

Study of Sperm Dna Fragmentation Index in Patients from Rural Areas of Wardha Region

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Abstract

Background: Sperm DNA fragmentation test is an advanced test for proper sperm function with respect to semen analysis, as semen analysis is used for motility and morphology assessment, it cannot explain sperm at molecular level and thus SDF test has been introduced to evaluate male fertility. The integrity of genetic material in sperm is crucial for successful fertilization & normal embryo development as this will lead to proper transmission of genetic information. Patients having varicocele, recurrent failure of ART techniques and improper lifestyle (radiation, smoking, alcohol, BMI, ageing, etc) are considered for sperm DNA fragmentation test. SDF test is useful in selecting patients for the ART procedures i.e. IVF/ICSI/IUI and highest pregnancy and live birth outcomes.

Objectives:

- To predict male infertility with normal semen parameters & its diagnostic values.
- To investigate the clinical factors related to sperm DNA fragmentation in male with infertility.
- To investigate the rate of sperm DNA fragmentation in male related to improper fertilization, embryo development and recurrent pregnancy loss.

Methods: The participants (n=40) with male fertility problems will be included in randomised control trial. All 40 participants will be randomly selected to evaluate their sperm DNA integrity from their respective semen samples using sperm chromatin dispersion test.

Discussion: Efficiency of the Sperm DNA Fragmentation test is assessed by performing sperm chromatin dispersion test on respective processed semen sample using Halosperm G2 test kit.

Keywords: Sperm DNA fragmentation (SDF), DNA fragmentation index (DFI), oxidative stress, genetic abnormalities, hyperthermia, gonadotoxins and endocrine abnormalities.

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Introduction

9% of couples are diagnosed with infertility out of which 20% is due to male factor.⁽¹⁾ Especially in males, infertility factor has become quite common. most of infertility cases are due to damage to sperm

DNA (i.e. sperm DNA fragmentation). At an age, as small quantity of sperm can achieve pregnancy, hence it becomes important to select healthy sperms for ICSI/IVF. However Semen analysis is used for morphology, motility and sperm concentration assessment, it cannot evaluate the sperm at molecular level and therefore SDF test is nowadays used to evaluate male infertility. SDF (sperm DNA fragmentation) term is used to indicate abnormal genetic material in sperm which can lead to male infertility. The integrity of genetic material in sperm is important for successful fertilization and normal embryo development, as proper DNA integrity will lead to proper transmission of genetic information.

DNA combination protein blocks

Chromatin

23 chromosomes in sperm head are made up of chromatin that consist of DNA and proteins. Thus intact chromatin is important for proper pregnancy outcomes.

Research has been done on new testing methods on sperm abnormalities in the past 30 years and sperm DNA damage has come to be an area of interest.

It has been observed that Sperm DNA Fragmentation is highly recognized as important cause of male infertility. The correlation between sperm damaged DNA and failed reproductive outcome led to introduce SPERM DNA FRAGMENTATION or Sperm DNA Integrity tests for the evaluation of male infertility. Sperm DNA fragmentation test is an advanced and valuable test for proper sperm function with respect to semen analysis and this test is utilized during evaluation of male fertility. The integrity of genetic material in sperm is crucial for successful fertilization, normal embryo development and proper reproductive outcome as good DNA integrity will lead to proper transmission of genetic information. SDF term is used to indicate abnormal genetic material in sperm which can lead to male infertility.



Fig: Factors associated with Sperm DNA Damage

Patients having varicocele, unexplained infertility, recurrent pregnancy loss, recurrent failure of assisted reproductive techniques (ART) and improper lifestyle/environmental exposures are considered for sperm DNA fragmentation test. SDF test is useful in selecting patients for the ART procedures i.e. IVF/ICSI/IUI and highest pregnancy and live birth outcomes. ⁽²⁾ As Sperm DNA Fragmentation (SDF) test is considered as an important tool for measuring the quality of sperm on basis of its DNA fragmentation rate values. ⁽⁴⁾ Hence this method is more conventional and significant than the parameters analysed from semen analysis. Semen analysis is used for motility and morphology assessment, it cannot explain sperm at molecular level and thus SDF test has been introduced other than semen analysis to evaluate male fertility. High SDF rate largely affects the embryo quality, improper fertilization, miscarriages and failed IUI/IVF/ICSI.

