

Effect of cigarette smoking on semen quality of infertile men

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ABSTRACT

Introduction: Defective sperm quality is a significant cause of infertility. It is known that cigarette smoking affects semen quality. Our aim was to compare the semen of infertile cigarette smokers with infertile non-smokers to study the effect of smoking on semen quality.

Methods: Semen samples of 100 cigarette smokers and 100 strictly non-smoking primary infertility patients were included in the study, following stringent exclusion criteria. Smokers were categorised as light, moderate and heavy smokers. Semen samples were examined for asthenozoospermia, oligozoospermia and teratozoospermia, according to World Health Organisation guidelines.

Results: 39 percent of non-smokers showed normozoospermia, while only three percent of smokers were normozoospermics. Light smokers predominantly showed asthenozoospermia. Heavy smokers showed asthenozoospermia, teratozoospermia and oligozoospermia. Statistical analysis using Fisher's exact test showed that the incidence of both isolated asthenozoospermia (p-value is 0.0015) and asthenozoospermia with teratozoospermia (p-value is 0.0106) among smokers was significant, in comparison to non-smokers. Overall impact of asthenozoospermia (p-value is less than 0.0001) and teratozoospermia (p-value is 0.0328) but not of oligozoospermia was observed on the semen quality in smokers, compared with non-smokers.

Conclusion: Asthenozoospermia, the most common semen variable in our study, can be an early indicator of reduction in quality of semen, as seen in light smokers. In addition,

heavy smoking produces teratozoospermia, which further reduces semen quality. Oligozoospermia may be due to factors other than smoking.

Keywords: asthenozoospermia, cigarette smokers, male infertility, semen quality, teratozoospermia

Singapore Med J 2007; 48(2):119-123

INTRODUCTION

Infertility is a common problem affecting one in six couples. It can be defined as the incapacity to fulfil pregnancy after a reasonable time of sexual intercourse with no contraceptive measures taken. In 30% of infertile couples, the male factor, in the form of defective sperm quality, is a major cause.⁽¹⁾ As a large number of men smoke worldwide, and the fact that cigarette smoke contains known mutagens and carcinogens, there has been much concern that smoking may have unfavourable effects on male reproduction.⁽²⁾ Several studies from different parts of the world have observed that cigarette smoking has an effect on the semen quality, especially in those who are heavy smokers or who have been smoking for many years.⁽²⁻⁷⁾

Measures of semen quality are used as surrogate measures of male fertility in clinical andrology.⁽⁸⁾ 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, liquefaction time, sperm motility and viability can be of help in assessing the overall sperm quality and its fertility potential.^(5,7) The aim of our study was to compare the various semen parameters of infertile men who were cigarette smokers with non-smoking infertile men, in order to ascertain the effect of cigarette smoking on the quality of seminal fluid.

METHODS

The present study was conducted at the Infertility Laboratory of the Post Graduate Department of Pathology, Himalayan Institute of Medical Sciences, Dehradun, Uttarakhand, India. Since our aim was to study the effect

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of cigarette smoke on semen quality, it was a primary requirement to lay down stringent patient-selection criteria in order to exclude as many co-existing factors as possible, as they may otherwise influence or modify the effect of cigarette smoke on semen parameters. For the same reasons, we did not compare smokers with healthy fertile controls, because we wanted to exclude any undiscovered factors present in infertile men, which might not be present in healthy fertile controls. By comparing infertile smokers with infertile non-smokers, such undiscovered factors could be nullified in both groups.

Only patients with primary infertility, who were either smokers or strict non-smokers, were selected. Patients labelled as having primary infertility were married at least for the past one year and none of them were using any contraceptive measures for the past one year or longer. Strict non-smokers were those men who had never smoked before. Informed consent was taken, as a routine procedure, from all the cases. Only one sample per patient was included in this study.

