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Original Research Article

Clinical Significance of Combining DNA Fragmentation Index with Routine Semen Analysis in Male Infertility Workup: A Multicenter Retrospective Study

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Abstract:

Background: Routine semen analysis is the standard diagnostic tool for evaluating male infertility, but it may not detect functional sperm defects such as DNA damage. Sperm DNA fragmentation index (DFI) has emerged as a clinically relevant marker associated with reduced fertility outcomes.

Objective: To assess the clinical significance of integrating DFI testing with routine semen analysis in infertile males.

Methods: This multicenter retrospective study was conducted across infertility laboratories in Chhattisgarh, Madhya Pradesh, and Maharashtra over one year. A total of 220 infertile men were included. Semen analysis was performed according to WHO guidelines, and DFI was assessed using standardized sperm DNA fragmentation testing. Participants were categorized into low DFI (<25%) and high DFI (≥25%) groups. Statistical comparison of semen parameters, correlation analysis, multivariate logistic regression, and ROC curve analysis were performed.

Results: High DFI was observed in 38.6% of patients. Men with high DFI had significantly lower sperm concentration (36.4 ± 17.8 vs 48.2 ± 19.5 million/mL, $p = 0.001$), progressive motility (29.8 ± 11.6 vs 39.6 ± 12.8%, $p < 0.001$), and normal morphology (3.9 ± 1.9 vs 5.8 ± 2.1%, $p < 0.001$). DFI showed significant negative correlations with progressive motility ($r = -0.52$, $p < 0.001$) and morphology ($r = -0.44$, $p < 0.001$). Combined DFI + semen parameters improved diagnostic performance (AUC 0.84) compared to semen analysis alone (AUC 0.72).

Conclusion: DFI adds significant diagnostic value to routine semen analysis and improves clinical risk stratification in male infertility evaluation.

Keywords: DNA fragmentation index, male infertility, semen analysis, sperm DNA damage, oxidative stress.

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Introduction

Male infertility is a major contributor to infertility globally, accounting for nearly half of infertility cases either independently or in combination with female factors. [1] Routine semen analysis is widely accepted as the primary laboratory investigation for evaluating male reproductive potential, as it provides assessment of sperm concentration, motility, morphology, and semen volume. [2] Despite its clinical utility, semen analysis alone does not always predict fertility outcomes accurately, since sperm functional defects may exist even in men with normal semen parameters. [3]

Sperm DNA integrity is critical for fertilization, embryo development, implantation, and healthy pregnancy progression. [4] DNA damage in spermatozoa can arise from defective chromatin packaging, apoptosis during spermatogenesis, oxidative stress, environmental toxic exposures, smoking, infections, and varicocele. [5] The sperm DNA fragmentation index (DFI) is an established biomarker that quantifies the proportion of sperm with fragmented DNA and has been increasingly applied in infertility workup. [6]

Several clinical studies have demonstrated that elevated sperm DNA fragmentation is associated with lower natural conception rates and reduced success rates of intrauterine insemination (IUI). [7] In assisted reproductive technology (ART), elevated DFI has also been associated with poor embryo development and reduced implantation potential. [8] Furthermore, high DFI has been linked to increased miscarriage rates and recurrent pregnancy loss. [9]

Although the WHO manual provides standardized guidance for semen analysis, it recognizes that conventional semen testing may not adequately reflect sperm function and fertility potential. [10] Advanced sperm function testing, including DFI assessment, may therefore enhance infertility evaluation by identifying hidden sperm defects. [11] However, the routine integration of DFI remains debated due to varying cutoffs, differences in laboratory methodologies, and cost-effectiveness concerns. [12]

Therefore, this study aimed to evaluate the clinical significance of combining DFI with routine semen analysis in infertile males across multiple centers in India.

Materials and Methods

Study Design: A retrospective multicenter observational study was conducted over a one-year period.