High SDF rates is found in male with both normal and abnormal semen parameters, improper lifestyle modifications, smoking, radiation, oxidative stress, etc. "The percentage of cells having fragmented DNA is represented by the **DNA FRAGMENTATION INDEX (DFI).**" DFI is recommended as element for prediction of fertility in males.

The Sperm DNA damage include defects in spermatogenesis (i.e. genetic abnormalities) and oxidative stress, hyperthermia, gonadotoxins and endocrine abnormalities).

Various techniques used to measure sperm DNA integrity include:

- the sperm chromatin structure assay (SCSA)
- the terminal deoxynucleotidyl transferase mediated deoxyuridine triphosphate nick end labelling (TUNEL) assay
- the single cell gel electrophoresis (Comet) assay & the sperm chromatin dispersion (SCD) test.

However, rate of sperm DNA fragmentation varies due to methods used for testing SDF and population. There are few studies which showed the correlation between clinical factors and sperm DNA damage.⁽³⁾

Clinical Factors/reasons leading to high sperm DNA damage are:

- lifestyle modifications (i.e. radiations, heat, airborne pollutants, chemicals and sexually transmitted infections)
- biological factors (i.e. increased body mass index, diabetes and male age)
- Infection (i.e. inflammation and male genital tract infection)
- Oxidative stress
- Alcohol exposure (lead to alteration of endocrine system/ toxic effect to testis/genetic defect)
- Smoking (cause poor semen quality and genetic damage to sperms)
- Increased testicular temperature, varicocele

Clinical significance of sperm DNA damage for pregnancy:

It includes assesment of couples struggling with infertility and counselling strategies for patients with high SDF. This include

- Natural conception
- Intrauterine insemination (IUI)
- In vitro fertilization (IVF)
- Intracytoplasmic sperm injection (ICSI)

Treatments

- Damage due to free radicals: change in lifestyle and diet designed to reduce oxidative stress that will simultaneously reduce the levels of DNA fragmentation.
- Antibiotics if have existing infection
- Say no to drugs, smoking and tobacco
- Improve lifestyle modifications i.e. less exposure to radiation, pollution, etc
- Varicocele surgery

- Less BMI and good diet i.e. fresh foods, particularly those containing antioxidants and vitamin C & E

- TESA: Testicular aspiration of sperm (DNA damage occurs at the post-testicular level, hence testicular sperm may have a better DNA integrity than ejaculated sperm)

- ICSI rather than IVF

Background/Rationale

1) There was study⁽⁴⁾ related to association of sperm DNA fragmentation with lifestyle factors and semen parameters of Saudi men. For this they selected total 94 couples for investigation with study parameters included were male age, body mass index, smoking, semen values, %sperm DNA fragmentation, fertilization rate and pregnancy outcome. In this they grouped semen samples on basis of % sperm DNA fragmentation into <15%, 15-30% and >30% i.e. low, moderate and high % spermDNA fragmentation respectively.

Results observed were, there was no difference in the ICSI outcome in low and moderate SDF. However in high SDF no patient achieved pregnancy. In this 53.19% Saudi men has low DFI, 32.98% moderate and 13.83% high DFI. It also showed that semen volume, sperm morphology and fertilization rate did not show any correlation with DNA fragmentation. But sperm concentration and motility has negative correlation with DFI category. Also,

- BMI- +ve correlation (moderate DFI)
- SMOKING- +ve correlation (low DFI)
- AGE- +ve correlation (moderate and high DFI)

The study says 14% of Saudi men had high DFI.

2) There was study⁽⁵⁾ to compare the result of TUNEL assay(i.e. technique used for SDF) applied to semen samples from males with proven fertile factor (n=47) and patient from infertile male population (n=66) to get discriminating threshold value. Results observed were that patients with male factor have high mean level of DNA fragmentation then proven fertile men.⁽⁵⁾ The calculated threshold value for TUNEL assay to distinguish between both was 20% i.e. high for

both positive value 92.8% and negative value 95.5%. (specificity-89.4%, confidence interval(CI)-95% and sensitivity-96.9%). This study demonstrated that SDF measured by TUNEL assay is highly valuable indicator of male infertility.⁽⁵⁾

Objectives:

- To predict male infertility with normal and abnormal both semen parameters & its diagnostic values.

