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Original Research Article

Evaluating the efficacy of Dfrag tablets on human sperm DNA fragmentation - insights from a prospective clinical study

Mala Raj[1]*, Roobini J.[2], Aarathy Kasturiraj[2]

[1]Infertility Specialist, Endoscopic Surgeon, Firm Hospitals, Chennai, Tamil Nadu, India
[2]Clinical Embryologist, Firm Hospitals, Chennai, Tamil Nadu, India

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***Correspondence:**
Dr. Mala Raj,
E-mail: drmalaraj@gmail.com

ABSTRACT

Background: Infertility affects approximately 8-10% of couples worldwide. Sperm deoxyribonucleic acid (DNA) fragmentation index (DFI) has emerged as a significant factor in infertility research, highlighting its importance in understanding reproductive health.
Methods: This prospective clinical study aimed to assess the impact of Dfrag® tablets, a unique nutraceutical combination containing vitamin D3 (600 IU), selenomethionine (40 mcg), coenzyme Q10 (100 mg), and astaxanthin (8 mg), on high sperm DFI over a 3-month period. The study utilized the sperm chromatin dispersion test (SCD) to measure DFI and examined semen parameters before and after the intervention.
Results: Dfrag® tablets were found to significantly improve semen volume, total sperm count, sperm concentration, and progressive motility within the 3-month treatment period. The study reported an average reduction of 36% in DNA fragmentation levels post-treatment with Dfrag® tablets. However, no significant changes were observed in total motility or sperm morphology.
Conclusions: This study demonstrates the potential of Dfrag® tablets in reducing sperm DNA fragmentation and improving key semen parameters associated with fertility.

Keywords: Early clinical exposure, Knowledge, Medical education, Medical students

INTRODUCTION

Infertility is a significant global health concern affecting approximately 8-10% of couples worldwide, with a substantial burden observed in India alone, where between 15 and 20 million couples suffer from infertility each year.[1-3] The World Health Organization (WHO) reports that one in every four couples in developing countries faces infertility issues.[4] Male factors contribute to approximately 40% of infertility cases, with sperm DNA fragmentation emerging as a recognized cause impacting fertility outcomes.[5,6]

Semen analysis, the cornerstone of male infertility evaluation, assesses parameters such as semen volume, sperm count, motility, and morphology. However, traditional semen analysis may not fully capture the underlying issues related to sperm DNA integrity, which plays a crucial role in fertilization and embryo development.[7] Evidence suggests that nearly 30% of cases of idiopathic infertility exhibit high levels of sperm chromatin/DNA anomalies, adversely affecting fertility and assisted reproductive technology (ART) outcomes.[6-8]

Sperm DNA fragmentation, characterized by anomalies in chromatin packing, sperm maturation, and DNA integrity, is associated with increased early embryo death, decreased cleavage rates, and poor embryo quality.[8-10] Various factors contribute to sperm chromatin and DNA anomalies, including aberrant chromatin packing during spermatogenesis, oxidative stress induced by reactive

oxygen species (ROS), genetic defects, environmental toxins, and lifestyle factors.[11-13]

To address sperm DNA damage, strategies such as avoiding ROS-inducing factors and supplementing with vitamins, antioxidants, and oligo elements have been proposed to improve sperm quality and protect DNA integrity.[14] This prospective clinical study aims to investigate the effects of a nutraceutical combination comprising vitamin D3, selenomethionine, coenzyme Q10, and astaxanthin over a 3-month period on high sperm DNA fragmentation index (DFI), providing valuable insights into potential interventions for male infertility.

This study emphasizes the importance of addressing sperm DNA fragmentation as a modifiable factor in male infertility management, with implications for improving fertility outcomes and guiding therapeutic interventions.

METHODS

Study type

This was a prospective single-center study conducted at FIRM Hospital, Chennai, Tamil Nadu, India.

Period

The study was carried out for a period of 2 years from January 2022 to December 2023.

Selection criteria

Fifty men, referred for infertility treatment and exhibiting DFI levels of ≥25% in semen analysis, were enrolled. Informed consent was obtained from all participants after detailed explanations of the study objectives, procedures, and potential risks.

Inclusion criteria

Males aged 21 to 45 years, with no medication usage (including antioxidants) for the past three months, were eligible. Physical measurements, including weight and height, were recorded. Baseline semen samples were collected and analyzed.