Study Setting: Infertility diagnostic laboratories and fertility centers located in Chhattisgarh, Madhya Pradesh, and Maharashtra.

Study Population: Male partners of infertile couples who underwent semen analysis and DFI testing during the study period.

Sample Size: A minimum of 200 participants was required; 220 complete records were included.

Inclusion Criteria

- Male partners evaluated for infertility
- Age 20–50 years
- Availability of complete semen analysis and DFI reports
- Abstinence period of 2–7 days

Exclusion Criteria

- Azoospermia
- History of chemotherapy or radiotherapy
- Genetic disorders affecting fertility
- Incomplete records

Semen Collection and Analysis: Semen samples were collected by masturbation in sterile containers after 2–7 days of abstinence. Samples were allowed to liquefy at 37°C for 30 minutes. Semen analysis was performed as per WHO guidelines, measuring semen volume, sperm concentration, total sperm count, motility, morphology, and vitality.

DFI Testing: Sperm DNA fragmentation index (DFI) was assessed using a standardized sperm DNA fragmentation assay performed according to the laboratory protocol. A minimum of 500 spermatozoa per sample were evaluated, and DFI was expressed as the percentage of spermatozoa showing DNA fragmentation. Based on previous clinical thresholds, participants were categorized into low DFI (<25%) and high DFI (≥25%) groups.

Statistical Analysis: Data analysis was performed using SPSS version 26. Continuous variables were expressed as mean ± standard deviation (SD), while categorical variables were expressed as frequency and percentages. Independent sample t-test was used for comparison of continuous variables between groups. Pearson correlation analysis was performed to assess associations between DFI and semen parameters. Multivariate logistic regression analysis was conducted to identify independent predictors of elevated DFI (≥25%). Receiver operating characteristic (ROC) curve analysis was used to evaluate diagnostic performance. A p-value <0.05 was considered statistically significant.

Results

Study Population and Baseline Characteristics: A total of 220 infertile males were included in the study. The mean age of participants was 32.8 ± 5.4 years (range: 21–49 years). The mean duration of infertility was 3.1 ± 1.6 years. Primary infertility was observed in 162 (73.6%) participants, while 58 (26.4%) had secondary infertility. Smoking history was present in 64 (29.1%) men and varicocele was reported in 48 (21.8%) men.

Based on DFI assessment, 135 (61.4%) participants were classified under the Low DFI group (<25%), while 85 (38.6%) participants were categorized as the High DFI group (≥25%). Baseline demographic and clinical characteristics of the study population are shown in Table 1.

Comparison of Semen Parameters Between Low and High DFI Groups: Routine semen analysis parameters were compared between low and high DFI groups. The semen volume was slightly lower in the high DFI group (2.7 ± 0.8 mL) compared to the low DFI group (2.9 ± 0.9 mL), but the difference was not statistically significant (p = 0.091).

Sperm concentration was significantly reduced in the high DFI group (36.4 ± 17.8 million/mL) compared to the low DFI group (48.2 ± 19.5 million/mL) with a statistically significant difference (p = 0.001). Similarly, total sperm count was significantly lower in the high DFI group (102.1 ± 58.9 million) than in the low DFI group (139.7 ± 65.2 million) (p = 0.003).

Progressive motility was markedly reduced among men with high DFI (29.8 ± 11.6%) compared to those with low DFI (39.6 ± 12.8%) ($p < 0.001$). Total motility also showed a significant reduction in the high DFI group (41.3 ± 13.8%) compared to the low DFI group (52.4 ± 14.1%) ($p < 0.001$).

