Original Research Article

Semen analysis in smokers and non-smokers

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Abstract

Background: 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. **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:** A total of 150 cases referred to Pathology department for semen analysis in Katuri Medical College & Hospital, from September 2016 to August 2018 were included in the study. The following criteria was used in the selection of the participants. **Results:** The overall quality of sperm was significantly decreased in smokers when compared with non-smokers. **Conclusion:** Semen parameters like volume, motility, count, morphologically normal spermatozoa were reduced in cigarette smokers when compared to Non-smokers, but only sperm count was found to have a high significant decrease in Smokers (P: <0.01) when compared to Non-smokers. In concordance with other researchers, results of present study support that cigarette smoking have a relatively significant effect on semen.

Key words: Semen, Reproduction, Sperm, Males

Introduction

Reproductive medicine has undergone tremendous advances. The concept of cloning humans from cells from a person's nose suggested in woody allensfilm 'Bananas' might not be as farfetched as it seemed in 1971 when the film was released. The advances in current reproductive technologies were made clear after cloning of sheep, with the birth of dolly.

Cloning of humans has not been accomplished and the concept remains highly controversial. Steady advances in assisted reproductive techniques such as blastocyst transfer, embryo biopsy and intra cytoplasmic sperm injection have been made. With the success of these high technology and high cost procedures, the complete evaluation of man can be bypassed. But basic semen analysis is still essential for these techniques [1]. 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.

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Materials and Methods

Study design: Observational study
Study setting: This study was conducted in Pathology department in Katuri Medical College &Hospital, from September 2016 to August 2018.

Study Participants: A total of 150 cases referred to Pathology department for semen analysis in Katuri Medical College & Hospital, from September 2016 to August 2018 were included in the study. The following criteria was used in the selection of the participants.

Inclusion criteria: All patients referred from Obstetrics and Urology and other departments were included.

Exclusion criteria: Those patients who had cryptorchidism, parotitis, testicular atrophy Proved by biopsy, history of systemic illness, tuberculosis, diabetes mellitus, testicular injury were excluded from the study.

Methods: Detailed history of age, marital status, and cigarette smoking was taken as per the proforma by questionnaire and detailed physical examination including genital examination was done.

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 immediatelyafter 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 22 mm 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.

Ethical considerations: The present study was approved by Institutional human ethical committee. Informed consent was obtained from all the participants. Confidentiality of the data was maintained.

Results

A total number of 150 cases were studied in the 2 year span. These cases were classified in to smokers and non-smokers. Smokers (person who smoked more than 1 cigarette/day for more than 6 months) and control group were non-smokers. Table 1 presents the distribution of semen quality among age groups. Table no 2 presents the distribution of semen quality of Non- smokers (controls) among age groups. Table no 3 presents the mean values of semen parameters in Non-

smokers (controlsTable no-4 presents the mean values of semen parameters in Smokers. Table no 5 presents the mean values of semen parameters in Smoker. Table no 6 presents Mean comparison of parameters between Smokers and Non-smokers.

Table no 7 presents distribution of semen quality among different types of Cigarette Smokers. Figure no1 presents the microphotograph of spermatozoa.

Table-1: Distribution of semen quality among age groups

Semen quality	Age groups (in years)				
	21-25	**26-30**	**31-35**	**36-40**	**41-45**
NZ	21	43	22	09	02
OZ	6	13	2	0	1
AZ	2	5	1	0	0
OAZ	5	8	3	1	0
OATZ	0	2	1	0	0
AZOO	2	0	1	0	0
Total	**36**	**71**	**30**	**10**	**03**

Table-2: The distribution of semen quality of Non- smokers (controls) among age groups.

Semen quality	Age groups(in years)				
	21-25	**26-30**	**31-35**	**36-40**	**41-45**
NZ	17	31	15	5	1
OZ	5	4	1	0	0
AZ	0	5	1	0	0
OAZ	2	5	3	1	0
OATZ	0	0	1	0	0
AZOO	1	0	1	0	0
Total	**25**	**45**	**22**	**6**	**1**

Table-3: Mean values of semen parameters in Smokers.

Parameters	Values
N	51
Age in years	29.13
Volume in ml	2.86
Liquefaction time	21.86
Sperm count	24.96
Total motility	59.80
Progressive motile	43.96
Morphologically normal sperms	84.49
Morphologically abnormal sperms	13.45
WBC(10˚6/ml)	0.27

Table-4: Mean values of smokers are stratified according to age groups.

Parameters	21-25	26-30	31-35	36-40	41-45
N	12	25	8	4	2
Volume in ml	2.58	2.8	3.18	3.3	3
Liquefaction time	23.7	21.4	18.1	26.2	22.5
Sperm count	22.7	21.3	36.5	30	27.5
Total motility	47.5	59.2	68.7	70	85
Progressive motile	32.5	41.8	55	56.2	70
Morphologically normal sperms	76.6	85.8	85.6	96	87.5
Morphologically abnormal sperms	15	14.2	13.7	4	12.5
WBC (10^6/ml)	0.41	0.36	0	0	0

Table-5: Mean values of semen parameters in non-smokers and smokers.

