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The Effects of Lifestyle Modofications on Sperm DNA Fragmentation Index

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background: Most of the men go through infertility due to DNA defects in sperm. DNA defects in sperm can be measured by sperm DNA fragmentation test. Sperm DNA splitting have been correlated with sperm viability and its major impact on male infertility. Many studies have shown that men with disturbed lifestyle habits such as smoking ,alcoholic consumption ,poor diet have very poor DNA quality compared to the men with proper habits like good diet , exercise and a well maintained routine. There has been many tests to measure the DNA breakage after conducting proper semen examination and infertility duration. This can be treated with proper medications and regular exercise.
Objectives: 1**.** To predict the reason for male infertility by evaluating routine semen parameters.
2. To study the effects of daily lifestyle habits associated with SDF in patients who are not fertile.
3. To investigate the amount of sperm DNA splitting in men who are not fertile. 4.To investigate the

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association between DFI and semen quality, between DFI and IVF, between DFI and IUI. 5.To estimate the outcomes of sperm DNA fragmentation index and allow the patients to seek post infertility treatments.

Methods: This non-invasive methodology includes recording of treatment history and the indications of infertility. Proper counselling for the patients were done. A proforma were filled along with their consent showing the accuracy of smoking, alcohol consumption, stress levels and other mentioned factors and hence calculating the sperm breakage levels.

Keywords: Sperm DNA splitting; infertile men; dna breakage; sperm chromatin dispersion test; alcohol consumption; smoking; oxidative stress; % SDF; BMI; psychological stress.

1. INTRODUCTION

Infertility is unable to get pregnant until one year even after having carefully planned unprotected sex. The reason behind this may be combination of factors that prevent pregnancy in either male partner or the female partner [1]. According to World Health Organization, most of the infertile couples in the world is suffering from primary infertility, which means the woman has never been able to conceive since sexual exposure without any contraceptive. Apart from this, a woman can suffer secondary infertility anytime in her life after the first pregnancy. Globally, millions of couples suffer from infertility issues in their reproductive lives [2]. In males, infertility factor has become quite common. In 30 to 50% of couples with infertility problems, the reason for the infertility lies in men due to sperm defects because of ageing, various other disorders or bad lifestyle habits [3].

In case of male infertility, the prior reason is sperm defects. Hence, during any ART **?** procedures, it is most important to first analyze the semen and select the healthy sperms for the further processes like **IVF** or **ICSI**. For this, semen analysis is always recommended as it will help a doctor to determine whether the man is infertile, having low sperm count, any morphological dysfunction or the other reason behind the male infertility [4]. However, it does not completely predict the outcomes after any particular treatment. Hence, in this area of interest, various researches has been conducted so that it can be more easy to determine direct measures of infertility. We know that ,the genetic composition of a new born is a combination of an oocyte and sperm DNA quality and is the vital factor effecting on further embryo development .Any type of damage in sperm DNA or oocyte DNA can interfere the whole reproduction process resulting in further embryo or foetal development [5,6]. Hence, the relation between damaged DNA and the increasing rate in male infertility has given rise to the initiation of sSperm DNA fragmentation iIndex or some other sperm DNA integrity tests for evaluating male infertility [7,8].

2. SPERM DNA FRAGMENTATION

Sperm DNA fragmentation is a term used to denote the integrity of the genetic material inside sperm on the basis of its fragmentation index value. The percentage of cells with fragmented DNA is represented by DNA fragmentation index. The men who are infertile has found to have a higher amount of defective DNA compared to the ones who are fertile. Therefore, DNA fragmentation index is impelled as an important segment for prediction of fertility in males.

There are numerous techniques used to measure sperm DNA splitting includes: 1.Terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate-nick end labeling (TUNEL Assay). 2. Comet Assay. 3. Sperm chromatin structure assay (SCD test). 4. Sperm chromatin structure assay **(**SCSA**)**

SCD test is a novel assay to detect the amount of DNA splitting in semen sample. The SCD assay is a very simple, affordable and the most authentic technique to detect sperm DNA fragmentation, hence delivering information regarding sperm quality and its reproductive capacity.

In this study, we measured the effects of lifestyle modifications on sperm DNA fragmentation. The parameters for study included: Age, BMI, Smoking habits, Alcohol consumption, % SDF, Psychological stress [8].

3. CLINICAL OUTCOMES OF SDF ON PREGNANCY

This section will give review for the evaluation of couples striving with long time infertility issues

and counselling policy for patients with high SDF and its importance which includes: Natural conception, intra uterine insemination (IUI), intracytoplasmic sperm injection (ICSI), effects of assisted reproductive techniques (ART) [9].

