

Effect Of Cigarette Smoking On Sperm Morphology Of Infertile Men

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Abstract:Background:Cigarette smoking is a serious health problem of most societies. Smoking has been suggested to contribute to a number of diseases including male infertility. The present study was aimed at studying effect of cigarette smoking on Sperm morphology of infertile men. Material And Methods: A total of one hundred infertile men (50 Non-smokers and 50 Smokers) between the age group 20-45 years were taken into study. The Sperm morphology in the infertile Non-smokers and infertile Smokers group were compared using Z Test. Infertile Smokers which were divided into Group A (≥ 1 and ≤ 10 cigarettes/ day), Group B (> 10 and < 20 cigarettes/ day) and Group C (≥ 20 cigarettes/ day) were analyzed for Sperm morphology by ANOVA Test. Result: We observed that Sperm morphology was significantly lower ($p < 0.01$) in infertile Smokers group than infertile Non-smokers group. We also observed that Sperm morphology was significantly decreased ($p < 0.01$) in accordance with the severity of smoking. Conclusion: Cigarette smoking adversely affect sperm morphology. [Gaisamudre K Natl J Integr Res Med, 2021; 12(3):49-53]

Key Words:Smokers, Non-smokers, Infertile, Sperm morphology.

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Introduction: Despite worldwide antismoking campaigns, cigarette smoking is very common. A large number of men smoke worldwide¹. The highest prevalence of smoking is observed in young adult males during their reproductive period. Smoking has been suggested to contribute to a number of diseases including male infertility. It has been reported that tobacco smoke contains some of the most deadly toxic chemicals. Smokers inhale directly and absorb the following substances: nicotine, carbon monoxide, nitrogen oxide, mutagenic pyrolysis-derived compounds and cadmium. Most of them are known to be mutagens and carcinogens, directly affecting male and female gametes and embryos².

Infertility is defined as the inability to achieve pregnancy after one year of unprotected intercourse³.

Male infertility plays a key role in conception difficulties of up to 40% infertile couples⁴. The term 'male infertility' does not constitute a defined clinical syndrome but rather a collection of different conditions exhibiting a variety of etiologies and varying prognosis⁵. Although in some men a specific disorder may be present, in majority no apparent reason for infertility could be found.

This has drawn attention to the impact of lifestyle and environmental factors especially diet, obesity, smoking, alcohol intake, recreational drug use and exposure to environmental toxins on reproductive health of such men⁴.

Semen analysis is a keystone in the clinical workup of the infertile male patient. Over the years undue importance has been given to sperm count, though it is meaningless without the required motility or normal sperm morphology.

In fact, other parameters like seminal fluid volume, sperm motility, sperm viability can be of help in assessing overall sperm quality and its fertility potential^{6,7,8}.

The aim of our study was to compare Sperm morphology of infertile men who were cigarette Smokers with Non-smokers, in order to ascertain the effect of cigarette smoking on the Sperm morphology.

Material & Methods: The present study was carried out in the Department of Physiology in collaboration with Department of Biochemistry. The study protocol was approved by the Institutional Ethical Committee. Before enrolment in the study, informed written consent was obtained from each subject. A total of one hundred infertile men (fifty Non smokers and

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fifty Smokers) between the age group 20-45 years were taken into study. The study was undertaken for duration of 12 months. Non-smokers were the men who had never smoked.

Smokers were the men who smoke cigarettes since 5 years or more and smoking till date. The Smokers were in turn divided into following groups:

- Group A (n = 28) - (≥ 1 and ≤ 10 cigarettes/ day)
- Group B (n = 17) - (> 10 and < 20 cigarettes/ day)
- Group C (n = 5) - (≥ 20 cigarettes/ day)
- n = Number of subjects

Exclusion Criteria: History of tobacco chewing and alcohol intake. History of injury to testes, varicocele, hydrocele or undescended testes. History of any chronic illness like Tuberculosis, diabetes, hypertension, thyroid disease. History of UTI, occupational exposure to chemicals or excess heat. Azoospermic Subjects. History of taking drugs like Vitamin E, Vitamin C or glutathione supplementation.

Sample Collection And Semen Analysis: Semen samples were collected by masturbation into a sterile, wide mouthed container, after at least 72 hours (3 days) of sexual abstinence. Samples were allowed to liquefy at room temperature (25°C) for at least 45 minutes. After liquefaction, samples were analyzed for Sperm morphology according to World Health Organization (WHO) guidelines⁹.

