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Original Research Article

Impact of cigarette smoking on semen quality and sperm DNA fragmentation in infertile men of Chhattisgarh, India

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ABSTRACT

Background: Cigarette smoking is a major risk factor for male infertility, primarily by reducing sperm count and motility, thereby impairing spermatogenesis and overall reproductive function. This study aimed to evaluate the effects of smoking on semen parameters and sperm DNA integrity in infertile men.
Methods: A total of 200 male partners of infertile couples attending an infertility clinic in Raipur, Chhattisgarh, from 2022 to 2024 were included. Detailed smoking histories were obtained, followed by semen analysis. Statistical comparisons between smokers and non-smokers were performed using appropriate software.
Results: Smokers had significantly lower semen volume (2.08±0.78 versus 2.45±0.79 ml), total sperm count (59.22±60.39 versus 94.12±91.12 million), total motility (44.82±24.66 versus 57.03±20.33%), progressive motility (24.67±24.19 versus 33.07±24.14%), vitality (46.46±20.18 versus 56.81±22.15%), and normal morphology (3.25±1.27 versus 3.84±1.42%), with higher DNA fragmentation index (21.33±10.52 versus 15.05±6.63%) compared with non-smokers. Moderate or occasional smokers exhibited intermediate values. Sperm concentration did not differ significantly. Normozoospermia was most frequent in non-smokers (46%), whereas daily smokers showed higher prevalence of asthenozoospermia, teratozoospermia, and combined abnormalities, suggesting a dose-dependent negative effect of smoking.
Conclusion: Cigarette smoking significantly impairs semen quality and increases sperm DNA fragmentation, contributing to male infertility. Even intermittent smoking adversely affects semen parameters, highlighting the importance of smoking cessation for fertility preservation.

Keywords: Sperm count, Motility, Smoking, Male infertility, DNA fragmentation

INTRODUCTION

Male infertility is the inability of a man to achieve conception with a fertile female and is diagnosed when semen parameters fall below the World Health Organization (WHO) reference limits.[1] Male-factor infertility is defined as altered sperm concentration, motility, and/or morphology in at least one of two semen analyses performed 1–4 weeks apart.[2] While female factors account for over half of infertility cases, male factors alone contribute to about 30% of couples and, together with mixed causes, play a role in roughly 50% of infertility cases.[3-5]

Despite extensive evaluation, 30–50% of male infertility remains idiopathic. Proposed contributors include advancing age, psychological stress, poor lifestyle habits, and environmental or occupational exposures.[6-11] Established causes encompass genetic defects, anatomic or hormonal abnormalities, genital tract infection, chronic systemic disease, cancer and its therapy, and sexual disorders that impair intercourse.[7]

Lifestyle factors—particularly tobacco use and alcohol intake—are increasingly recognized as important. Tobacco smoking is a significant source of reactive oxygen species (ROS) and is associated with leucocytospermia, oxidative stress, and impaired sperm concentration, motility, morphology, DNA integrity, and viability.[10,12-15] World Health Organisation (WHO) data show that in 2020, 22.3% of the global population used tobacco, including 36.7% of men.[16] India alone has an estimated 120 million smokers and accounts for about 12% of the world's smoking population; approximately 267 million Indians use some form of tobacco.[17,18] Multiple studies link heavy tobacco consumption to decreased semen volume, concentration, motility, viability, and normal morphology, whereas others report no significant effect.[19-22] These inconsistent findings, coupled with a paucity of data from Chhattisgarh, underscore the need for regional studies. Therefore, the present study evaluates semen parameters of smokers compared with non-smokers among infertile men attending a tertiary clinic in Chhattisgarh, India.

METHODS

Study design and participants

This retrospective observational study was conducted at the Infertility Clinic, Raipur, Chhattisgarh, India, between 2022 and 2024. A total of 200 male partners of infertile couples were initially considered. Written informed consent was obtained from all participants, and a standardized questionnaire was administered to collect clinical and smoking histories.

Inclusion criteria

Men of reproductive age with primary or secondary infertility were included.

Exclusion criteria

Patients with azoospermia were excluded, resulting in a final cohort of 195 participants.

Based on smoking status, participants were divided into non-smokers (control, n=108) and smokers (n=87). Smokers were further categorized as occasional/moderate (1–4 cigarettes/day) and daily smokers (≥5–15 cigarettes/day).

Collection of semen samples

Semen samples were collected by masturbation after 2–7 days of sexual abstinence into wide-mouthed, sterile, leak-proof containers in a designated collection room.

