operating Characteristic (ROC) curve of sperm DFI.

III. Results

S.No	Age Group	Sample number	Percentage
1	Less than 35 years	54	54%
2	35-45 years	39	39 %
3	Greater than 45 years	7	7 %
4	Total	100	100

Table 1: Age wise distribution of cases

The average of sperm concentration and progressive motility was maximum in group two with age between 35 to 45 years. The percentage of normal forms declined with increasing age as well as DFI values increased with increasing age, from 9.2±7.7 in younger age group to 19.1±9.3 in > 45 years age group.

S.No	Parameters	Value
1	Sperm count	35.21 ±14.3
2	Progressive motility	38.2 ±12.4
3	Normal morphology	9.6 ±8.4
4	age	35.6±42.7
5	Years of marriage	7.3±5.3

Table 2: Comparison of sperm parameters with DFI

Even normozoospermia is having significantly high DNA fragmentation rate

Effect of medicine is more early rejected by DNA fragmentation test than normal semen analysis. So DNA fragmentation has more prognostic value.

Both the normal report (WHO guided semen analysis report, DNA fragmentation rate) can determine and assure high pregnancy rate (natural, IUI, IVF), less pregnancy loss.

High age group males (> 45 years), patient with Diabetes, Obesity, are having more DFI, may be cause of reduced fertility in elderly.

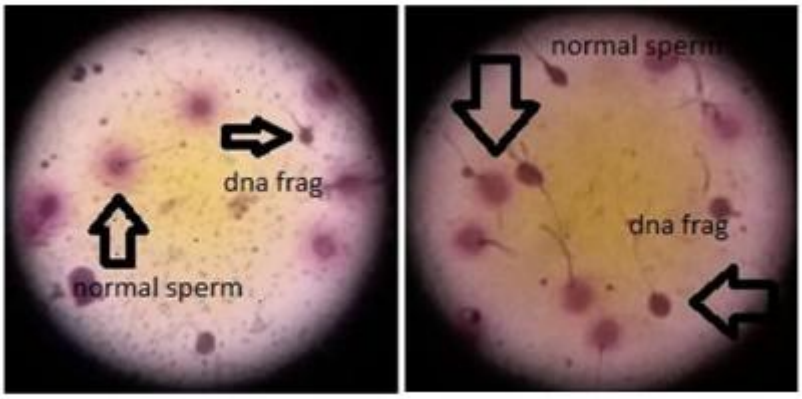

Figure 1: Photo by microscope during Sperm DNA Fragmentation analysis

Characteristics	DFI% (P Value)		
	<15	**15-30**	**>30**
Semen Volume (mL)	-0.05 (0.67)	0.208 (0.258)	0.18 (0.554)
Sperm Con. (106/mL)	-0.02 (0.88)	-0.134 (0.475)	-0.009 (0.976)
Progressive Motility (%)	-0.35 (0.008)	-0.334 (0.06)	-0.334 (0.264)
Sperm Morphology (%)	-0.096 (0.509)	-0.018 (0.925)	0.198 (0.517)
Fertilization Rate (%	-0.022 (0.878)	0.05 (0.790)	0.497 (0.084)
Age (Yrs.	-0.273 (0.366)	0.603 (0.001)	0.548 (0.001)
BMI	0.21 (0.167)	0.42 (0.041)	0.562 (0.073)
Smoking	0.291 (0.040)	0.035 (0.851)	0.340 (0.256)

Table 3: The correlations between sperm DFI, semen parameters, and fertilization rate and lifestyle factors of the sub fertile men

Among the environmental factors, BMI, smoking and heat to the scrotum are major factors causing DNA fragmentation. In the present study, BMI was significantly correlated with the moderate and total DFI categories. This is in agreement with a study conducted in USA, where multiple linear regression detected a significant association between obesity and sperm DNA fragmentation. It found men with BMI higher than 25 to have less DNA integrity, thus, patients should be advised to reduce their body weight in order to achieve maximum possible fertility. This is inconsistent with the findings of a study conducted in Czech, where no significant association between BMI and DFI was reported. The smoking was significantly correlated with each of the high and total DFI categories. Smoking can affect the sperm by losing their ability to fight off free oxygen radicals in the seminal fluid which make the sperm more sensitive to oxidative stress. Therefore, the increases in the free radicals in the seminal fluid affect DFI, motility and fertilization.