- To investigate the clinical factors related to sperm DNA fragmentation (SDF) in male with infertility.

- To investigate the rate of sperm DNA fragmentation in male related to improper fertilization, embryo development and recurrent pregnancy loss.

- To investigate the relationship between Sperm DNA fragmentation index (DFI) and normal and abnormal both semen parameters and pregnancy rate of In Vitro Fertilization (IVF) and Intracytoplasmic sperm injection (ICSI).

- Prediction of outcome by evaluating increased SDFI (mean 95% confidence interval) and allowing with post infertility treatments.

Methods

Study Design:

POPULATION: male partner attending wardha test tube baby centre (AVBRH).

INTERVENTION: performing Sperm Chromatin Dispersion Test to evaluate Sperm DNA Fragmentation Index in male partner registered for SDF test with specific clinical parameters.

COMPARISON: Sperm DNA Fragmentation Index (DFI) with different clinical factors will be compared among patients.

OUTCOME: prediction of male infertility will be done by evaluating primary outcome i.e. increased sperm DNA fragmentation index (mean difference with 95% confidence interval) of patients and allowing male partner with post infertility treatments and success pregnancy rate.

Aim:

“To Study Dna Fragmentation Index In Patients Of Rural Area From Vidharbha Region With Different Clinical Parameters.”

Methodology

Study Setting:

The trial will be carried out at Wardha Test Tube Baby Centre, AVBRH, Sawangi (Meghe), Wardha, after approval of Institutional Ethics Committee (IEC) of Datta Meghe Institute Of Medical Sciences (DU)

Study Design & Sample Size:

The design of the study is a randomized controlled

trial of a male attending Wardha Test Tube Baby Centre, AVBRH, Sawangi (meghe), wardha. The number of participants enrolled in this experimental study is 40 (n = 40). Eight Groups are made considering 5 males in each group. Only single group of five people will be allowed for SDF test on particular day. The selected participants will be randomly allowed for collection of semen samples (well labelled with name, date & time of collection). Before inclusion, the participants will be explained about the objectives and approaches of the study, and written patient consent forms will be signed by them. The study procedure is shown in Figure 1

Fig 2: Patients Schedule, Interventions & Assessments.

Patient no./name	Male age (yrs ± SD)	Initial concentration of sample (M×10 ⁶ /ml ± SD)	Initial motility (M% ± SD)	DFI (M% ±SD)
1.				
2.				
3.				
4.				
5.				

(test to be performed for 40 patients in group of 5)

M= mean, SD= Standard Deviation, DFI= DNA Fragmentation Index (by SCD test).

Participants

The inclusion criteria for the participants are as under:

1. Infertile male partner with poor semen quality.
2. Patients addicted to alcohol, tobacco and smoking.
3. Patient from rural area of wardha region with different lifestyle parameters (i.e. radiation, heat, air borne pollutants, etc)

4. Patient with IVF implantation failure.

Patient with IVF blastocyst formation failure.

Variables: This clearly defines all outcomes, exposures, potential confounders & effect modifiers. This will provide diagnostic criteria, if applicable.

Data sources/ measurement: For each variable of interest will provide sources of data & details of method of measurement.

The exclusion criteria for the participants are as under:

1. Patient not giving consent for treatment.

2. Patients having infections like HIV, HbsAG etc.

3. The cases with female infertility were excluded.

Sample Size: 40 infertile male

Statistical methods: Describe all statistical methods, including those used to control for confounding. Describe any methods used to examine subgroups and interactions.

Methodology

This study will be done in wardha test tube baby centre AVBRH (SAWANGI) WARDHA. Relevant data on the demographics and treatment history as well as the indications will be recorded. Counselling of all participants for research work will be done. The routine protocol in our set up will be followed and history of patient will be taken.⁽⁶⁾

Patients will be asked for medical history, physical examinations, conventional semen analyses, and blood tests including assessments of luteinizing hormone, follicle-stimulating hormone, and testosterone and for smoking and alcohol drinking status.⁽⁶⁾

Hormonal Analysis

LH, FSH and testosterone level will be analysed at Central pathology lab of AVBRH, Sawangi Meghe, Wardha.

Physical Examination

A general physical examination will be done. Patient will also be examined including examination of the penis i.e.