Semen analysis

After the liquefaction of the semen sample at 37°C for 20 min to 1 hour, the samples were analyzed for pH, count, motility, morphology, vitality, and DNA fragmentation index (DFI) according to the World Health Organisation guidelines 2021. The pH of the semen is measured using pH paper (pH: 6-10) by evenly spreading one drop of semen onto the pH paper, and after 30 seconds, the color change is compared with the calibration strip to read the pH. Sperm count and motility were calculated in 10 µl of liquefied semen by Makler chamber under the microscope. Motility is graded as per the WHO laboratory manual for the examination and processing of human semen, 6th edition, 2021.[23]

Assessment of sperm morphology

The sperm morphology was evaluated according to strict criteria, using pre-stained slides for sperm morphology assessment of the QwikCheck™ method. After thoroughly mixing the liquefied semen samples, a small drop (3-4 µl) was put on the pre-stained slide and allowed to slide for 15-20 minutes. After that, evaluate the number of normal and abnormal spermatozoa under the microscope with the aid of a laboratory counter as per the guidance of the WHO laboratory manual for the examination and processing of human semen, 6th edition (WHO 2021).[23]

Calculation

$$Percentage\ of\ normal\ sperm = \frac{(Number\ of\ normal\ forms) \times 100}{Total\ number\ of\ cells\ counted}$$

Assessment of sperm vitality

Vitality was done by the Eosin Nigrosine Test QwikCheck™ 1-Step Vitality Stain MES India solution. 10µl of ejaculate was mixed with 10µl of 0.5% aqueous yellowish Eosin Y solution. The mixture was covered with a cover slide, then evaluated after 3–5 min by distinguishing between the dead spermatozoa (red stained) and live spermatozoa (not stained). A total of 200 spermatozoa from each slide were evaluated per slide under a phase-contrast microscope.

DNA fragmentation index (DFI)

DFI was done by the sperm 360 sperm processor, laboratory chemicals and procedure. After staining the slide, count the fragmented DNA and non-fragmented DNA in the sperm. A total of 200 sperm were evaluated in the slide, and sperm with a large halo and medium halo were considered as non-fragmented DNA sperm, sperm with a small halo were considered as fragmented DNA, and sperm with no halo were considered as degraded sperm. DFI was calculated as per the given formula:

$$DFI = (Number\ of\ sperm\ with\ fragmented\ DNA + number\ of\ sperm\ with\ degraded\ sperm \times 100) / Total\ number\ of\ sperm\ evaluated$$

RESULTS

In the present study, 200 male participants were involved; however, 195 participants fulfilled the criteria. Out of which 87 were smokers (daily smokers and occasional/moderate smokers) and 108 participants were non-smokers (Table 2). Baseline characteristics of the smokers and non-smokers in mean±SD of age, BMI, and duration of infertility are shown in Table 1. The number and percentage of primary infertility, secondary infertility, male factor, female factor, both factors, and unexplained factor were also shown in Table 1.

Table 1: Smoker and non-smoker characteristics.

Characteristics	Smoker (87)	Non-smoker (108)
Age (in years)	34.66±5.15	35.50±10.85
Weight	65.74±6.98	68.52±9.33
BMI	23.46±2.530	23.76±2.79
Duration of infertility (years)	4.64±3.388	3.91±2.72
Infertility factor primary infertility (%)	72 (83)	89 (82)
Secondary infertility (%)	15 (17)	19 (18)
Male infertility factor (%)	36 (41)	36 (33)
Female infertility factor (%)	5 (6)	22 (20)
Both factor (%)	36 (41)	26 (24)
Unexplained (%)	10 (11)	24 (22)

Values are in mean±SD

Comparison between smokers and non-smokers

The sperm parameters were compared between smokers and non-smokers, as shown in Table 2. The result revealed that significantly lower semen volume (2.08±0.78 versus 2.45±0.79), reduced pH (7.30±0.26 versus 7.52±0.90), decreased total sperm count (59.22±60.39 versus 94.12±91.12), total motility (44.82±24.66 versus 57.03±20.33), progressive motility (24.67±24.19 versus 33.07±24.14), vitality (46.46±20.18 versus 56.81±22.15), morphology (3.25±1.27 versus 3.84±1.42), and higher DFI (21.33±10.52 versus 15.05±6.63) were found in smokers as compared to non-smokers.

Comparison of daily smokers, occasional/moderate smokers, and non-smokers

The baseline characteristics of the smokers, occasional smokers, and non-smokers in mean±SD of age, BMI, and Duration of infertility are shown in Table 3. The number and percentage of primary infertility, secondary infertility, male factor, female factor, both factors, and unexplained factor were also shown in Table 3.

