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EFFECTS OF CIGARETTE SMOKING ON SEMEN PARAMETERS OF MEN

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ABSTRACT: Addicted smokers Semen analysis from a cross section of population of Anantapuramu district, Andhra Pradesh, India was done as it provides a useful profile of the function of the male reproductive health. Seventy two (72) men were drown from a cross section of smokers and divided in to two age groups namely 25-30 years and 31-40 years. In both age groups persons were selected basing as how many cigarettes they smoked in a day. Different semen parameters like volume, liquefaction, alkalinity, sperm count, motility and morphology were analysed. Incidences of Hypospermia in 25, Teratozoospermia in 27, Oligozoospermia in 54, Oligoasthenozoospermia in 20, and Oligoasthenoteratozoopermia in 6 persons were observed. Abnormalities were seen in both age groups and in persons who smoke either moderately or heavily.

INTRODUCTION: Infertility is one of the most alarming of all marital problems. It has been reported that in about 40% of infertility couples, male are at fault while remaining 60% are due to female partners. Unfortunately in most married couples infertility is a social stigma. There are many factors which effect male reproductive health such as environmental issues, lifestyle, sleep deprivation etc [1, 2]. Added to these smoking, which causes harmful effects to probably every tissue on the human body [3]. Effects various parameters of male reproduction like sex hormones [4, 5]. Semen parameters were also known to be effected due to smoking [6, 7]. It was estimated that there are over 1 billion smokers across the globe of which more than 80% are known to be from developing and under developed countries [8].

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WHO mentions that India accounts for 12% of the global smokers with highest number from the state of Jammu and Kashmir [9, 10]. The same survey [9], further showed that there are 275 million tobacco users in India. In the state of Andhra Pradesh, where the present investigation was carried out, National statistics have shown 47% men and 14% women aged 15 years or above either smoked or chewed tobacco, which is about 29% of the total population.

Cigarette smoking is a broadly recognised has health hazards and a major cause mortality[11]. The maximum prevalence of smoking was observed in young adult males who are in the age group of 20-39 years, the age being active reproductive period [12].

Hence the present investigation was carried out by surveying of sample population between age group of 25-40 years in the district of Anantapuramu, Andhra Pradesh, India. Cigarette smoking may be associated with sub-fertility in men and my result in decreased sperm concentration, lower sperm

motility, and a reduced percentage of morphologically normal sperm [13, 14]. Some studies have reported that the association between man smoking and semen quality was stronger in healthy men than in the infertile population [15, 16].

MATERIALS AND METHODS:

One hundred healthy human males, who are involved in cigarette smoking, were interviewed particularly regarding age and the number of years they were smoking. From this sample seventy two (72) persons were selected and divided into two groups. Each group consisted of thirty six (36) men who are involved in these two groups. Out of this thirty six, eighteen is moderate smoking (10±3 Cigarettes/day) and eighteen heavy smoking (20±3 Cigarettes/day) of the age group of 25-30 and same in the age group of 31-40 years. The study protocol was approved by the Institutional Animal Ethical Committee. Before enrollment in the study, written consent was obtained from volunteers.

The selected men were invited to clinical laboratory and semen sample was collected by masturbation and ejaculated into a clean wide mouth glass container. Care was taken to see that the sample was collected after a minimum of two days and maximum of seven days sexual abstinence. The semen sample collected was kept at room temperature (20°C-37°C) to avoid any effect on spermatozoa. Container was labeled with person's name, identification number, date and time of collection. WHO guidelines were followed in collection and analysis of semen sample [17]. The following investigations were carried out in the samples.

Colour, volume and pH: Colour of the semen was observed immediately after collection and the volume was measured using graduated test tube. The semen reaction was observed by measuring its pH.

Liquefaction: Immediately after ejaculation into the collection vessel sample was kept at room temperature and time of liquefaction was observed to 90 min. Semen was typically a semisolid coagulated mass first and within a few minutes at room temperature, the semen usually begins to liquefy (become thinner). The time taken to liquefy was noted.

Sperm count and motility:

Sperm count and motility were made using the above liquefied sample under the microscope. Total sperm count (Mill/ml) was calculated by using neubauer chamber [17]. Briefly the liquefied semen was diluted 1:20 with sodium carbonate and this diluted sample was placed on the neubauer chamber and counted under the microscope (Labomed). Motility was determined by counting the number of motile and immotile spermatozoa from the same slide in several randomly selected fields under 20X objective until at least 200 spermatozoa were counted. The minimum of five microscopic fields were examined.