Sperm Morphology:

Diff-Quik Method: Requirements- Liquefied semen, normal saline, fixative reagent (Triarylmethane dye dissolved in Methanol), staining solution 1 (Eosinophilic Xanthene) and staining solution 2 (Basophilic Thiazine).
Procedure-

Preparation Of Smear: The semen sample was mixed well. 0.5ml of liquefied semen in the test tube was taken. 1ml of normal saline was added to it and mixed well. Test tube was kept in the centrifuge at 2000 rpm for 2 to 3 min. The supernatant was discarded with the help of pipette. A drop from the pellet was taken on the Nonsmokers group and infertile Smokers group. Sperm morphology in all three groups of infertile Smokers were compared using one way ANOVA

glass slide. The semen drop spreads along the back edge of the angled slide and was pulled forwards over the slide to form the smear. The slide was air dried.

Fixing The Air-Dried Semen Smear: Slides were immersed in Triarylmethane fixative for 15 seconds. The excess solution was drained by placing slides vertically on absorbent paper.

Staining The Fixed Semen Smear: The slides were sequentially immersed in:

- a) Rapid stain solution 1 for 10 seconds
- b) Rapid stain solution 2 for 5 seconds
- c) Running tap water for 10 to 15 times to remove excess stain

The excess solution at each step was drained by placing slides vertically on absorbent paper. The slides were observed under oil-immersion microscope and the results were noted as:

- Number of sperms with normal morphology - %
- Number of sperms with abnormal morphology- %

The normal morphology includes sperm with a head occupied by 40-65% area by acrosome, neck and a tail.

Abnormal forms includes abnormalities in the head, acrosome, neck (size and insertion) and tail deformities.

The lower reference limit for normal forms is 4%. In this study sperm morphology is % of sperms with normal morphology.

Results: In the present study, all the calculations and statistics were done using Microsoft Excel 2007 and "graph pad prism 5 software" version 5.01 was used. A 'p' value of less than 0.05 ($p < 0.05$) was considered to be statistically significant. A 'p' value of less than 0.01 ($p < 0.01$) was considered to be statistically highly significant.

For Sperm morphology, the mean value and standard deviation were calculated. Z test was applied to study the difference between infertile (analysis of variance) test. The observations and results of the present study were tabulated as below:

Table 1: Comparison of Sperm morphology in infertile Nonsmokers and infertile Smokers group

Parameter	Nonsmokers (Mean ± Sd) N=50	Smokers (Mean± Sd) N=50	P Value	Significance (S/Ns)
Sperm Morphology (%)	32.68 ± 7.47	27.88 ± 9.63	<0.01	S

S = Significant, NS = Non significant, SD = Standard Deviation, n = Sample Size

Graph 1: Comparison Of Sperm Morphology In Infertile Non-smokers And Infertile Smokers Group

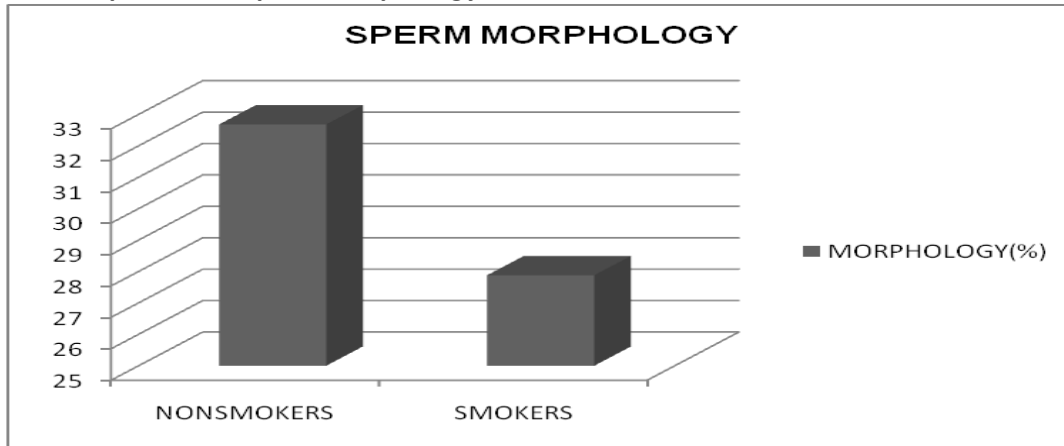


Table 2: Comparison Of Sperm Morphology In Three Groups Of Infertile Smokers

Parameter	Group	N	Mean ±Sd	P Value	Significance (S/Ns)
Morphology (%)	A	28	31.32±8.77	<0.01	S
	B	17	26.05±8.62		
	C	5	14.8±2.77		