The following were excluded from the study group:

1. Patients suffering from secondary infertility, as presence of other co-factors may have interfered with our observations.
2. Ex-smokers, to avoid any persistence of effects of smoking.
3. Patients with history of tobacco/betel nut chewing, "bidi" smoking and alcohol intake.
4. Patients with occupational exposure to chemicals or excessive heat, e.g. cases working at petrol pumps, chemical factories, and bakeries.
5. Patients with history of injury to the testes, varicocele, hydrocoele, undescended testis or its corrective surgery and vasectomy-reversal surgery.
6. Patients with history of any chronic illness, such as tuberculosis, diabetes mellitus, hypertension, thyroid diseases, mumps or any ailment for which long-term medication was being given.
7. Patients with leucocytospermia, frank pyospermia, haemospermia or chronic urinary tract infection.
8. Patients with history of intake of non-proprietary medications or tonics.
9. Patients in whom semen fructose test was negative.
10. Azoospermics.
11. Patients above 45 years of age, to avoid effects of ageing on sperm variables.

Thus, the selected study group of 100 smokers and 100 strict non-smokers had only one known factor which differentiated them, i.e. cigarette smoking. The smokers were categorised further, based on the number of cigarettes smoked per day, into: light, moderate and

Table I. Smoking status of cases included in the study.

Smoking status	Number of cigarettes/day	Number of cases (n)
Non-smokers		100
Smokers		100
Light	1–20	49
Moderate	21–40	28
Heavy	≥ 41	23

heavy smokers (Table I).⁽⁹⁾ All patients had abstinence of 4–6 days.⁽¹⁰⁾ Samples were collected in wide-mouthed sterile container by masturbation. Samples with partial spillage were rejected. Such patients were asked to come again after 4–6 days' abstinence. Only one sample per patient was included in the study. All samples were kept at 37 ± 2 °C and processed immediately after complete liquefaction.

All semen samples were analysed for ten primary semen parameters: liquefaction time, volume, viscosity, amorphous particulate matter, agglutination, motility, viability, sperm density, morphology (normal forms), and headless spermatozoa, as per the recommended guidelines according to the World Health Organisation manual.⁽¹¹⁾ These parameters, when taken together, indicated the presence or absence of the three main semen variables: asthenozoospermia (A), teratozoospermia (T) and oligozoospermia (O), which acted as pointers to specific need for further specific evaluation.

Samples showing A were those which had less than 25% spermatozoa showing linear forward progression; samples of O had less than 20 million spermatozoa/ml of ejaculate; and samples of T showed more than 70% of morphologically abnormal spermatozoa.⁽¹¹⁾ These three variables were present either individually or in various combinations such as A+T, A+O, O+T, and A+O+T. Samples with normozoospermia (N) were those which had all the parameters within the recommended ranges and were thus categorised separately.⁽¹⁰⁻¹²⁾

Semen samples were microscopically examined for sperm density, sperm motility, sperm vitality, sperm morphology, presence of agglutination and particulate matter. Sperm morphology was studied on Papanicolaou-stained smears, counting a minimum of 200 spermatozoa using 100× magnification oil-immersion lens. Sperm vitality was assessed in wet mount smears after supravital staining with Eosin.⁽¹²⁾ The data was analysed by Fisher's exact test and chi-square test to find out the p-values. A p-value of < 0.05 was taken as being statistically significant.

Table II. Semen variables among different groups of smokers in comparison to non-smokers.

Diagnosis	Smokers				Non-smokers	
	Light n (%)	Moderate n (%)	Heavy n (%)	Total		p-value*
N	2 (4)	1 (3.5)	0	3	39	1.009×10 ⁻¹⁰
A	20 (41)	7 (25)	0	27	9	0.0015
A + O	4 (8)	3 (11)	4 (17)	11	7	0.2297
A + T	11 (22)	6 (21.5)	6 (26)	23	10	0.0106
A + O + T	5 (11)	7 (25)	9 (39)	21	12	0.1266
O	3 (6)	2 (07)	2 (9)	7	8	1.0000
O + T	2 (4)	1 (3.5)	0	3	8	0.2134
T	2 (4)	1 (3.5)	2 (9)	5	7	0.7673
Total	49 (100)	28 (100)	23 (100)	100	100	

N: Normozoospermia; A: Asthenozoospermia; O: Oligozoospermia; T: Teratozoospermia; *p-value by Fisher's exact tests

Table III. Semen variables in smokers and non-smokers.

