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Original Research Article

Examination of Sperm DNA Fragmentation in Male Partners of Infertile Couples

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Abstract:

Background and Objectives: Infertility affects 15% couples of reproductive age in those 35% with male factor. Semen analysis is cornerstone to evaluate male factor infertility. Sperm chromatine dispersion test is light weight and fast test Normal sperm creates sperm halo. If sperm DNA fragments exceeds 30% sperm quality is significantly reduced. To study the Role of DNA fragmentation in male partner of infertile couple.

Results: The mean age of cases was 31.71 years and that of control was 32.63 years with majority of cases (43.3%) were in age group 31-35 years and controls were (56.7%) in age group 25-30 years. The age difference was statistically non- significant as p-value is greater than 0.05. The mean BMI in cases was 22.9 kg/m^2 and that of control was 24.5 kg/m^2 with majority of cases (73.33%) and controls (53.3%) had BMI between 18.5-24.9 kg/m^2. The difference was statistically non- significant. Here in cases 56.7% were tobacco chewer followed by 30% were smoker, 26.7% were alcoholic, 6.7% had other habits and 1% had no personal history. In control 33.33% were tobacco chewer followed by 23.3% were smoker, 6.7% were alcoholic and 36.7% had no personal history. In cases 83.3% had DFI greater than equal to 30 and 16.7% had DFI less than 30. In control 20% had DFI greater than equal to 30 and 80% had DFI less than 30. The mean DFI in cases was 37.26 andthat of control was 23.64. The difference was statistically significant

Conclusion: Sperm DNA integrity measurement is more reproducible and more objective than conventional parameters. Ourstudy indicates that the levels of sperm DNA fragmentation in men with subnormal semen parameters were significantly higher compared to the levels in men with normal semen parameters. SDI may reveal a hidden abnormality of sperm nuclear DNA in infertile men classified as idiopathic based on apparently normal standard sperm parameters. This test has an important diagnostic and prognostic value in the evaluation of male infertility, particularly in relation to assisted reproductivetechnologies.

Keywords: DNA fragmentation, DFI.

Introduction

Infertility is a disease of the reproductive system defined bythe failure to conceive a clinical pregnancy after 12 months or more of regular unprotected sexual intercourse. Infertilityaffects 15% couples of reproductive age, of these, 40 to 50% associated with females. Male factor infertility plays a role in approximately 50% of infertility cases with male factor as a sole cause in about 35%. [1] Semen analysis is cornerstone of the laboratory evaluation of the infertile male and helps to define the severity of malefertility. With absolute normal semen analysis parameter it may not be necessary to shift to specialized tests early butin cases with borderline parameters or with history of fertilization failure in past it becomes necessary to do a battery of tests to evaluate different parameters of spermatozoa. Various sperm function tests are proposed andendorsed by different researchers in addition to the routine evaluation of fertility. These tests detect function of a certain part of spermatozoon and give insight on the events in fertilization of the oocyte. [2] In 1980, concept of DNA fragmentation introduced. Sperm DNA damage can occur as a result of intrinsic factors, such as protamine deficiency and mutations affecting DNA compaction, or from extrinsic factors, such as heat, radiation, and gonadotoxins. The term "DNA fragmentation" refers to denatured or damaged sperm DNA that cannot be repaired. Indications for DNA fragmentation testing: unexplained or persistent infertility, failure to conceive after 5-6intrauterine insemination (IUI) cycles despite good count and motility, low fertilization rates or poor embryo quality in IVF cycles, recurrent miscarriage, prolonged stay in an environment that exposes to reproductive toxins, abnormal semen analysis and advancing male age (>45 years).

The diagnostic test used today (SCD) is a

lightweight and fast test based on the sperm chromatin dispersion. Normal sperm creates DNA halo zones. [3] If sperm DNA fragments exceeds 30% sperm quality is significantly reduced. Based on the percentage of DNAfragmentation it is possible to choose the right technique and improve pregnancy outcome.

Material and Methods

A comparative prospective study on DNA fragmentation index was done in sub normal Semen and normal semen parameters of participants semen analysis of all study participants was done according to WHO criteria 2010. Men undergoing study selected on basis of inclusion and exclusion criteria. Written and informed consent of the patient was taken prior to study. Institute review board and ethical committee approval was taken.

Inclusion Criteria

Male partners of infertile couples

Couple giving written and informed consent

Exclusion Criteria

Any infection (UTI, prostatitis) Abstinence period >3 days

Drugs affecting spermatogenesis in past 3-6 months

Male partners of infertile couple attending DMCH Laheriasarai, Darbhanga. semen analysis was done according to WHO criteria 2010.30 cases with subnormal semen parameters and 30 controls with normal parameters randomly recruited.

And DNA Fragmentation Index analysed by sperm chromatin dispersion test with grade 1 and grade 2 was taken as normal and 3 &4 was taken as abnormal.

$$\text{DNA fragmentation index will be calculated: } \frac{\text{Sperms without halo}}{\text{Sperm with halo + without halo}} = \text{Total sperm count}$$

A minimum of 500 spermatozoa per sample were scored into four groups (Fig-2):

Spermatozoa with large halos (halo width is similar or larger than the minor diameter of the core) + spermatozoa with medium size halos (halo size is between those with large and very small halo).

1. Spermatozoa with very small-size halo (halo width is similar or smaller than 1/3 of the minor diameter of the core).
2. Spermatozoa without a halo.
3. Degenerated spermatozoa (halo weakly or irregularly stained.