The comparison of daily smokers, occasional smokers, and non-smokers (control) is shown in Table 4. The results revealed that there was no significant difference between daily smokers and moderate/occasional smokers in semen volume and pH; however, significant differences were observed with non-smokers. The sperm concentration did not significantly differ between daily smokers and moderate/occasional smokers; however, higher sperm counts were observed in non-smokers.

The total sperm count per ejaculate was found to be higher in non-smokers and significantly different from daily smokers, but not from occasional smokers. Total sperm motility was found to be significantly higher in non-smokers as compared to daily smokers, but not significantly higher with occasional smokers. Similarly, sperm vitality and morphology were found to be significantly higher in the non-smokers group compared to daily smokers, but not significantly higher with occasional smokers. The DFI was found to be significantly lower in non-smokers as compared to daily smokers, but not significantly higher with occasional smokers.

Distribution of semen abnormalities

The distribution of semen abnormalities differed according to smoking status. Normozoospermia was most frequent among non-smokers (46%) compared with daily smokers (24%) and moderate/occasional smokers (25%). Daily smokers exhibited a higher prevalence of asthenozoospermia (25%), teratozoospermia (10%), and mixed abnormalities, including oligoasthenozoospermia (24%), asthenoteratozoospermia (8%), and oligoasthenoteratozoospermia (6%), than non-smokers. Moderate or occasional smokers showed intermediate frequencies of abnormal semen parameters. These findings indicate that smoking, particularly daily use, is associated with an increased risk of single and combined semen abnormalities, suggesting a dose-dependent negative effect of tobacco on sperm quality (Table 5).

Table 2: Semen parameters in smokers and non-smokers.

Parameters	Smoker	Non-smoker	Significance (P value)
Semen volume in ml	2.08±0.78	2.45±0.79	0.0010
Semen pH	7.30±0.26	7.52±0.90	0.0001
Sperm concentration (million/ml)	27.74±26.18	36.10±29.19	0.0389
Total sperm count (per ejaculate)	59.22±60.39	94.12±91.12	0.0024
Total motility %	44.82±24.66	57.03±20.33	0.0002

Continued.

Parameters	Smoker	Non-smoker	Significance (P value)
Progressive motility %	24.67±24.19	33.07 ±24.14	0.0167
Vitality %	46.46±20.18	56.81±22.15	0.0009
Morphology (normal forms) %	3.25±1.27	3.84±1.42	0.0029
DFI	21.33±10.52	15.05±6.63	0.0001

Values are in mean±SD; Level of significance = p≤0.05.

Table 3: Characteristics of smokers, occasional/moderate smokers, and non-smokers.

Characteristics	Daily smoking (≥5 to 15)	Moderate smoker (1-4 in a day, or occasionally smoker)	Non-smoker (control)
Age (in years)	35.11±5.11	33.50±5.19	35.50±10.85
Weight	66.04±7.47	64.96±5.56	68.52±9.33
BMI	23.72±2.69	22.79±1.91	23.76±2.79
Duration of infertility (years)	4.96±3.71	3.81±2.17	3.91±2.72
Primary infertility (%)	51 (81)	21 (33)	89 (82)
Secondary infertility (%)	12 (19)	3(5)	19 (18)
Male factor (%)	29 (46)	7 (11)	36 (33)
Female factor (%)	2 (3)	3 (5)	22 (20)
Both factor (%)	26 (41)	10 (16)	26 (24)
Unexplained (%)	6 (10)	4 (6)	24 (22)

Values are in mean±SD

Table 4: Semen parameters in smokers, occasional smokers, and non-smokers.

Parameters	Daily smoker (≥5-15)	Moderate/occasional smoker (1-4)	Non-smoker	Signifi-cance
Semen volume (ml)	[2.10±0.792]^{a}	[2.01±0.76]^{b}	[2.45± 0.79]^{c}	p<0.05
Semen pH	[7.29±0.267]^{a}	[7.31±0.25]^{a}	[7.45 ± 0.28]^{b}	p<0.05
Sperm concentration (million/ml)	[27.99±27.074]^{a}	[27.08±24.22]^{a}	[36.10±29.19]^{a}	NS
Total sperm count (per ejaculate)	[58.13±56.24]^{a}	[62.05±71.39]^{a}	[94.12±91.12]^{b}	0.0126
Total motility %	[43.31±23.76]^{a}	[48.75±27.03]^{a}	[57.03±20.33]^{b}	0.0010
Vitality %	[46.23 ± 19.41]^{a}	[47.04±2.50]^{a}	[55.17±23.40]^{b}	0.0058
Morphology (normal forms) %	[3.22±1.12]^{a}	[3.33±1.61]^{a}	[3.84±1.42]^{b}	0.012
DFI %	[22.40±10.2]^{a}	[18.52±10.97]^{a}	[15.05±6.63]^{b}	0.0010

*Values within square parentheses indicate mean±SD in each group. Averages with different superscripts within columns are significantly different at the 5% level of significance (p≤0.05)

Table 5: Distribution of semen abnormalities in daily smokers, occasional smokers, and non-smokers.