Semen variable	Smokers				Non-smokers	
	Light (n=49) n (%)	Moderate (n=28) n (%)	Heavy (n=23) n (%)	Total (n=100)	(n=100)	p-value*
Asthenozoospermia (A)+(A+T)+(A+O) + (A+O+T)	40 (81.6)	23 (82.1)	19 (82.6)	82	38	<0.0001
Teratozoospermia (T)+(A+T)+(O+T) + (A+O+T)	20 (40.8)	15 (53.6)	17 (73.9)	52	37	0.0328
Oligozoospermia (O)+(A+O)+(O+T) + (A+O+T)	14 (28.6)	13 (46.4)	15 (65.2)	42	35	0.3090

A: Asthenozoospermia; O: Oligozoospermia; T: Teratozoospermia; *p-value by chi-square test

RESULTS

Of the semen samples analysed in our infertility laboratory from January 1, 2001 to June 30, 2004, a total of 100 smokers and 100 strict non-smokers who qualified under the stringent selection criteria, as ascertained by direct interviews, were included in the study. Based on their detailed smoking history, the smokers (n = 100) were divided into categories according to the number of cigarettes smoked daily. Those who smoked 20 or less cigarettes per day were designated light smokers (n = 49); those who smoked 21–40 cigarettes per day were moderate smokers (n = 28); and those who smoked 41 or more cigarettes per day were heavy smokers (n = 23) (Table I).

In order to determine the contribution of each of the three main semen variables, viz. A, O and T, light, moderate and heavy smokers as well as non-smokers were distributed according to the presence of individual semen variables or their various combination observed during semen analysis (Table II). Only three samples from smokers had semen parameters consistent with N, while 39 samples from non-smokers showed N (Table II).

Among smokers, the most dominant semen variable

was A, individually (n = 27) or in combination with other variables. Among non-smokers, isolated A was seen only nine times (Table II). 41% of samples from light smokers showed isolated A, compared with 25% from moderate smokers, and none from heavy smokers. In contrast, heavy smokers (39%) and moderate smokers (25%) had more samples with A+O+T than light smokers (Table II). Statistical analysis showed that the incidence of both isolated A and A+T among smokers was statistically significant, in comparison to non-smokers. The p-values for other sub-groups were not statistically significant, mainly due to their small sample size (Table II).

Among smokers, A was observed in 82 samples, T in 52 samples and O in 42 samples (Tables II & III). Corresponding totals for non-smokers were 38, 37 and 35 samples, respectively. On analysis using the chi-square test, the impact of A and T but not of O were significant in smokers, compared with non-smokers (Table III).

DISCUSSION

Smoking is a lifestyle hazard for both active and passive smokers. Although much is known now about the

carcinogens in tobacco cigarette smoke and their resultant effects on organs like lungs and urinary bladder, their effects on fertility status have been less documented. In our study, 39 non-smokers had N with their semen parameters falling within the normal ranges. In contrast, samples from only three smokers qualified as N (Table II). This finding underscores the fact that smoking certainly has an adverse influence on the semen quality, as concluded in several other studies.^(2,3,6,7) A was the most dominant semen variable contributing to the semen quality of smokers (n = 82) as well as non-smokers (n = 38), individually as well as in combination with other variables like teratozoospermia (A+T), oligozoospermia, (A+O) and (A+O+T) (Table III). A appears to be a premier factor contributing to the infertile status of a male.⁽¹³⁾ Viable and morphologically normal spermatozoa, if they are not actively motile, showing linear forward motion in the seminal fluid, they will fail to fulfil their prime function of traversing the complex route through the female genital tract to seek and fertilise an ovum. In assessing the semen quality of an individual, emphasis has always been on the sperm count and sperm morphology.⁽⁵⁻⁷⁾