Sperm morphology:

This was determined with the help of smears made from semen samples using feathering technique. A clean glass slide was taken, washed in 70% ethanol and dried. A small drop of semen (5 to 20 µl) was taken onto the slide. The edge of a second slide was placed on the first, at an angle of 45° and the semen drop was dragged along the surface to make a thin smear. These were then air dried and fixed. Sperm morphology was evaluated using hematoxylin and eosin stain. Normal and the abnormal sperms were observed under 100X oil immersion microscope. Each of the spermatozoa was examined for head, mid–piece and tail defects. A total of 200 spermatozoa were observed for defects and expressed in percentage. Loose heads were counted (as abnormal forms), while free tails were not counted. Structures without any head anterior to the basal plate were not counted.

RESULTS:

Data on analysis of semen carried out in men addicted to smoking are given in tables 1 to 3. In this investigation we found that all men had white and alkaline semen. The volume of semen measured was less than the normal values in twenty five (25) men out of seventy two (72). In men whose was addicted of high smoking nine persons in the age group of 25-30 have shown hypospermia (**Table 1**). Six persons in the age group of 31-40 who are moderate smokers and men ten persons in

the age group of 31-40 who are heavy smokers have shown hypospermia (**Table 2).**

Liquefaction time of semen was observed in men involved in all Two groups was within the time given by WHO in all age groups (**Table 1-2**). With regard to sperm count it was less than the normal values in Fifty four (54) men out of seventy two (72) men who were examined. In men whose was addicted of moderate smoking eleven persons in the age group of 25-30 and in heavy smoking men fifteen persons in the age group of 25-30 have shown oligozoospermia (**Table 1**). In men who addicted in moderate smoking, twelve persons in the age group of 31-40 and in heavy smoking men sixteen persons in the age group of 31-40 have shown oligozoospermia (**Table 2**).

TABLE 1: ANALYSIS OF SEMEN IN MEN WHO HAVE BEEN SMOKING FOR MORE THAN FIVE YEARS.

S.No	Parameters Examined	Normal Values	Age:25-30 10±3[a]	Age:25-30 20±3[a]
1	Colour	White	White	White
2	Reaction	Alkaline	Alkaline	Alkaline
3	Volume	1.5-5ml	1.94±0.37 (1.5-2.5)	1.62±0.69 (1-3)
4	Liquefaction	15-60mins	23.88±6.31 (20-40)	34.16±21.43 (20-90)
5	Sperm count	39-150mill/ml	31.66±13.27 (12-50)	24.83±13.04 (15-57)
6	Total motility	32%	42.27±15.43 (15-60)	41.50±14.60 (20-60)
7	Morphology	4%	3.77±0.42 (3-4)	3.27±0.46 (3-4)

Note: Values are mean ± SD (n=18), Minimum and maximum values.
[a]Number of cigarettes smoked per person/day.

TABLE 2: ANALYSIS OF SEMEN IN MEN WHO HAVE BEEN SMOKING FOR MORE THAN FIVE YEARS.

S. No	Parameters Examined	Normal Values	Age:31-40 10±3[a]	Age:31-40 20±3[a]
1	Colour	White	White	White
2	Reaction	Alkaline	Alkaline	Alkaline
3	Volume	1.5-5ml	1.48±0.50 (1-3)	1.36±0.39 (1-2.5)
4	Liquefaction	15-60mins	33.05±19.18 (20-90)	42.50±25.90 (15-90)
5	Sperm count	39-150mill/ml	30.61±11.21 (4-45)	19.55±13.28 (0-56)
6	Total motility	32%	40.44±14.18 (20-60)	38.88±16.14 (0-60)
7	Morphology	4%	3.83±0.51 (3-5)	3.77±0.64 (3-5)

Note: Values are mean ± SD (n=18), Minimum and maximum values.
[a]Number of cigarettes smoked per person/day.

Oligoasthenozoospermia have been noticed in Twenty men (20) out of seventy two (72) men examined. In men whose was addicted of moderate smoking Twenty seven persons in the age group of 25-30 and in heavy smoking men six in the age group of 25-30 have shown Oligoasthenozoospermia (**Table 1**). In men who was addicted in moderate smoking, three persons in the age group of 31-40 and in heavy smoking men eight persons in the age group of 31-40 have shown Oligoasthenozoospermia (**Table 2**).

TABLE 3: ABNORMALITIES OBSERVED IN PERSONS OF TWENTY SEVEN DIFFERENT AGE GROUPS DUE TO SMOKING.