- 1) location of urethral meatus
- 2) Presence of both the vas deferens and epididymis
- 3) Palpation of the testes and their size
- 4) Presence of a varicocele (Varicoceles were diagnosed during scrotal examinations with the patient in a standing position)

Semen Collection and Analysis

Initially Patient will be asked to undergo for semen

analysis. Patients were asked to have at least five days of abstinence before semen analysis. Semen sample will be collected at AVBRH IVF laboratory in semen collection room in a sterile plastic container by masturbation (acc. To WHO 2010). Sample is allowed to liquefy for 15 minutes prior to examination. Sample having less than 5 million/ml will be excluded, as sperm chromatin dispersion test require concentration of 5-10 million/ml.

Sperm chromatin dispersion test

After analyzing semen sample, adequate sample will undergo sperm chromatin dispersion test Halosperm G2 kit. All the result from the test will be analysed and recorded to check DNA FRAGMENTATION INDEX % in patients.

Scope:

· Currently used sperm selection methods have been found to effectively eliminate sperm with damaged DNA, as a consequence of which the risk of using a genetically incompetent sperm for medically assisted reproductive technologies seems to be low and will increase the pregnancy outcome rate.

Limitations:

Ø One of the factor for assessing male infertility in my study is FSH, LH level measured/ testosterone conc. measured/varicocele daignosis and because cost many of the patient may not be willing for investigation.

Expected Outcomes/Results:

Participants: Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed. Give reasons for non-participation at each stage. Consider use of a flow diagram.

Descriptive data: Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders. Indicate number of participants with missing data for each variable of interest.

Outcome data: Report numbers of outcome events or summary measures.

Main results: Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included. Report category boundaries when continuous variables were categorized. If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period.

Other analyses: Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses.

Discussion:

Efficiency of the Sperm DNA Fragmentation test is assessed by performing sperm chromatin dispersion test on respective semen sample (processed semen sample used)

using Halosperm G2 test kit (Halotech DNA madrid spain). Fragmentation in DNA of sperms are evaluated through no/small halos i.e. Sperm with DNA fragmentation and medium/large halos i.e. sperm free from DNA fragmentation.

Key results: Summarise key results with reference to study objectives.

Limitations: Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias.

Interpretation: Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence.

Generalisability: Discuss the generalisability (external validity) of the study results.

1. Ethical clearance :
2. Source of funding: Self

3. Conflict of interest: Nil

References

1. Panner Selvam MK, Agarwal A. A systematic review on sperm DNA fragmentation in male factor infertility: Laboratory assessment. *Arab J Urol*. 2018;16(1):65-76. Published 2018 Jan 17. doi:10.1016/j.aju.2017.12.001
2. Majzoub A, Agarwal A, Esteves SC. Clinical utility of sperm DNA damage in male infertility. *Panminerva Med*. 2019;61(2):118-127. doi:10.23736/S0031-0808.18.03530-9
3. Agarwal A, Majzoub A, Esteves SC, Ko E, Ramasamy R, Zini A. Clinical utility of sperm DNA fragmentation testing: practice recommendations based on clinical scenarios. *Transl Androl Urol*. 2016;5(6):935-950. doi:10.21037/tau.2016.10.03
4. Al Omrani B, Al Eisa N, Javed M, Al Ghedan M, Al Matrafi H, Al Sufyan H. Associations of sperm DNA fragmentation with lifestyle factors and semen parameters of Saudi men and its impact on ICSI outcome. *Reprod Biol Endocrinol*. 2018;16(1):49. Published 2018 May 19. doi:10.1186/s12958-018-0369-3
5. Sergerie M, Laforest G, Bujan L, Bissonnette F, Bleau G. Sperm DNA fragmentation: threshold value in male fertility. *Hum Reprod*. 2005;20(12):3446-3451. doi:10.1093/humrep/dei231
6. Komiya A, Kato T, Kawauchi Y, Watanabe A, Fuse H. Clinical Factors Associated with Sperm DNA Fragmentation in Male Patients with Infertility. *Sci World J*. 2014;2014.
7. Yang H, Li G, Jin H, Guo Y, Sun Y. The effect of sperm DNA fragmentation index on assisted reproductive technology outcomes and its relationship with semen parameters and lifestyle. *Transl Androl Urol*. 2019;8(4):356-365. doi:10.21037/tau.2019.06.22