Semen abnormalities	Daily smoker (≥5-15 per day) (63) (%)	Moderate/ occasional smoker (1-4) (24) (%)	Non-smoker (108)
Normozoospermia	15 (24)	6 (25)	50 (46)
Oligozoospermia	1 (2)	2 (8)	1 (1)
Asthenozoospermia	16 (25)	7 (29)	19 (18)
Teratozoospermia	6 (10)	0 (0)	3 (3)
Oligoasthenozoospermia	15 (24)	5 (21)	15 (14)
Oligoteratozoospermia	1 (2)	-	-
Asthenoteratozoospermia	5 (8)	1 (4)	8 (7)
Oligoasthenoteratozoospermia	4 (6)	3 (13)	12 (11)

*Values within square parentheses indicate mean ±SD in each group. Values are presented as number and percentage (%). Daily smokers showed a higher prevalence of mixed semen abnormalities compared to non-smokers, indicating a negative impact of smoking intensity on semen quality.

DISCUSSION

Our study shows that cigarette smoking—particularly daily smoking—has a clear negative effect on male reproductive health. Smokers had significantly lower semen volume, total sperm count, motility, and vitality, as well as a higher DFI compared with non-smokers. These findings are consistent with earlier reports showing reduced semen volume, sperm concentration, and motility among smokers, with stronger effects in men smoking

more than 20 cigarettes per day.[24-26] Kunzle et al found a 17.5% decline in total sperm count and a 16.6% decline in total motile sperm in smokers compared with non-smokers.[25] Merino et al reported similar adverse changes even in men smoking fewer than 10 cigarettes daily, suggesting that light smoking also poses a risk.[27]

The mechanisms are thought to involve oxidative stress and accumulation of toxicants. Cigarette smoke contains reactive oxygen species (ROS) and heavy metals, such as cadmium, lead, and arsenic, that concentrate in seminal plasma, leading to oxidative stress and sperm DNA damage.[28-33] Our finding of higher DFI among smokers supports this explanation. Elevated ROS can disrupt membrane integrity, impair mitochondrial function, and trigger apoptosis in germ cells, all of which reduce sperm quality and fertilization potential.[28-31]

Trace elements also play a role. Smokers frequently have lower seminal zinc concentrations; a factor associated with diminished sperm motility and morphology.[34] Zinc acts as an antioxidant and stabilises sperm membranes; deficiency increases susceptibility to ROS. In addition, reduced expression of checkpoint kinase 1 (Chk1) in smokers may impair DNA repair, leading to higher sperm apoptosis and lower semen quality.[35,36]

Although occasional smokers in our cohort showed only a non-significant increase in DFI, their semen parameters trended lower than those of non-smokers. This suggests that even moderate exposure can be harmful, echoing Merino's observation of altered semen quality in men smoking fewer than 10 cigarettes per day.[27] Zhang et al further demonstrated that superoxide dismutase activity—an important antioxidant defence declines with smoking frequency and duration, reinforcing a dose-response relationship between smoking and oxidative stress.[28]

Our results contribute to a growing body of evidence that smoking adversely affects multiple aspects of male fertility, including sperm count, motility, morphology, and DNA integrity. Given the high prevalence of tobacco use in India, where an estimated 267 million individuals use some form of tobacco, these findings have important public-health implications. Counselling infertile men to quit smoking should be a key component of fertility management.

Limitations

Limitations of this study include reliance on self-reported smoking habits and the cross-sectional design, which precludes establishing causality. Nevertheless, our findings, combined with consistent data from other populations, strongly support a harmful role of smoking in male infertility. Future prospective studies should explore the reversibility of sperm DNA damage after smoking cessation and evaluate potential protective interventions such as antioxidant therapy.

CONCLUSION

The study concluded that daily smoking has a significant impact on male infertility, as it reduces semen quality in terms of count, motility, volume, and morphology, while also significantly increasing the DFI. Additionally, infrequent smoking negatively impacts male fertility. It is advisable for men seeking to conceive to discontinue smoking to improve their fertility outcomes.

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