Parameters	Values	
	Non-smokers	smokers
n	99	51
Age in years	28.8	29.13
Volume in ml	2.92	2.86
Liquefaction time	20.91	21.86
Sperm count	41.76	24.96
Total motility	65.85	59.80
Progressive motile	46.86	43.96
Morphologically normal sperms	86.26	84.49
Morphologically abnormal sperms	11.71	13.45
WBC(10^6/ml)	0.27	0.27

Table 6: Mean comparison of parameters between Smokers and Non-smokers.

Parameter	Mean	SD	P-Value	Inference
Volume	2.93	0.769	0.605	NS
	2.86	0.693		
liquefaction time	20.92	7.744	0.52	NS
	21.86	9.745		
Sperm count	41.77	28.269	<0.01	*HS
	24.96	20.59		
Total motility	65.86	30.373	0.245	NS
	59.8	29.55		
Progressive motility	46.87	29.913	0.563	NS
	43.96	27.507		
Normal	86.26	20.79	0.638	NS
	84.49	23.741		
Abnormal	11.72	16.736	0.58	NS
	13.45	20.566		

Table-7: Distribution of semen quality among different types of Cigarette Smokers.

Type ofcigarette smoker	Semen quality					
	NZ	OZ	AZ	OAZ	OATZ	AZOO
Less than 5/day	8	1	0	0	0	1
Less than 10/day	17	8	2	3	1	0
More than 10/day	3	3	0	3	1	0

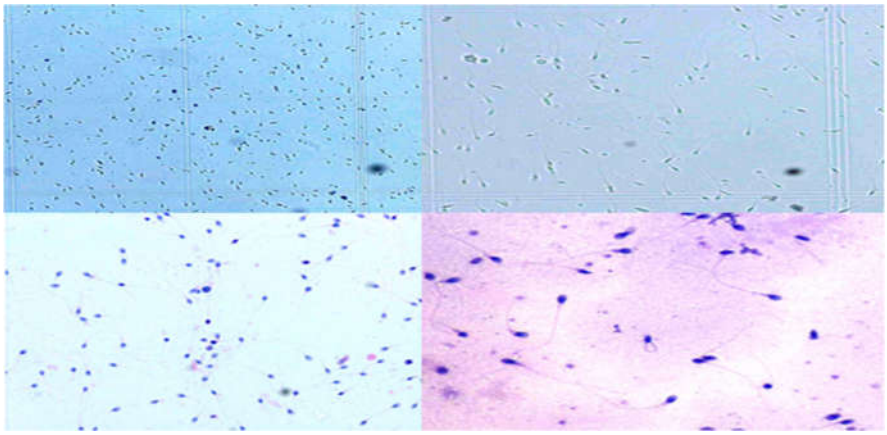

Figure-1: Microphotograph of spermatozoa

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 51 cases of smokers, maximum cases (31) 60.78%. belong to smokers who smoke less than 10 cigarettes/ day. Of which 17 cases showed Normozoospermia and 14 cases showed abnormal semen quality among them 8 cases showed Oligozoospermia, 3 showed Oligoasthe-nozoospermia, 2 showed Asthenozoospermia and 1 oligosthenoteratozoospermia. Smokers who smoked more than 10 cigarettes/ day were 10/51, of which majority 7% showed abnormal semen quality reported as Oligozoospermia and Oligoasthenozoospermia and Oligoasthenoteratozoo-spermia.

Maximum number of cases belong to age group 26-30 (n=25), 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. Examination of semen parameters amongall the 3 categories, persons who smoked more than 10 cigarettes/day were showing decrease in semen volume, High liquefaction time, Least sperm count, Least total and progressive motility, high abnormal sperm percentage and Majority of morphological defects. It was reported that, cigarette smoking was associated with a significant decrease in sperm density, total sperm count and total number of motile sperms [5]. 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 [10,11]. Abnormal morphology of sperms was reported in the smokers when compared with non-smokers [12]. 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 [13]. Tobacco consumption directly damages the germ cells in both males and females through the chemical constituents present in it [14]. 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 [14-16].

It was reported that overall quality of sperm decreased in smokers [17]. Vine et al. reported negative correlation between smoking and the quality of sperm [18]. 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 [19]. The nicotine was reported to decrease the motility by damaging the flagella [20].

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.

Limitations: The study was conducted at one center. Hence, the results cannot be generalized.

Conclusion

Semen parameters like volume, motility, count, morphologically normal spermatozoa were reduced in cigarette smokers when compared to Non-smokers, but only sperm count was found to have a high significant decrease in Smokers (P: <0.01) when compared to Non-smokers. In concordance with other researchers, results of present study support that cigarette smoking have a relatively significant effect on semen.

Addition to existing knowledge: The study highlights the hazardous effects of smoking on reproductive functions which increase the awareness of general public about the smoking and its dangerous effects. It also helps researchers to understand further the association of smoking and reproductive functions which may help to develop effective management methods.

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