Original Article

The relationship between age and DNA fragmentation index: Use of sperm chromatin dispersion assay to evaluate the sperm DNA fragmentation

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ABSTRACT

Objective: To evaluate sperm DNA fragmentation using the sperm chromatin dispersion (SCD) assay in a large retrospective cohort and to assess age-related patterns of DNA damage. **Methods:** This retrospective observational study included 456 semen samples obtained from male partners of infertile couples. Sperm DNA fragmentation was assessed using the SCD method, and the DNA fragmentation index (DFI) was calculated as the percentage of spermatozoa showing the absence of halo formation and degraded nuclei. Participants were stratified into four age groups (≤30, 31–35, 36–40, and >40 years). Mean DFI values and the prevalence of abnormal DFI (≥25% and ≥30%) were analyzed across the age groups. **Results:** Mean DFI values did not differ significantly among age groups (p=0.637). Correlation analysis showed a weak, non-significant association between age and DFI. However, the proportion of samples with abnormal DFI (≥25%) increased progressively with age, reaching 55.4% in men older than 40 years. A similar trend was observed for severe DNA fragmentation (DFI ≥30%). **Conclusion:** Although mean DFI values were comparable across different age groups, advancing age was associated with a higher prevalence of clinically significant sperm DNA fragmentation. These findings support threshold-based interpretation of DFI and highlight the clinical utility of SCD testing in routine male infertility evaluation, particularly in older individuals.

Key words: Male infertility assessment, Age groups, Sperm DNA fragmentation, Sperm chromatin dispersion assay.

Infertility affects approximately 10-15% of couples worldwide, with male infertility contributing to nearly half of all cases [1-4]. Conventional semen analysis, which evaluates sperm concentration, motility, and morphology, remains the cornerstone of male fertility assessment. However, a substantial proportion of men with normal semen parameters continue to experience infertility, recurrent implantation failure, or pregnancy loss, highlighting the limitations of routine semen analysis alone [5,6]. Sperm DNA integrity plays a critical role in fertilization, embryo development, and maintenance of pregnancy. Sperm DNA fragmentation (SDF), characterized by single- or double-strand DNA breaks, may arise from defective chromatin remodeling, oxidative stress, apoptosis, or post-testicular damage [5,6].

While limited DNA damage can be repaired by the oocyte, excessive fragmentation overwhelms repair mechanisms and adversely affects reproductive outcomes [10-13]. Several assays are available to assess SDF, including the sperm chromatin structure (SCSA) assay, terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) assay, comet assay, and sperm chromatin dispersion (SCD) assay [8,9].

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Among these, the SCD test has gained widespread clinical acceptance due to its simplicity, cost-effectiveness, and suitability for routine laboratory use. The method is based on halo formation following controlled denaturation and protein removal, allowing visual identification of spermatozoa with fragmented DNA [9]. Accumulating evidence indicates that an elevated sperm DNA fragmentation index (DFI) is associated with unexplained infertility, poor assisted reproductive technology (ART) outcomes, and increased risk of miscarriage [10-13]. Importantly, many men with normal semen parameters exhibit high DFI, emphasizing the independent diagnostic value of sperm DNA testing [5,6,8]. Although various DFI thresholds (15-30%) have been proposed, cut-offs around 25-30% are most associated with adverse reproductive outcomes [8,10,13].

Retrospective SCD-based studies provide valuable real-world insight into the prevalence and clinical distribution of sperm DNA fragmentation. However, population-specific data remain limited, particularly regarding age-related trends [14,15].

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The present retrospective study evaluates sperm DNA fragmentation using the SCD method in a large cohort of infertile men and examines the relationship between age and DFI. The findings aim to strengthen evidence supporting the incorporation of SCD testing into routine male infertility assessment.

MATERIALS AND METHODS

The present retrospective observational study was conducted at AMPATH Diagnostic Laboratory, Hyderabad, a diagnostic and infertility testing center in Western India, where DFI assessment using the SCD assay is routinely performed as a part of the evaluation of male infertility [7,9]. For the study, the data were retrieved from the andrology laboratory records for all patients who underwent semen analysis along with sperm DFI testing between January 2025 and [insert end month/year] . During the study period, a total of 456 semen samples with concurrent DFI assessment were included for analysis. Patients were instructed to provide semen samples by masturbation following approximately 3 days of sexual abstinence. The collected semen samples were allowed to liquefy at room temperature before analysis.

Each semen sample was divided into two portions. One portion was used for routine semen analysis, including assessment of sperm concentration, while the second portion was utilized for sperm DNA fragmentation testing. Conventional semen parameters, including sperm concentration, motility, and morphology was determined by microscopic examination using a counting chamber, whereas spermatozoa were quantified within defined grid areas. Conventional semen parameters were interpreted according to the World Health Organization (WHO) reference values for human semen characteristics [3]. Participants were stratified into four age groups: ≤30 years, 31-35 years, 36-40 years, and >40 years. Mean DFI values and the prevalence of abnormal DFI (≥25% and ≥30%) were analyzed across age categories [8,10,13]. Demographic and clinical information, including age and available laboratory parameters, were extracted from routine laboratory records using a standardized data collection form and entered into Microsoft Excel for analysis.