S.No	Addiction of smoking	Age group in years	Abnormalities in number of men
1	Group-1	25-30 10±3 Cigarettes/day	Oligozoospermia-11, Oligoasthenozoospermia-3, Teratozoospermia-4
2	Group-2	25-30 20±3 Cigarettes /day	Hypospermia-9, Teratozoospermia-13, Oligozoospermia-15, Oligoasthenozoospermia-6, Oligoasthenoteratozoopermia-5.
		25-30 and 31-40 10±3 Cigarettes /day	Hypospermia-6, Oligozoospermia-12, Teratozoospermia-4, Oligoasthenozoospermia-3.
		31-40 20±3 Cigarettes /day	Hypospermia-10, Teratozoospermia-6, Oligozoospermia-16, Oligoasthenozoospermia-8, Oligoasthenoteratozoopermia-1.

Oligozoospermia (54) **:** Sperm count is less than 39 Mill/ml.
Hypospermia (25) **:** Semen volume less than 1.5 ml.
Teratozoospermia (27) **:** When less than 4% of the normal sperms show abnormal morphology.
Oligoasthenozoospermia (20) **:** Combination of low sperm count (less than 39 Mill/ml) and sperm motility (less than 32%).
Oligoasthenoteratozoospermia (6): Combination of low sperm count, motility and abnormal morphology (less than 4% of normal forms).

Oligoasthenoteratozoospermia have been noticed in six men (6) out of seventy two (72) men examined. In men whose was addicted of heavy smoking five persons in the age group of 25-30 have shown Oligoasthenoteratozoospermia (**Table 1**). In men who was addicted in heavy smoking, one person in the age group of 31-40 have shown Oligoasthenoteratozoospermia (**Table 2**).

Morphologically abnormal sperms have been noticed in three men (27) out of seventy two (72) men examined. In men whose was addicted of moderate smoking four persons have shown Teratozoospermia in the age group of 25-30 and in heavy smoking men thirteen persons in the age group of 25-30 have shown Teratozoospermia (**Table 1**). In men who was addicted of moderate smoking four persons have shown Teratozoospermia in the age group of 31-40 and in heavy smoking men six persons in the age group of 31-40 have shown Teratozoospermia (**Table 2**).

DISCUSSION: Despite worldwide campaign against smoking and printing of caution of health hazard on cigarette boxes smoking is still common. Reproductive health has been one of the most effected systems in humans. Relationship between cigarette smoking and human sexual function have also been established. Semen color was observed initially and it was found to be white color in all the smokers of both groups. There was no change with regard to alkalinity of the semen also. Smoking does not seem to have a recognizable effect on these two parameters. Our study have shown decrease in the volume of semen in twenty five (25) men. Men who smoke moderately or heavily are affected. It was shown earlier that reduction in semen volume was in proportion the number of cigarettes smoked [18]. Significantly smaller of semen volume in smokers than nonsmokers was also reported by other researchers [19, 20, 21].

Semen is a thick gel at the time of ejaculation and normally becomes liquid within 20 minutes (or 15 to 60 mins) after ejaculation. The thick gel is formed by proteins from the seminal vesicles. It was shown that liquefaction occurs only in a pH range of 6.8-8.8, at which pepsin is not active [22]. If liquefaction is delayed it will be difficult for sperm to break thick semen. Also the semen must liquefy quickly for sperm to swim out of the acidic vagina. All men examined from two different groups exhibited liquefaction time within the normal time range.

Reduction in sperm count was observed in fifty four (54) smokers which is quite dangerous. It was shown earlier that antisperm antibody increase in

smokers is the reason for reduction of sperm count [23]. Smokers were also known to possess lower concentrations of non methyltetrahydrofolate in semen which leads to reduced sperm count[24]. The findings are in conformity with earlier studies who have shown decreased to sperm count due to smoking[25, 26, 27].

Sperm motility has been shown to be a good predictor of human male fertility *in vivo* and *in vitro* [28] and as such has also been found to be strongly associated with the probability of conception[29, 30]. Reduced testosterone level leads to disturbed epididymal function which results in reduced sperm motility [31]. Though testosterone was not estimated in the present study this could be contributing to reduced sperm motility. CAT (Choline acetyl transferase) is known to facilitates sperm motility [32] and cigarette smoke condensates possess inhibitors of CAT. These could have also lead to reduced sperm motility. Strong relationship between decline sperm motility number of cigarettes smoked per day were shown by [19]. Reduction of sperm motility was also shown by other researchers in smokers[33, 34].

Normal sperm has an oval head and long tail. Abnormality of sperm could be defective heads/tails. If semen sample contains 4% of morphologically normal forms, it is considered fit. It is observed in this study that two persons in the age group of 31-40 years have shown morphologically abnormal sperm. In the case of semen morphology, some reports have shown that semen morphology correlated with smoking, while others have not. Semen morphology was shown to be an important parameter related to pregnancy[35].

CONCLUSION: The findings of the present study underscores the fact that smoking certainly has an advance influence on semen quality, as concluded by other studies. 74.1% of smokers have shown observed morphology [7].

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