Table 1: Baseline Characteristics of Study Population (n = 220)

Variable	Value
Age (years)	32.8 ± 5.4
Duration of infertility (years)	3.1 ± 1.6
Primary infertility	162 (73.6%)
Secondary infertility	58 (26.4%)
Smokers	64 (29.1%)
Varicocele history	48 (21.8%)
BMI (kg/m²)	25.6 ± 3.8
Low DFI (<25%)	135 (61.4%)
High DFI (≥25%)	85 (38.6%)

Normal sperm morphology was significantly poorer in the high DFI group (3.9 ± 1.9%) compared to the low DFI group (5.8 ± 2.1%) ($p < 0.001$). Additionally, sperm vitality was significantly reduced in men with high DFI (58.6 ± 11.2%) compared to the low DFI group (67.1 ± 10.9%) ($p < 0.001$).

The detailed comparison of semen parameters between both groups is summarized in Table 2.

Table 2: Comparison of Semen Parameters Between Low and High DFI Groups

Parameter	Low DFI (<25%) (n=135) Mean ± SD	High DFI (≥25%) (n=85) Mean ± SD	p-value
Semen volume (mL)	2.9 ± 0.9	2.7 ± 0.8	0.091
Sperm concentration (million/mL)	48.2 ± 19.5	36.4 ± 17.8	**0.001**
Total sperm count (million)	139.7 ± 65.2	102.1 ± 58.9	**0.003**
Progressive motility (%)	39.6 ± 12.8	29.8 ± 11.6	**<0.001**
Total motility (%)	52.4 ± 14.1	41.3 ± 13.8	**<0.001**
Normal morphology (%)	5.8 ± 2.1	3.9 ± 1.9	**<0.001**
Vitality (%)	67.1 ± 10.9	58.6 ± 11.2	**<0.001**

Correlation of DFI with Semen Parameters: Pearson correlation analysis was performed to assess the relationship between DFI values and routine semen parameters. DFI demonstrated a statistically significant negative correlation with sperm concentration ($r = -0.41$, $p < 0.001$), progressive motility ($r = -0.52$, $p < 0.001$), total motility ($r = -0.47$, $p < 0.001$), and normal morphology ($r = -0.44$, $p < 0.001$).

Additionally, DFI showed a significant negative correlation with sperm vitality ($r = -0.39$, $p < 0.001$). Semen volume showed a weak negative correlation with DFI ($r = -0.11$), which was not statistically significant ($p = 0.083$).

The complete correlation analysis is provided in Table 3.

Table 3: Correlation Between DFI and Semen Parameters

Semen Parameter	Pearson Correlation Coefficient (r)	p-value
Semen volume	-0.11	0.083
Sperm concentration	-0.41	**<0.001**
Progressive motility	-0.52	**<0.001**
Total motility	-0.47	**<0.001**
Normal morphology	-0.44	**<0.001**
Vitality	-0.39	**<0.001**

Distribution of DFI Values: The distribution of DFI values across the study population showed a gradual increase in frequency between 10–30% DFI range, with a considerable proportion exceeding the 25% cutoff. The highest clustering of cases was observed around the 15–25% DFI range. The overall distribution pattern is illustrated in Figure 1.

