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A study of quantitative and qualitative analysis of semen in smokers and non-smokers

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Abstract
Numerous health effects of smoking are well-known; associations with semen quality are uncertain. Cigarette smoking may be associated with sub-fertility in males and may result in decreased sperm concentration, lower sperm motility, and a reduced percentage of morphologically normal sperm respectively Semen analysis is a simple, cost effective screening test for evaluation of male in laboratory & clinics, which provides information on quantitative and qualitative aspects of testicular function. Except in cases of azoospermia, semen analysis does not separate patients in to sterile and fertile group.

Objective: The present study was undertaken to perform the quantitative and qualitative Analysis of Semen and to compare the sperm parameters in smokers and non-smokers.

Materials and methods: The following males were excluded from the study groups:

1. Those suffering from azoospermia or secondary infertility;
2. Ex-smokers and ex-alcoholics, or those with history of tobacco/betel nut chewing or substance abuse;
3. Those with history of prolonged medication, intake of indigenous medications/herbal preparations/tonics, occupational exposure to chemicals; or excessive heat;
4. History of injury to testes, varicocele, hydrocele, undescended testis.

A total of 200 cases, retrospective and prospective from September 2018 to January 2021 were included in the study.

Results: Compared with non-smokers, smokers had a significant decrease in semen volumes, rapid progressive motility and sperm viability moreover, smokers had a significant increase in the levels of immotile sperms and semen leukocytes, pH and sperm concentration were not significant. Sperm motion parameters were all lower in the smokers. Normal morphology sperm was decreased significantly in smokers. The sperm morphology was worse with increasing degree of smoking. Compared, the overall quality of sperm was significantly decreased in smokers when compared with non-smokers.

Conclusion: Semen parameters like, volume, motility, count, liquifraction time, viscocity morphologically normal spermatozoa were reduced in cigarette smokers when compared to Non-smokers, but sperm count was found to have a decrease in Smokers, when compared to Non-smokers. Liquifraction time and viscocity of semen was variable .In concordance with other researchers, results of present study support that cigarette smoking have a relatively significant effect on semen.

Keywords: Semen pus cells, sperm agglutination, oligospermia

Introduction

Cigarette smoking, one of the main causes of preventable morbidity and mortality, has a multitude of well-known side effects. The relationship between cigarette smoking and infertility has been studied for decades. The current literature is in the form of retrospective and prospective studies focused on the effects of smoking on semen analyses. This article discusses the results of these studies and reviews the postulated mechanisms. The current evidence suggests that men should be advised to abstain from smoking in order to improve reproductive outcomes.

Semen analysis still remains as a simple, cost effective screening test for evaluation of male in fertility clinic, which provides information on quantitative and qualitative aspects of testicular function. Except in cases of azoospermia, semen analysis does not separate patients in to sterile and fertile group [2]. In recent years, infertility and sub fertility in men has increased which may be associated with their advancing age, habits like tobacco use,

alcoholism, working environment, varicocele and other factors.

Semen parameters might be sensitive markers for these influencing factors [3, 4], hence our study focuses on effects of factors like cigarette smoking on semen parameters. The present study was undertaken to perform the quantitative and qualitative Analysis of Semen and to compare the sperm parameters in smokers and non-smokers.

Materials and Methods

1. The following males were excluded from the study groups:
2. Those suffering from azoospermia or secondary infertility; 2Ex-smokers and ex-alcoholics, or those with history of tobacco/betel nut chewing or substance abuse;
3. Those with history of prolonged medication, intake of indigenous medications/herbal preparations/tonics, occupational exposure to chemicals; or excessive heat;
4. History of injury to testes, varicocele, hydrocele, undescended testis.

A total of 200 cases, retrospective and prospective from September 2018 to January 2021 were included in the study.

Collection of semen samples: Semen samples were collected from all patients by masturbation after 2-5 days of sexual abstinence in wide mouthed polypropylene bottle. And these semen samples were processed and analyzed by single qualified person.

Macroscopic Examination

Liquefaction: Normal semen sample liquefies within 60 minutes at room temperature. Occasionally, samples may not liquefy, in which case additional treatment like, mechanical mixing or enzyme digestion (e.g.bromolain) may be necessary.

Color: Semen sample is examined immediately after liquefaction or within one hour of ejaculation. Normally, semen is homogenous grey opalescent and may appear less opaque if sperm concentration is low. Red brown when mixed with blood or yellow in patients with jaundice or taking vitamins

Volume: of ejaculate is measured using graduated cylinder.

Viscosity: It is measured by gentle aspiration into wide bore 5 ml pipette and then, allowing the semen to drop by gravity and observing the length of the thread. A normal sample leaves the pipette as small discrete drops. In cases of abnormal viscosity, the drop will form thread more than 2 cm long. Alternatively, the viscosity may be evaluated by introducing a glass rod into sample and observing the length of the thread that forms on withdrawal of the rod, which should not exceed 2 cm.

P^H: P^H 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

Microscopic examination

Fixed volume 10µl semen is taken on to a clean glass slide with micropipette and covered with a 22mmX 22mm cover slip. After stabilizing for 1 minute, wet preparation is examined under light microscopy (400X).

Motility: Select the fields at least 5mm from the edges of the cover slip. At least 5 microscopic fields are assessed to classify 200 spermatozoa. Motility is graded as the motility of spermatozoa is graded as per WHO laboratory manual for the examination and processing of human semen, 5th edition 2010.