Exclusion criteria

Participants with hypogonadism, vasectomy, undescended testis, prostate cancer, varicocele, hydrocele, chemotherapy, radiation therapy, or a history of azoospermia were excluded to ensure study reliability.

Procedure

Participants received Dfrag® tablets orally twice daily for three months. Throughout the treatment, a sexual abstinence period of 2-4 days before semen sample collection was advised. Any side effects were monitored and documented.

Primary outcome

The primary outcome was changes in DFI in sub-fertile males post-treatment.

Secondary outcomes

Secondary outcomes included changes in total semen volume, sperm motility, and sperm morphology post-treatment.

Evaluation

At the end of the treatment period, comprehensive evaluations were conducted. Semen samples were collected and analyzed to assess changes in standard semen parameters and DFI compared to baseline. DFI was evaluated using the sperm chromatin dispersion (SCD) test with the Sperm Chroma Kit from SAR Healthline.

Informed consent was taken from the patients.

Statistical analysis

IBM statistical package for the social sciences (SPSS) statistics 27.0.1 was used for statistical analysis. Mean and standard deviation were calculated for normal distribution, while median and interquartile range were used for abnormal distribution. Paired t tests were employed for group comparisons, with a significance level set at $p \leq 0.05$.

RESULTS

Patient demographics were provided in Table 1. The study included male patients seeking fertility treatment. Most of them were in the age group of 21 to 45 yrs. The findings from this study suggest that Dfrag® tablets may effectively improve semen parameters and reduce DNA fragmentation in individuals with fertility concerns. The results were given in Table 2. The mean semen volume at baseline was 2.35 ml (range: 1.3-5.0 ml). After 3 months of treatment with Dfrag®, the mean semen volume increased to 2.89 ml (range: 1.5-4.5 ml). This increase was statistically significant ($p<0.001$), indicating that the given Dfrag® tablets led to a significant improvement in semen volume. The mean total sperm number at baseline was 132.5 million (range: 45-400 million). Post-treatment, the mean total sperm number increased to 170.2 million (range: 55-333 million). This increase was statistically significant ($p<0.001$), suggesting that treatment with Dfrag® resulted in a significant increase in total sperm number. The mean sperm concentration at baseline was 56 million per ml (range: 45-102 million per ml). After 3 months of treatment, the mean sperm concentration increased slightly to 58 million per ml (range: 30-100 million per ml). The increase was statistically significant ($p<0.001$), indicating that Dfrag® tablets led to a

significant improvement in sperm concentration. The mean total motility at baseline was 70.04% (range: 20-97%). Post-treatment, the mean total motility remained relatively stable at 70.50% (range: 24-97%). There was no statistically significant change observed post-treatment (p=NS), indicating that Dfrag® tablets did not significantly affect total motility. The mean progressive motility at baseline was 16.74% (range: 0-39%). After treatment, the mean progressive motility increased to 19.04% (range: 5-29%). This increase was statistically significant (p<0.001), suggesting that Dfrag® tablets led to a significant improvement in progressive motility. There were no significant changes observed in sperm morphology between baseline and post-treatment assessments (p=NS), with the mean morphology remaining at 2.58%. The mean DFI percentage at baseline was 43.26% (range: 20-14%). After treatment, the mean DFI percentage decreased significantly to 26.54% (range: 10-47%). This decrease was statistically significant (p<0.001), indicating that Dfrag® tablets led to a significant reduction in sperm DNA fragmentation (Figure 1).

Overall, these results suggest that Dfrag® tablets effectively improved parameters related to sperm quality, including semen volume, total sperm number, sperm concentration, progressive motility, vitality, and average reduction of 36% in DNA fragmentation levels post-treatment with Dfrag®, within a 3-month treatment period. However, no significant changes were observed in total motility or sperm morphology.

Table 1: Patient demographics.

Age	Number of patients	Percentage (%)
20-30	15	30
30-40	22	44
40-45	13	74

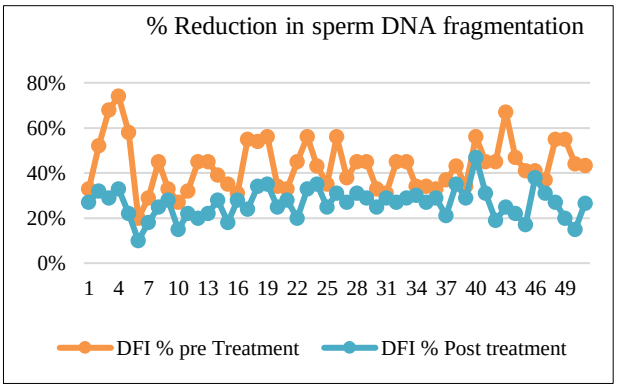

Figure 1: Percentage reduction in sperm DNA fragmentation post Dfrag® tablets.