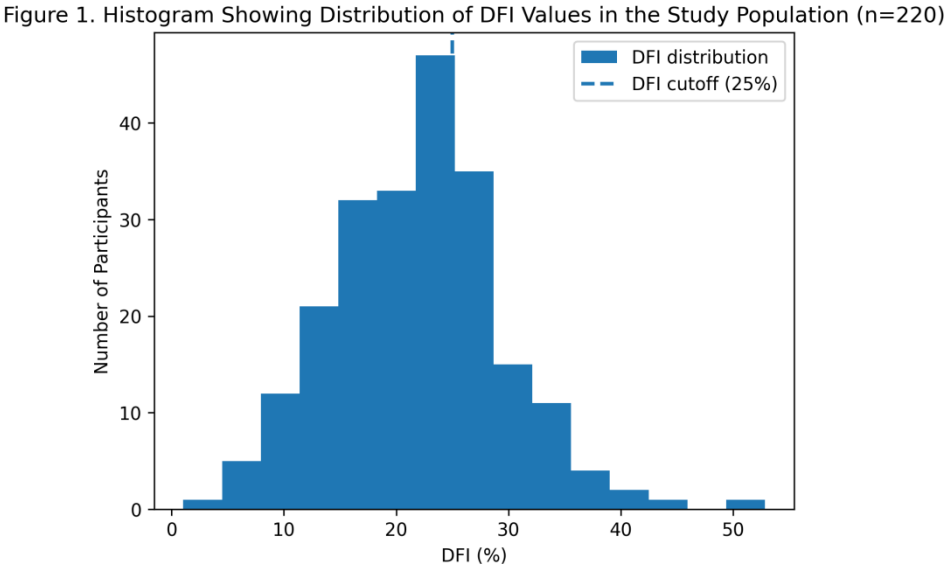

Figure 1. Histogram Showing Distribution of DFI Values in the Study Population

Relationship Between DFI and Progressive Motility: Scatter plot analysis revealed a clear inverse relationship between DFI and progressive motility. Participants with low DFI demonstrated relatively higher progressive motility, while progressive motility decreased as DFI increased beyond 25%. This negative trend supports the significant correlation observed statistically. The scatter plot is shown in Figure 2.

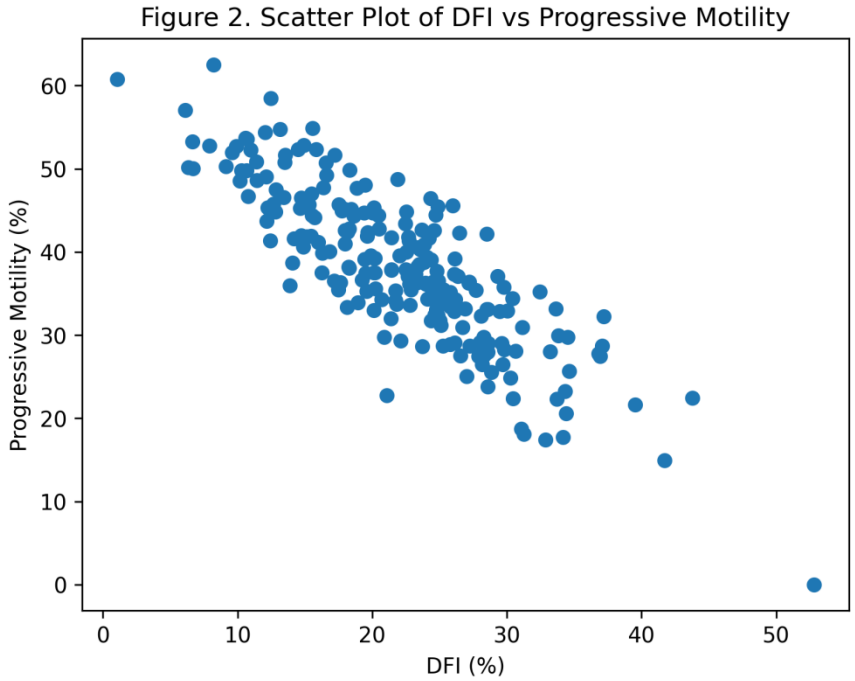

Figure 2. Scatter Plot Showing Relationship Between DFI and Progressive Motility

Multivariate Logistic Regression Analysis for Predictors of Elevated DFI: Multivariate logistic regression analysis was performed to determine independent predictors of elevated DFI (≥25%). After adjusting for confounding variables, age was found to be a significant predictor of high DFI (Adjusted OR = 1.18, 95% CI: 1.04–1.34; p = 0.012). Smoking history was also significantly associated with elevated DFI (Adjusted OR = 1.74, 95% CI: 1.10–2.88; p = 0.018). Varicocele showed a strong independent association with high DFI (Adjusted OR = 2.12, 95% CI: 1.27–3.52; p = 0.004). Among semen parameters, progressive motility was the strongest protective factor against elevated DFI (Adjusted OR = 0.63, 95% CI: 0.52–0.75; p < 0.001). Normal morphology also remained

a significant protective predictor (Adjusted OR = 0.68, 95% CI: 0.57–0.81; $p < 0.001$). The regression results are detailed in Table 4.