3.1 Background/ Rationale

A study conducted by Basmah Al Omrani in June 2015- June 2016 regarding association of sperm DNA disintegration in regards to everyday lifestyle pattern sss. They grouped all the semen samples on basis of % SDF. It was observed that there is no variation in the ICSI. Nevertheless, no patient achieved pregnancy in high SDFI. Overall, it was concluded that 53.19% Saudi men has low DFI. It also showed that sperm concentration and motility has negative correlation with DFI category. Also, BMI; +ve correlation (moderate DFI), Smoking-; +ve correlation (low DFI), Age; +ve correlation (average to high DFI).

Overall conclusion of this study was that maximum percentage of Saudi men had high DFI [10]. In a research conducted by M. Sergerie et al to compare the results of TUNEL assay (I.e. technique used for SDF) with semen samples from men with proven fertile factor (n=47) and men with infertile factor (n=66) to get different threshold value. The computed threshold value for TUNEL assay to differentiate among them was 20% i.e high for both positive value 92.8% and negative value 95.5%. (Specificity: 89.4%; confidence interval (CI); 95%; sensitivity:96.9%). This overall study demonstrated that SDF measured by TUNEL assay is efficient marker of male infertility [11].

In July 2010, a study was conducted on clinical significance of sperm DNA damage in field of infertility outcomes by Luke Simon, Deborah Lutton, Sheena E.M. Lewis. The motto of this study was to discover the benefits of DNA disintegration to predict assisted reproductive technique outcomes.

Many couples were taken into investigation (IVF ? and ICSI) in which their DF ? was calculated by Comet assay followed by gradient centrifugation and contrast with implantation rate and other factors. Formamidopyridine DNA glycosylase as used to measure modified bases (MBV) to translate them them into strands. The outcomes observed were as follows: DNA breakage seems to be increased in processed semen sample, and the couples who had higher DF had failed to achieve clinical pregnancy(CP) than that of couples with succesful pregnancy. Also, the nucleic acid breakage seems to be markedly higher when modified bases are added to existing strands. No correlations is observed between DNA disintegration and fertilization rate, embryo cumulative scores and clinical pregnancy. The study concluded that the nucleic acid breakage in sperm can predict and affect ART outcome for IVF. Converting modified bases into further DNA strand breaks increased the test vulnerability and shown negative correlations in between DF & CP for ICSI as well as IVF [12].

In November 2017, a study was conducted by R.M.Mostafa, Ashok Agrawal, M.M. Hassan regarding the outcome of smoking on human semen quality, chromatin structure of sperm and precipitation. This study id directed to evaluate the outcomes of smoking cigarettes on semen variables and sperm DNA fragmentation. Total 95 men were included taken into investigations who were distributed into non-smokers (45) and smokers (50). All the smokers has been splitteds into modest,adequate and heavy smokers. Semen analysis, SDF tests and sperm viability tests results were compared between infertile non-smokers and infertile smokers, a notable fall off has been observed in sperm density, viability, % of regular forms and motility. The results suggested that semen parameters were highly affected by smoking [13].

4. OBJECTIVES

Several objectives to study the effects of lifestyle modifications on sperm DNA fragmentation index were included. They were as follow: 1. To predict the reason for male infertility by evaluating routine semen parameters. 2. To study the effects of daily lifestyle habits associated with SDF. 3.To investigate the rate of sperm DNA disintegratation index in sterile men. 4. To investigate the association between DFI and semen quality, between DFI and IVF, between DFI and IUI. 5. To estimate the outcomes of sperm DNA disintegration index and allow the patients to seek post infertility treatments [1].

5. HYPOTHESIS

We hypothesized that the increased SDFI may be related to lifestyle habits such as alcohol consumption, regular smoking , psychological stress, ageing, exposure to chemical environment ,poor diet, etc . Thus, the above

mentioned factors can be treated among individuals resulting in reducing the SDF and male infertility rate.

6. METHODS

6.1 Study Design, Observational Study and Methodology

This study was done in Wardha Test Tube Baby centre AVBRH (SAWANGI) WARDHA. Apposite data on the demographics and treatment history as well as the indications was recorded. Counselling of all participants for research work were done. Both verbal along with written consent was taken from the participants. The routine protocol in our set up for sperm chromatin dispersion testis as follows:

Patients suffering from male infertility and having sperm count more than 5 million/mL were included included in a study and those having sperm count less than 5million/mL were as the SCD test needs minimum of 5-10 million/ml concentration of semen sample.