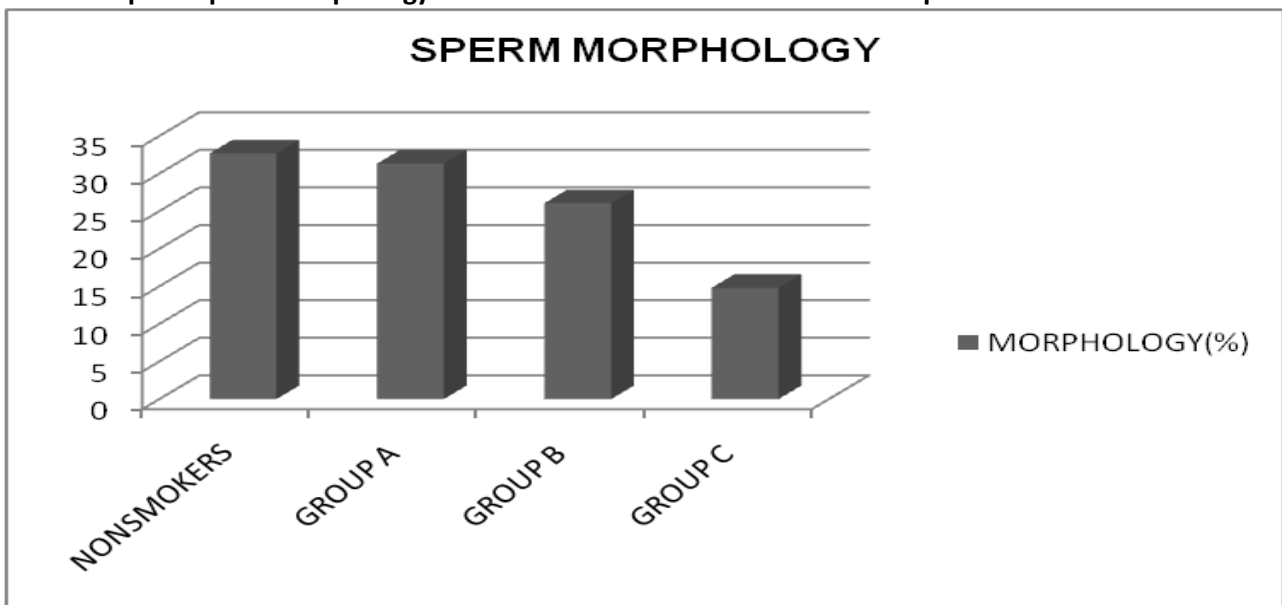
S=Significant, NS=Non significant, SD=Standard Deviation, N = sample size

Group A: Mild smokers (≥1 and ≤10 cigarettes/ day)

Group B: Moderate smokers (>10 and <20 cigarettes/ day)

Group C: Heavy smokers (≥20 cigarettes/ day)

Graph 2: Sperm Morphology In Infertile Nonsmokers And Three Groups Of Infertile Smokers



Discussion: The results obtained in the present study showed that mean ± SD of the Sperm morphology in infertile Smokers group was 27.88 ± 9.63 and in infertile Nonsmokers group the

value was 32.68 ± 7.47. The Sperm morphology was decreased in infertile Smokers group. The difference of the Sperm morphology in both the groups was statistically significant (p<0.01).

The mean values of Sperm morphology in different groups of infertile Smokers were:

- Group A (Mild smokers) - 31.32 ± 8.77
- Group B (Moderate smokers) - 26.05 ± 8.62
- Group C (Heavy smokers) - 14.8 ± 2.77

The number of morphologically normal sperms were decreased in accordance with severity of smoking and the differences between these values were statistically significant ($p < 0.01$). The effects of cigarette smoking on Sperm morphology were also evaluated in several studies.

Kulikauskas et al¹⁰ found that cigarette smoking has been associated with decreased sperm count, alterations in motility and an overall increase in the number of abnormal sperm.

Kunzle et al⁶ showed that the percentage of normal forms of sperms was significantly reduced in smokers.

Mostafa¹¹ reviewed the studies regarding relationship between cigarette smoking and male infertility showed that smoking reduces sperm morphology through increased seminal oxidative stress and DNA damage. Liu et al¹² found significant decrease in sperm morphology among smokers in comparison to nonsmokers.

Tobacco smoke consists of approximately 4,000 compounds such as alkaloids, nitrosamines and inorganic molecules. Many of these substances are reactive oxygen or nitrogen species¹³.

Smoking increases Reactive Oxygen Species (ROS) levels¹⁴. ROS causes oxidative damage to normal sperm DNA, proteins & lipids which may be related to sperm abnormalities¹⁵. Heavy smoking was found to have detrimental effects on tail of the spermatozoon¹⁶. Infertile cigarette smokers have abnormal retention of sperm cytoplasmic droplets¹⁷. Smoking also produces teratozoospermia⁷. The clinical significance of the present finding is to develop effective interventions aimed at helping patients to stop smoking for the benefits of their general health and fertility. Hence we suggest that every smoker should be encouraged to stop smoking especially if pregnancy is planned.

Conclusion: It is concluded that cigarette smoking adversely affects Sperm Morphology. The

number of morphologically normal sperms were decreased in accordance with severity of smoking.

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