Table 2: Baseline and post-treatment values for various sperm parameters.

Parameter	Baseline (%)	Post-treatment 3 months (%)	P value
Semen volume (ml)	2.35 (1.3-5.0)	2.89 (1.5-4.5)	<0.001 significant
Total sperm number (x million per ejaculate)	132.5 (45-400)	170.2 (55-333)	<0.001 significant
Sperm concentration (million per ml)	56 (45-102)	58 (30-100)	<0.001 significant
Total motility (PR+NP)	70.04 (20-97)	70.50 (24-97)	NS
Progressive motility (PR)	16.74 (0-39)	19.04 (5-29)	<0.001 significant
Sperm morphology	2.58 (1-4)	2.58 (1-4)	NS
DFI percentage	43.26 (20-14)	26.54 (10-47)	<0.001 significant

DISCUSSION

Number of studies have tried to elucidate the effect of antioxidant supplementation on different semen parameters. In a previous study by Agarwal et al indicated that generally combined antioxidants seemed to be more beneficial than single antioxidant treatment.[15] Varying levels of ROS is found in seminal plasma, and it originates from both endogenous and exogenous sources. Oxidative stress occurs when the amount of ROS overwhelms the natural antioxidant defence. This is known to cause damage on the spermatozoa, by damaging its DNA and by lipid peroxidation, affecting sperm membrane function.[15] In cases where it is likely that oxidative stress could be a part of the aetiology of the male subfertility, antioxidant supplementation has been proposed as a possible solution, both to increase the chance for natural conception and also to increase the success rate of assisted reproduction.[15] DNA fragmentation testing is crucial in assessing male fertility as it provides insights into the integrity of sperm DNA. High levels of DNA fragmentation are associated with reduced sperm quality and fertility potential. Hence, interventions targeting DNA fragmentation can potentially improve male fertility outcomes. Emerging evidence suggests that vitamin D and other antioxidants may play a role in reducing DNA fragmentation in sperm. Vitamin D deficiency has been linked to increased DNA damage in sperm, while supplementation with vitamin D has shown promising results in reducing DNA fragmentation. Oxidative stress, caused by harmful molecules called reactive oxygen species (ROS), can damage sperm. Antioxidants might counteract this damage, making natural conception more likely. They could be particularly helpful for men with conditions like obesity, smoking, or varicocele, which increase oxidative stress. The study involving 50 men showed that treatment with Dfrag® tablets led to significant improvements in semen parameters. Specifically, there were notable increases in

semen volume, total sperm number, sperm concentration, and progressive motility, along with a reduction in DNA fragmentation index percentage. However, there were no significant changes observed in total motility and sperm morphology. These findings suggest that Dfrag® tablets may offer substantial benefits in enhancing male fertility by positively affecting key semen parameters and DNA integrity. This suggests we need more research to understand the full effects of antioxidants on male fertility better. However, caution is needed in interpreting these results, as longer treatment duration may be necessary to observe significant improvements.

Limitations

This study had few limitations.

Single-center design

Conducting the study at a single center may limit the generalizability of the findings to a broader population. Results may not fully represent the diversity of patients seen in other healthcare settings.

Sample size

The relatively small sample size of 50 participants might affect the statistical power of the study, potentially limiting the ability to detect smaller treatment effects or associations.

Treatment duration

The study's three-month treatment duration might not be sufficient to observe long-term effects or changes in fertility outcomes beyond the immediate post-treatment period.

Outcome measures

While the study focused on changes in DFI and other semen parameters, other relevant outcomes such as pregnancy rates or live birth rates were not assessed, limiting the comprehensive evaluation of treatment efficacy.

CONCLUSION

The study finding suggests that Dfrag® tablets may effectively improve semen parameters and reduce DNA fragmentation in individuals with fertility concerns. This study reported an average reduction of 36% in DNA fragmentation levels post-treatment with Dfrag® tablets. These results indicate the potential of Dfrag® tablets as an effective treatment option for improving male fertility outcomes, though further research is warranted to validate these findings and explore long-term effects.

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Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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