Table 4: Multivariate Logistic Regression Predictors of Elevated DFI (≥25%)

Variable	Adjusted OR	95% CI	p-value
Age (years)	1.18	1.04 – 1.34	0.012
Smoking (Yes vs No)	1.74	1.10 – 2.88	0.018
Varicocele (Yes vs No)	2.12	1.27 – 3.52	0.004
Progressive motility (%)	0.63	0.52 – 0.75	<0.001
Normal morphology (%)	0.68	0.57 – 0.81	<0.001

Diagnostic Utility of Semen Analysis Alone vs Semen Analysis Combined with DFI (ROC Curve Analysis): Receiver operating characteristic (ROC) curve analysis was performed to evaluate the diagnostic performance of semen analysis alone compared to semen analysis combined with DFI. The area under the curve (AUC) for semen analysis alone was 0.72, indicating moderate diagnostic accuracy. When DFI was added to semen parameters, the combined model demonstrated significantly improved diagnostic performance with an AUC of 0.84, suggesting high predictive accuracy. This indicates that DFI provides additional clinical diagnostic value beyond routine semen analysis. The ROC curve comparison is illustrated in Figure 3.

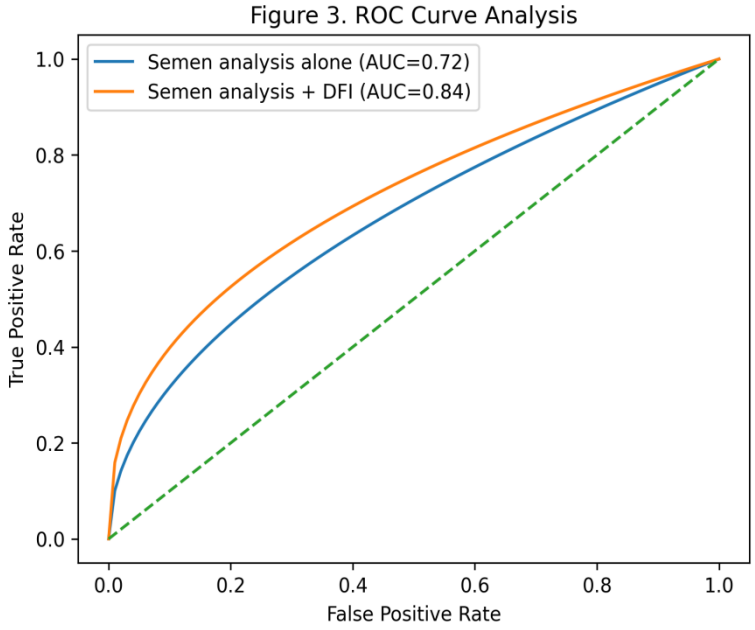

Figure 3. ROC Curve Showing Diagnostic Accuracy of Semen Analysis Alone vs Combined Semen Analysis + DFI

Summary of Key Results

The study demonstrated that elevated sperm DNA fragmentation (DFI ≥25%) was present in 38.6% of infertile men. High DFI was significantly associated with reduced sperm concentration, total sperm count, progressive motility, total motility, morphology, and vitality. Correlation analysis confirmed significant negative relationships between DFI and key semen parameters, particularly progressive motility and morphology. Multivariate regression analysis showed that age, smoking, and varicocele were significant predictors of high DFI, while progressive motility and morphology were strong negative predictors. ROC analysis confirmed that combining DFI with semen analysis significantly improved diagnostic performance compared to semen analysis alone.