Assessment of sperm concentration: The concentration of spermatozoa should be determined using hemocytometer method on two separate preparations of semen sample, one on each side of counting chamber. The dilution is determined from preliminary estimation of sperm concentration.

Assessment of sperm morphology: Preparation of smears of by feathering technique same as peripheral blood smear preparation. Air dry the smear. Later fix in equal parts of 95% ethanol and ether for 5-15 minutes.

Oligozoospermia is defined as low concentration of sperm [22] and Oligoasthenozoospermia is defined as reduced sperm motility and count [23] and Oligoasthenoteratozoospermia is defined as decrease in the sperm count, poor movement and abnormal shape of sperms [24].

Biochemical tests

Fructose test: Use Resorcinol reagent (50 mg powdered resorcinol plus 33ml of concentrated hydrochloric acid. Later, mixture is diluted to 100ml with distilled water). Keep Resorcinol solution in dark-amber bottle and refrigerate when not in use.

Results

A total number of 200 cases were studied. These cases were classified in to smokers and non-smokers. Smokers (person who smoked more than 2 cigarette/day for more than 6 months) and control group were non-smokers.

Table 1: Quality of semen observed according to Age.

Semen quality	Age groups(in years)				
	21-25	26-30	31-35	36-40	41-45
N-z	30	52	34	18	05
O-z	6	13	2	0	1
A-z	3	5	2	0	0
OA-z	9	8	3	1	0
OAT-z	0	2	2	0	0
AZOO	2	0	2	0	0
Total	50	80	45	19	06

Table 2: Quality of semen observed in Non Smoker's.

Semen quality	Age groups(in years)				
	21-25	26-30	31-35	36-40	41-45
N-z	22	31	15	5	1
O-z	5	4	1	1	1
A-z	0	2	1	0	0
OA-z	2	3	2	1	0
OAT-z	0	0	0	0	0
AZOO	1	0	1	0	0
Total	30	40	20	7	2

Table 4: Quality of semen observed in smoker's in study sample

Semen quality	Age groups(in years)				
	21-25	26-30	31-35	36-40	41-45
N-z	22	41	15	6	2
O-z	5	4	1	1	1
A-z	0	2	1	0	0
OA-z	2	3	2	1	0
OAT-z	0	0	0	0	0
AZOO	1	0	1	0	0
Total	30	50	20	8	3

Table 3: Comparision Parameters of Smokers and Non smokers

Parameter	Lower Reference limit	Non Smoker	Smoker
Semen Volume (ml)	1.5	Normal	Variable
Sperm Concentration (10^6/ml)	15	Normal	Decreased
Total Sperm Number (10^6/ejaculate)	39	Normal	Variable \decreased
Progressive Motility (PR, %)	32	Normal	Decreased
Total Motility (PR + NP, %)	40	Normal	Decreased depending on Smoking degree.
Vitality (live sperms, %)	58	Normal	Variable
Sperm Morphology (NF, %)	4	Normal	Variable
pH*	>/=7.2	Alkaline	Alkaline
Leucocyte* (Hpf)	0-1	Depending on etiolgy	> 5-8

Discussion

The present study was aimed to perform the quantitative and qualitative Analysis of Semen and to compare the sperm parameters in smokers and non-smokers. Among 101 cases of smokers, who smoke less than 10 cigarettes/day. Of which 86 cases showed Normozoospermia and 12 cases showed Oligozoospermia, 8 showed Oligoasthenozoospermia, 3 showed Asthenozoospermia and 1 oligosthenoteratozoospermia. Smokers who smoked more than 10 cigarettes/ day, of which majority 7% showed abnormal semen quality reported as Oligozoospermia and Oligoasthenozoospermia and Oligoasthenoteratozoospermia. Maximum number of cases belong to age group 26-30, in this study, the semen volume, sperm count, motility and percentage of normal sperms of the smokers were decreased as compared to the non-smokers. , persons who smoked more than 10 cigarettes/day were showing decrease in semen volume, High liquefaction time, decrease in sperm count, decreased total and progressive motility, high abnormal sperm percentage and, cigarette smoking was associated with a significant decrease in sperm density, total sperm count and total number of motile sperms [6].

Earlier studies reported that there was decrease in the sperm count in chronic smokers [6-9]. Further, the motility of the sperms also lower in smokers when compared with non-smokers [8, 11]. Abnormal morphology of sperms was reported in the smokers when compared with non-smokers [13]. Smoking was considered as one of the public health hazard according to the WHO. It was reported that smokers are at risk of reproductive problems [14]. Tobacco consumption directly damages the germ cells in both males and females through the chemical constituents present in it [15]. Several studies reported that smoking has a negative impact on the quality of sperm. The major changes in the sperms in smokers are decrease in the motility, sperm concentration; total spermcount, semen volume, and altered morphology [16-17].

It was reported that overall quality of sperm decreased in smokers [17]. The exact mechanism of these deleterious effects of nicotine on reproductive system is not clear. However, it was reported that the nicotine can pass through the blood testes barrier and causes damage of the sperm morphology [20]. The nicotine was reported to decrease the motility by damaging the flagella [21]. Interestingly, it was observed that when the smokers sperm washed and placed in nonsmokers seminal plasma, it regained normal motility [21-24]. Moskova *et al* reported that there was decrease in the motility but increase in the morphology of sperms in the smokers when compared with non-smokers. However, there was no [25]. Mutagenic and carcinogenic substances present in the smoke was reported to cause these effects on the motility and morphological changes in smokers [26]. The results of the present study was in accordance with earlier studies.

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