Results

The mean age of cases was 31.71 years and that of control was 32.63 years with majority of cases (43.3%) were in age group 31-35 years and controls were (56.7%) in age group 25-30 years. The age difference was statistically non- significant as p-value is greater than 0.05. The mean BMI in cases was 22.9 kg/m^2 and that of control was 24.5 kg/m^2 with majority of cases (73.33%) and controls (53.3%) had BMI between 18.5-24.9 kg/m^2. The difference was statistically non-significant Here in cases 56.7% were tobacco chewer followed by 30%were smoker, 26.7% were alcoholic, 6.7% had other habits and 1% had no personal history. In control 33.33% were tobacco chewer followed by 23.3% were smoker, 6.7% were alcoholic and 36.7% had no personal history. In cases 83.3% had DFI greater than equal to 30 and 16.7% had DFI less than 30. In control 20% had DFI greater than equal to 30 and 80% had DFI less than 30. The mean DFI incases was 37.26 and that of control was 23.64. The difference was statistically significant

Discussion

In present study we compared DNA fragmentation in patients with normal semen parameters and subnormal semen parameters. We found that mean age of cases was 31.71 years and that of control was 32.63 years with majority of cases (43.3%) were in age group 31-35 years and controls were (56.7%) in age group 25-30 years. The age difference was statistically non-significant as p-value is greater than 0.05. In cases, 83.3% had DFI greater than equal to 30 and in control 20% had DFI greater than equal to 30 and 80% had DFI less than. The mean DFI in cases was 37.26% and that of control was 23.64%. The difference was statistically significant. Khamar T et al [4] found that mean age of patients was 34.21± 4.14 years and the mean sperm DFI was 34.69 ± 19.13%. In addition, 45 patients (45%) had sperm DFI greater than 30%. Heidari M et al [5] in Tehran showed that the mean sperm DFI in infertile men was 73.77%. In our study difference in mean DFI was statistically significantly high in cases when compared to controls. It was also found that Mean DFI increases as age of patients increases. Vaamonde D et al [6] studied that Training load affects oxidative stress; as such, superoxide dis-mutase (SOD) capacity has been reported to be increased in elite athletes which can affect the testicular environment. Physically active men, in comparison to higher level athletes, have been shown to have higher levels of seminal antioxidant compounds and lower levels of seminal reactive oxygen species (ROS), oxidative stress and sperm DNA fragmentation. Erectile dysfunction has also been reportedto be less common in physically active subjects (non-athletes) than in sedentary; moreover, this condition has been seen to improve as a result of unhealthy lifestyle modification like becoming physically active. In our study mean BMI in cases was 22.9 kg/m^2 and that of control was 24.5 kg/m^2 with majority of cases (73.33%) andcontrols (53.3%) had had BMI

between 18.5-24.9 kg.m^2. The difference was statistically non-significant. The difference in mean DFI was statistically significantly high in cases and compared to controls. As BMI increases the mean DFI increases. Omrani et al [7] found that among the environmental factors, BMI, smoking and heat to the scrotum are major factors causing DNA fragmentation. In their study, BMI was significantly correlated with the moderate and total DFI categories. This is in agreementwith 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. Here in cases 56.7% were tobacco chewer followed by 30%were smoker, 26.7% were alcoholic, 6.7% had other habits and 1% had no personal history. In control 33.33% were tobacco chewer followed by 23.3% were smoker, 6.7% were alcoholic and 36.7% had no personal history. Thedifference in mean DFI was statistically significantly high in cases and compared to controls.

Table 1: Distribution of Subjects According to Age

Age Group(in yrs)	Cases		Controls	
	No.	%	No.	%
25 - 30	11	36.70	17	56.70
31 - 35	13	43.30	3	10.00
36 - 40	5	16.70	10	33.30
>40	1	3.30	0	0.00
Total	**30**	**100.00**	**30**	**100.00**
Mean ± SD (in yrs)	31.71 ± 4.70		32.63 ± 4.61	

Table 2: Distribution of Subjects According to BMI

BMI (in kg/m^2)	Cases		Controls	
	No.	%	No.	%
18.5 - 24.9	22	73.33	16	53.30
25 - 29.9	8	26.67	13	43.30
>30	0	0.00	1	3.30
Total	**30**	**100.00**	**30**	**100.00**
Mean ± SD (in yrs)	22.9 ± 4.5		24.5 ± 2.2	

p = 0.2011

Table 3: Distribution of Subjects According to Personal History

Personal History	Cases		Controls	
	No.	%	No.	%
Alcohol	8	26.70	2	6.70
Smoking	9	30.00	7	23.30
Tobacco	17	56.70	10	33.30
Other	2	6.70	0	0.00
No History	4	1.00	11	36.70

Table 4: Distribution of Subjects According to DFI

DFI	Cases		Controls	
	No.	%	No.	%
<30	5	16.70	24	80.00
≥30	25	83.30	6	20.00
Total	**30**	**100.00**	**30**	**100.00**
Mean ± SD (in yrs)	37.26 ± 6.97		23.64 ± 6.46	

Conclusion

Sperm DNA integrity measurement is more reproducible and more objective than conventional parameters. Our study indicates that the levels of sperm DNA fragmentation in men with subnormal semen parameters were significantly higher compared to the levels in men with normal semen parameters. SDI may reveal a hidden abnormality of sperm nuclear DNA in infertile men classified as idiopathic based on apparently normal standardsperm parameters. This test has an important diagnostic andprognostic value in the evaluation of male infertility, particularly in relation to assisted reproductive technologies.

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