Sperm DNA fragmentation was assessed using the SCD method employing the CANfrag DNA Fragmentation Kit (Candore Biosciences, Belgium), following the manufacturer's instructions [9]. Briefly, semen samples were mixed with 1% low-melting-point agarose, followed by placing them onto slides, and allowed to solidify. The slides were subsequently treated with lysis solution (Reagent I) for 7 min, followed by neutralization and secondary lysis (Reagent II). After washing, slides were dehydrated, stained, and examined under light microscopy. For each sample, 500 spermatozoa were evaluated to assess halo size and dispersion patterns, and spermatozoa were classified as having intact or fragmented DNA based on established criteria described by Fernandez et al. [9]. The percentage of spermatozoa lacking halo formation or exhibiting degraded nuclei was recorded as the DFI. Based on DFI values, samples were categorized into 3 groups: <15%, 15–25%, and ≥25%. In accordance with established guidelines, DFI ≤25% was considered within acceptable limits, whereas values >25% were regarded as indicative of compromised reproductive potential [8,10,13].

Statistical analysis was performed using SPSS version 26 and Microsoft Excel. Continuous variables were expressed as mean ± standard deviation. Differences in mean DFI among age groups were evaluated using one-way analysis of variance (ANOVA). Correlation between age and DFI was assessed using Pearson and Spearman correlation coefficients. A p-value <0.05 was considered statistically significant. The present retrospective study was conducted in accordance with the Declaration of Helsinki. As the analysis involved anonymized laboratory records and did not require direct patient contact, formal institutional ethics committee approval was waived. All patient identifiers were removed before data analysis to ensure confidentiality.

RESULTS

A total of 456 semen samples were included in the analysis. After calculating DFI, the participants were categorized into four age groups: ≤30 years (n = 86), 31-35 years (n = 171), 36-40 years (n = 134), and >40 years (n = 65). The mean DFI values (mean ± SD) for these groups were found to be 22.50 ± 8.53%, 24.09 ± 9.68%, 23.92 ± 10.32%, and 23.92 ± 9.78%, respectively. One-way analysis of variance revealed no statistically significant difference in mean DFI values among the age groups (p = 0.637). Correlation analysis also demonstrated a weak and non-significant association between age and DFI.

However, threshold-based analysis showed a clear age-related increase in the prevalence of abnormal sperm DNA fragmentation. Using a DFI cut-off of ≥25%, elevated DFI was observed in 27 (31.4%) men aged ≤30 years, 80 (46.8%) men aged 31-35 years, 56 (41.8%) men aged 36-40 years, and 36 (55.4%) men older than 40 years. A similar trend was noted for severe DNA fragmentation (DFI ≥30%), with prevalence increasing from 30.2% in the ≤30-year group to 55.4% in the >40-year group. Overall, although mean DFI values were comparable across age categories, advancing age was associated with a progressively higher proportion of men exhibiting clinically significant sperm DNA damage.

DISCUSSION

In this retrospective cohort of 456 semen samples, sperm DNA fragmentation was assessed by the SCD assay, which demonstrated substantial inter-individual variability. Although mean DFI values did not differ significantly across the age groups, a clear age-associated increase in the proportion of men

with clinically abnormal DFI was observed, with more than half of individuals older than 40 years exhibiting DFI ≥25%, consistent with previous reports [11-13]. This discrepancy between mean DFI values and threshold-based prevalence highlights an important clinical concept. Age-related sperm DNA damage is influenced by cumulative oxidative stress, impaired chromatin packaging, and reduced DNA repair capacity. However, these processes progress heterogeneously among individuals [5,6,11,12].

As a result, mean-based comparisons may underestimate clinically relevant patterns, whereas cut-off-based interpretation better reflects reproductive risk [8,10,13]. Previous studies using SCD, SCSA, and TUNEL assays have similarly reported weak correlations between age and mean DFI but consistently demonstrate higher rates of abnormal DFI in older men [11-13]. Elevated DFI above 25-30% has been associated with reduced fertilization, impaired embryo development, and increased miscarriage in both natural conception and ART cycles [10-13].

Therefore, the higher prevalence of abnormal DFI observed in older individuals in this study carries direct clinical relevance. The weak correlation between chronological age and DFI further emphasizes that age alone is insufficient to predict sperm DNA integrity. Lifestyle factors, environmental exposures, and underlying testicular pathology also contribute significantly to DNA fragmentation [1,5,6]. This supports the clinical value of direct SDF testing rather than reliance on age or conventional semen parameters [5,6,8]. From a practical perspective, the SCD assay proved useful for identifying men with elevated DFI in routine diagnostic settings [9,15].

Such information can guide patient counseling and management, including lifestyle modification, antioxidant therapy, or selection of ART strategies [7,10]. In couples with repeated treatment failure or unexplained infertility, SCD testing may provide critical additional prognostic insight [10,13]. This study is limited by its retrospective design and single-center setting. In addition, ART outcomes were not available for correlation with DNA fragmentation index values. Despite these limitations, the large sample size and standardized laboratory methodology strengthen the clinical relevance of the findings.

Figure1

CONCLUSION

In this large retrospective cohort, although mean sperm DNA fragmentation index (DFI) values did not differ significantly across age groups, advancing age was associated with a higher prevalence of clinically abnormal DFI. These findings emphasize the importance of threshold-based interpretation of sperm DNA fragmentation and support the routine clinical use of sperm chromatin dispersion testing in the evaluation of male infertility, particularly in older men.

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