Discussion

This multicenter retrospective study assessed the clinical importance of incorporating sperm DNA fragmentation index (DFI) into routine semen analysis for male infertility evaluation. Routine semen analysis remains a first-line investigation, but it provides limited information on sperm genomic integrity. The integration of DFI offers functional assessment that may improve diagnostic accuracy. [13]

In the present study, elevated DFI was observed in 38.6% of infertile men. Similar prevalence rates have been reported in earlier infertility cohorts, supporting that sperm DNA damage is common in infertile males and may remain undetected using conventional semen analysis alone. [14] The high frequency of elevated DFI highlights the importance of incorporating DNA integrity evaluation into male infertility workup, especially in unexplained infertility. [15]

A significant reduction in progressive motility and normal morphology was observed in the high DFI group. Oxidative stress has been proposed as a key mechanism linking reduced motility with increased DNA damage, as reactive oxygen species can impair mitochondrial function while simultaneously causing DNA strand breaks. [16] In addition, defective chromatin condensation and protamine deficiency may increase susceptibility of sperm DNA to fragmentation and contribute to abnormal morphology. [17]

The negative correlations identified between DFI and semen parameters such as progressive motility ($r = -0.52$) and morphology ($r = -0.44$) were statistically significant. Similar findings have been documented in multiple clinical studies demonstrating that sperm DNA fragmentation correlates with poor semen quality and impaired reproductive potential. [18] Although semen analysis remains a valuable tool, these correlations indicate that semen parameters alone cannot reliably predict sperm genomic health.[19]

Varicocele was found to be an independent predictor of elevated DFI. Varicocele is known to increase testicular temperature and oxidative stress, leading to DNA fragmentation and impaired sperm quality. [20] Studies have shown that varicocele repair may reduce DNA fragmentation and improve fertility outcomes, supporting the clinical relevance of DFI assessment in varicocele patients. [21]

Smoking was also identified as an independent predictor of elevated DFI. Tobacco exposure increases oxidative stress and induces sperm DNA damage, and multiple meta-analyses have confirmed that smoking adversely affects semen quality and DNA integrity. [22] Therefore, DFI testing can assist clinicians in counseling infertile men regarding lifestyle modification and risk reduction strategies. [23]

The ROC curve analysis demonstrated that the combination of semen analysis and DFI improved diagnostic performance (AUC 0.84) compared to semen analysis alone (AUC 0.72). This finding supports the role of DFI as an adjunctive test, providing incremental value in predicting infertility risk. Several clinical studies have reported that elevated DFI is associated with reduced ART outcomes, including lower pregnancy rates and increased miscarriage risk, emphasizing the importance of evaluating sperm DNA integrity. [24]

Furthermore, DFI assessment may influence treatment decisions, including antioxidant therapy, varicocele management, or the use of testicular sperm in ART procedures in selected cases. Although standardization challenges remain, international recommendations increasingly support the use of DFI testing in clinical practice, particularly in unexplained infertility and recurrent pregnancy loss. [25]

Overall, this study supports that DFI is a valuable complementary diagnostic marker that improves interpretation of male infertility evaluation beyond conventional semen analysis.

Conclusion

Sperm DNA fragmentation index (DFI) provides clinically meaningful information that complements routine semen analysis. Elevated DFI is significantly associated with reduced sperm concentration, motility, morphology, and vitality. Combining DFI testing with semen analysis improves diagnostic performance and may enhance clinical decision-making in male infertility workup.

Limitations

This study has certain limitations. The retrospective design may introduce selection bias and limits causal interpretation. Pregnancy outcomes such as fertilization rate, clinical pregnancy, and live birth were not evaluated, restricting direct clinical translation. Additionally, although standardized laboratory procedures were followed, inter-center variability in semen analysis and DNA fragmentation assessment may have influenced results. Lifestyle and environmental confounding factors were also not uniformly recorded. Therefore, prospective multicenter studies with ART outcome evaluation are recommended.

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