IV. Discussion

We define the fertile men were those who have normal semen parameter (concentration, motility, and morphology), even though the subsequent sub fertility condition might have correlated with the male factor that cannot be seen in semen analysis result, such as body mass index (BMI). There are emerging facts confirming that obesity negatively affects male reproductive potential not only by reducing sperm quality, but in particular, by altering the physical and molecular structures of germ cells in the testes, and ultimately by affecting the maturation and functions of sperm cells. Anifandis, et al., found that BMI of men did not correlate with sperm parameters, but influenced the quality of the produced embryos which, in turns, influenced the pregnancy rates. Similar result was also found by Petersen, et al., that showed couples with both partners having BMI >25

kg/m2had the lowest odds of live birth when compared to couples with both partners having BMI <25 kg/m2in IVF. In contrast with those studies, Kupka, et al., retrospectively analyzed data retrieved from the National German IVF Registry, which covered 12 years and included 650,452 cycles, and found that the highest clinical pregnancy rates for both IVF and ICSI were seen in normal weight females with obese male partners (P= 0.0028). However, because none of those studies were randomized controlled trials, several potential confounders and biases might have influenced the findings.

V. Conclusion

It can be concluded that DNA fragmentation evaluation can be one of the sensitive tests in male infertility (MI), when it is combined with normal procedure of semen analysis the accuracy in assessment of MI is more precise. It is expected DFI can be a routine and compulsory procedure in selection of sperm specially when sperm is selected for recurrent ART failure or even in first time ART.

References

[1]. Saleh RA, Agarwal A, Nelson DR, Nada EA, El-Tonsy MH, Alvarez JG, et al. Increased sperm nuclear DNA damage in normozoospermic infertile men: a prospective study. Fertil Steril. 2002;78(2):313–8.
[2]. Nallella KP, Sharma RK, Aziz N, Agarwal A. Significance of sperm characteristics in the evaluation of male infertility. Fertil Steril. 2006;85(3):629–34.
[3]. Barratt CL. Semen analysis is the cornerstone of investigation for male infertility. Practitioner. 2007;251(1690):8–10, 12, 15-7.
[4]. Riddell D, Pacey A, Whittington K. Lack of compliance by UK andrology laboratories with World Health Organization recommendations for sperm morphology assessment. Hum Reprod. 2005;20(12):3441–5.
[5]. Dam AH, Feenstra I, Westphal JR, Ramos L, van Golde RJ, Kremer JA. Globozoospermia revisited. Hum Reprod Update. 2007;13(1):63–75.
[6]. World Health Organization. 5th edn. Switzerland: WHO press; 2010. WHO Laboratory Manual for the Examination and Processing of Human Semen.
[7]. Agarwal A, Allamaneni SS. Sperm DNA damage assessment: a test whose time has come. Fertil Steril. 2005;84(4):850–3.
[8]. Spano M, Seli E, Bizzaro D, Manicardi GC, Sakkas D. The significance of sperm nuclear DNA strand breaks on reproductive outcome. Curr Opin Obstet Gynecol. 2005;17(3):255–60.
[9]. Lin MH, Kuo-Kuang Lee R, Li SH, Lu CH, Sun FJ, Hwu YM. Sperm chromatin structure assay parameters are not related to fertilization rates, embryo quality, and pregnancy rates in in vitro fertilization and intracytoplasmic sperm injection, but might be related to spontaneous abortion rates. Fertil Steril. 2008;90(2):352–9.
[10]. Lewis SE, Agbaje I, Alvarez J. Sperm DNA tests as useful adjuncts to semen analysis. Syst Biol Reprod Med. 2008;54(3):111–25.

Dr. Sankar Kr. Das, et. al. "Clinical Correlation of Sperm DNA Fragmentation Index (DFI) With Seminal Profile in Male Infertility." *IOSR Journal of Dental and Medical Sciences (IOSR-JDMS)*, 19(6), 2020, pp. 06-09.