The samples were taken and kept for incubation at for 37°C for at least 20 minutes. The samples will be diluted to per 10^7 ml to obtain suspensions. Then, those suspensions were added to 1% agarose mixing well. We will perform a DNA directed denaturation to bring about controlled ssDNA patterns from DNA breakouts. The slides were then treated with HCl (0.8N) at room temperature for 7 minutes followed by placing it in a lysis solution for at least 15 minutes at room temperature. This will remove the membrane with a nucleus and a surrounding halo of scattered DNA loops and nuclear proteins. DNA halos were exceeding in membranes with non-fragmented nucleic acid. Then, were cleanse the slides under abundant distilled water for 5 minutes. We performed silver staining using staining solution. To stop the staining, we will use 1% acetic acid solution. The slides were then be washed and dried at room temperature. We interpreted the results by observing the slides under a compound light microscope at 40X.

The SDFI was is calculated by the following formula:

SDFI (%) = 100 × (no. Of sperms with fragmented DNA)/ (count of sperm).

Sperm with large or medium halos will be contemplated to be healthy without any DNA damage present. Sperm with extremely small or absent halos will be considered to have a DNA damage present. Mortified sperm were analysed to be with sperm DNA disintegration.

Reference range of DFI

Reference Value	Sperm DNA quality QUALITY
≤15% DFI	Very Good
>15 TO < 25% DFI	Good
>25 TO <50% DFI	Fair
≥50% DFI	Poor

6.2 Setting

Location: Wardha test tube baby centre, Avbrh, Sawangi.

6.3 Relevant dates, Including Periods of Recruitment

August 2020 – August 2022.

6.4 Participants

6.4.1 Inclusion criteria

- Infertile male partner with poor semen quality.
- Patients addicted to alcohol, tobacco and smoking.
- Patient with IVF implantation failure.

6.4.2 Exclusion criteria

- Patient not giving consent for treatment.
- Patients having infections like HIV, HbsAG etc.

6.5 Sample size

50 infertile couple

$$N = \frac{א2 * N * p(1-p)}{C2(N-1) + א2p(1-p)}$$

Population = N= 120 during 36 months
$א^{2=}$Chi-square value for 1 degrees at some expected probability level. This is 3.84 at 5% level of importance.
P=50% proportion
Q= 100 – p

=50
C= Confidence interval of the one choice(95% CI)
=0.05

$$N = \frac{3.84 * 120 * 0.5 * 0.5}{(0.05)2 * 24 + 3.84 * (0.5 * 0.5)}$$

6.6 Outcomes

50 men were included. SCD were conducted to check the sperm nucleic acid breakage. Taking into consideration, all the factors or indications which shows the cause for DNA breakage and causing infertility were recorded. The results were used to determine the amount of damaged DNA in the men and whether it can be treated or not according the examen.

7. DISCUSSION

A number of studies reflect on related issues [14-20]. The aim of conducting sperm DNA fragmentation test is to measure the DNA damage in men so that the cause of infertility can be known. According to some studies, men who tends to have smoking and alcoholic activities on regular basis have clearly damaged DNA resulting into infertility. Sometimes, stress also plays a major role in both male and female factor sterility. Lifestyle habits have a very great impact on one's DNA. By performing sperm chromatin dispersion test , we can clearly observe the damaged DNA as it is based on the concept that sperm with broken DNA do not form the characteristic halo of scattered DNA loops which is observed in sperm with non-fragmented DNA along with removal of nuclear proteins and acid denaturalize.

8. CONCLUSION

After this test, one should be able to treat the fragmentation by providing those patients with some antioxidants, vitamins, etc. which can treat DNA breakage [21-28].

CONSENT

As per international standard or university standard, patients' written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Majzoub A, Agarwal A, Esteves SC. Sperm DNA fragmentation: A rationale for its clinical utility. Transl Androl Urol. 2017;6(Suppl 4):S455–6.
2. Larsen U. Primary and secondary infertility in sub-Saharan Africa. 2000;285–91.
3. Balasch J. Ageing and infertility : An overview. 2010;26(December):855–60.
4. Moghaddam FS, Hosseini H, Mohammadi S, Amirahmadi M, Hosseini MS. Effect of abstinence time on semen parameters among male patients referring to urology clinic. Journal of Pharmaceutical Research International. 2019;31(3):1-7.
   DOI: 10.9734/jpri/2019/v31i330300
5. Nallella KP, Sharma RK, Ph D, Aziz N. Significance of sperm characteristics in the evaluation of male infertility. 2006; 85(3).
6. Meseguer M, Santiso R, Garrido N, García-Herrero S, Remohí J, Fernandez JL. Effect of sperm DNA fragmentation on pregnancy outcome depends on oocyte quality. Fertil Steril. 2011;95(1):124–8.
7. Agarwal A, Allamaneni SSR. The effect of sperm DNA damage. 2004; 56:235–45.
8. 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. 2019;8(4):356–65.
9. Cissen M, Wely M Van, Scholten I, Mansell S, Peter J, Bruin D, et al. Measuring sperm DNA fragmentation and clinical outcomes of medically assisted reproduction : A Systematic Review and Meta- Analysis; 2016.
10. 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):1–6.