In comparison, less number of cases showed contribution of T and O among smokers but their numbers were still higher than in non-smokers (Table III). This again shows that smoking contributes to the deterioration of the semen quality of smokers when compared with non-smokers. Isolated A was seen in 41% of light smokers and 25% of moderate smokers, while no such case was detected among heavy smokers (Table II). Since among light smokers, even this “mild smoking could produce a reduction in the sperm motility in 41% of cases, it appears that there is no “safe” quantity of cigarette smoking that may not affect the semen quality. In 22% of light smokers, T was also present in addition to A. Thus, T appeared to be the next anomaly to develop in the spermatozoa of light smokers, after A. Overall, 27 cases showed isolated A, followed by 23 cases showing both A+T, among smokers (Table II).

In contrast, in heavy smokers, presence of all the three variables, A+O+T, were found in 39% of cases in comparison to light smokers (11%), indicating that heavy smoking appears to have a significant contribution in the development of T and O in addition to A.⁽²⁻⁴⁾ Observations similar to heavy smokers were made among moderate smokers (Table II). A study conducted on voluntary men of reproductive age showed that after ejaculation, sperm motility deteriorated much more rapidly in heavy smokers, in comparison to non-smokers.⁽¹⁴⁾ Researchers have variously concluded that toxins in cigarette smoke reach the male reproductive system, and their effects, though still under research, are mainly due to their direct interaction with seminal fluid components and the accessory glands, which contribute their secretions

to the seminal fluid, leading to its increased viscosity, reduced seminal volume and delayed liquefaction time, thus reducing forward linear progression of spermatozoa, manifesting as A.⁽²⁻⁷⁾ In studies conducted on fertile men, it was observed that those who were smokers showed a reduction in semen volume in comparison to non-smokers; and this reduction in semen volume was in proportion to the number of cigarettes smoked per day.^(14,15)

Direct exposure of spermatozoa to the toxins in cigarettes smoke probably tilts the delicate balance of reactive oxygen species (ROS) that are produced by spermatozoa for their special functions like decapitation. Increased quantities of ROS have been shown to be detrimental to the DNA of spermatozoa, thus producing a negative effect on the viability and morphology of spermatozoa.⁽¹⁶⁾ Thus, smoking plays a role in producing A in otherwise normal and viable spermatozoa, and can be a very subtle “early indicator” of deterioration in semen quality. Since 38% of non-smokers too showed A (Table III), many of these non-smokers may be innocent “passive smokers” or may be affected by environmental pollutants, chemicals and other unknown factors awaiting discovery.^(2,17) A higher level of research in non-smokers cases may unmask the influence of these additional factors.

In our study, among light smokers, cases with A+T were much more than cases with A+O (Tables II & III). Thus, after A, a defect in sperm morphology, i.e. T, is the second factor that appears in semen, further reducing its quality. In contrast, O may also result from other aetiological factors like chronic inflammatory or infective processes which need further exploration. In our study, more cases with pure T, pure O and their combination (O+T), were recorded among non-smokers than in smokers (Table II). Statistical analysis of the results also underscored our observations that the impact of A and T in the semen samples of smokers was significant, in comparison to non-smokers.

In conclusion, asthenozoospermia is the most common anomaly of semen, whether present individually or in combination with teratozoospermia and/or oligozoospermia. The presence of asthenozoospermia can be a very subtle “early indicator” of reduction in the semen quality of an individual, which frequently gets ignored, if the semen sample shows adequate sperm count and normal morphology. Smoking does affect semen quality. Deterioration in semen quality appears in direct proportion to the number of cigarettes smoked. There is no “safe” quantity of cigarette smoking as reflected by predominance of asthenozoospermia in light smokers. Heavy and moderate smoking reduce semen quality further by also producing teratozoospermia. Oligozoospermia may be a result of other aetiological factors besides smoking, and this needs further exploration.

ACKNOWLEDGEMENT

We are thankful to Dr Subodhsingh Chauhan, Assistant Professor in Obstetrics and Gynaecology, West Virginia University, Charleston, West Virginia, USA, for his valuable suggestions in this study.

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