11. Sergerie M, Laforest G, Bujan L, Bissonnette F, Bleau G. Sperm DNA fragmentation: Threshold value in male fertility. Hum Reprod. 2005;20(12):3446–51.
12. Simon L, Brunborg G, Stevenson M, Lutton D, McManus J, Lewis SEM. Clinical significance of sperm DNA damage in assisted reproduction outcome. Hum Reprod. 2010;25(7):1594–608.
13. Mostafa RM, Nasrallah YS, Hassan MM, Farrag AF, Majzoub A, Agarwal A. The effect of cigarette smoking on human seminal parameters, sperm chromatin structure and condensation. Andrologia. 2018;50(3):1–8.
14. Bhainsora RS, Patil PS, Ghogare AS, Vankar GK. A cross-sectional study of prevalence and types of sexual dysfunction among married male patients with alcohol dependence syndrome attending tertiary healthcare center from Central Rural India. Journal of Education and Health Promotion. 2021; 10(1).
15. Tayawade A, More A. A Successful ART Treatment of severe asthenoteratozoospermia with donor sperms: A case study at wardha test tube baby centre, India. Journal of Pharmaceutical Research International 2021;33(37B).
16. Ahire AM, Parwe S, Nisargandha M. A comparative evaluation of efficacy of mustadi yapan bastiand baladi yapan basti in the management of oligozoospermia-study protocol. Journal of Pharmaceutical Research International. 2021; 33(31A):208–16.
17. Kinkar JS, Jameel PZ, Kumawat BL, Kalbhor P. Heterozygous deletion in exon 6 of STEX gene causing ataxia with oculomotor apraxia type 2 (AOA-2) with ovarian failure. BMJ Case Reports. 2021;14(6).
18. Abbafati, Cristiana, Kaja M. Abbas, Mohammad Abbasi, Mitra Abbasifard, Mohsen Abbasi-Kangevari, Hedayat Abbastabar, Foad Abd-Allah, et al. Five Insights from the global burden of disease study 2019. Lancet. 2020; 396(10258):1135–59.
19. Gondivkar SM, Indurkar A, Degwekar S, Bhowate R. Evaluation of gustatory function in patients with diabetes mellitus type 2. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology. 2009;108(6): 876-80.
20. Prasad N, Bhatt M, Agarwal SK, Kohli HS, Gopalakrishnan N, Fernando E, Sahay M, Rajapurkar M, Chowdhary AR, Rathi M, Jeloka T. The adverse effect of COVID pandemic on the care of patients with kidney diseases in India. Kidney international reports. 2020; 5(9):1545-50.
21. Walia IS, Borle RM, Mehendiratta D, Yadav AO. Microbiology and antibiotic sensitivity of head and neck space infections of odontogenic origin. Journal of maxillofacial and oral surgery. 2014;13(1):16-21.
22. Lohe VK, Degwekar SS, Bhowate RR, Kadu RP, Dangore SB. Evaluation of correlation of serum lipid profile in patients with oral cancer and precancer and its association with tobacco abuse. Journal of oral pathology & medicine. 2010; 39(2):141-8.
23. Korde S, Sridharan G, Gadbail A, Poornima V. Nitric oxide and oral cancer: A review. Oral oncology. 2012;48(6):475-83.
24. Gondivkar SM, Gadbail AR. Gorham-Stout syndrome: A rare clinical entity and review of literature. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology. 2010; 109(2):e41-8.
25. Gadbail AR, Chaudhary M, Gawande M, Hande A, Sarode S, Tekade SA, Korde S, Zade P, Bhowate R, Borle R, Patil S. Oral squamous cell carcinoma in the background of oral submucous fibrosis is a distinct clinicopathological entity with better prognosis. Journal of Oral Pathology & Medicine. 2017;46(6):448-53.
26. Gadre PK, Ramanojam S, Patankar A, Gadre KS. Nonvascularized bone grafting for mandibular reconstruction: myth or reality?. Journal of Craniofacial Surgery. 2011;22(5):1727-35.
27. Sorte K, Sune P, Bhake A, Shivkumar VB, Gangane N, Basak A. Quantitative assessment of DNA damage directly in lens epithelial cells from senile cataract patients. Molecular vision. 2011; 17:1.

28. Basak S, Rajurkar MN, Mallick SK. Detection of Blastocystis hominis: A controversial human pathogen. Parasitology research. 2014;113(1):261-5.

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