

“A prospective Study on Effects of Tobacco Consumption on Semen Parameters”

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ABSTRACT

Background: Tobacco consumption in any form is a well known health hazard and a major cause of mortality. This may affect male reproductive function and conveniently evaluated by semen analysis. Thus semen parameters of the subjects with habits of consumption compared within cases and control groups.

Methods: Subjects referred for semen analysis & volunteers were studied. Detailed history of personal, occupational and tobacco consumption was obtained. Semen analysis performed as per the WHO guideline 2010, 5th edition. We included tobacco smokers, chewers and non-addicted subjects. Tobacco consumers further stratified according to type, amount and duration of consumption and various semen parameters from these groups compared by using ANOVA and unpaired-t test.

Result: Out of 157 subjects, 25 as control, 32- tobacco smokers, 87- tobacco chewers, 13 with both habits, further stratified according to amount and duration of consumption. There is significant reduced semen parameters of tobacco consumers obtained in relation to its amount of consumption were- sperm concentration, motility and morphology, while not affected with duration of consumption. Oligozoospermia was higher in tobacco consumers.

Conclusion: Results of study found that tobacco consumption in men is associated with reduced sperm quality (including count, motility & morphology). This concludes tobacco consumption is one of the risk factor in male infertility and subfertility.

Keywords: Tobacco Consumption, Semen Parameters, Reactive Oxygen Species, Male Infertility

Introduction

Tobacco consumption is one of the widely accepted undesirable habits by the people. It has been long documented that tobacco use contributes to high rates of morbidity and mortality across the world.^[1] In India, Smoking tobacco consumed as “bidis,” hookah, chuttas, cigarettes, and cigars and chewed extensively in the form of paan masala, gutka and other locally prepared mixtures of tobacco, areca nut, and additives called ‘mava- masala’. Chewing of paan with tobacco is a popular habit that has been integrated into customs and traditions in rural India.^[2,3] In addition, social norms, availability, acceptability, and advertising campaigns also influence tobacco use, particularly among males.^[4]

In current scenario infertility is one of most distressful of all marital problems estimated to affect 10%–15% of all couples. Male infertility plays a key role in conception difficulties of up to 40% infertile couples. Male infertility is not a definitive diagnosed disease, in some men causing factors are evaluated but most of the time reason for infertility could be unknown. It’s making worthy to draw attention towards the impact of lifestyle and environmental

factors, like diet, obesity, smoking, alcohol intake, recreational drug use, and exposure to environmental toxins, on the reproductive health. The male factors in terms of reduced sperm quality is the major cause.^[5]

Although tobacco abuse in any form is a widely recognized health hazard and a major cause of mortality, people continue to consume it on a regular basis. According to the world health organization (WHO) approximately one third of world population older than 15years, are consuming tobacco.^[6,7] Tobacco contains toxic substance like nicotine, carbon monoxide and other carcinogens and mutagens like radioactive polonium, benzopyrene, dimethyl-nitrosamine which may disrupt the testicular microcirculation and cause DNA or chromosomal damage in germinal cells. Many recent studies found tobacco in men is related to a decrease in overall sperm quality (including count, motility and morphology). According to the study, the prevalence of oligoasthenoteratozoospermia (a condition that includes decreased sperm motility, count and abnormal sperm morphology) is very high. Studies have also shown tobacco consumption detrimentally affects sperm concentration, volume, motility and morphology and damage the DNA.^[8-13]

Semen analysis used as key measures of male fertility.^[14,15] Conventionally importance has been given to sperm count, though without desirable motility or normal sperm morphology, male unable to achieve the reproductive function. 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.^[6,14,15]

Semen parameters of tobacco chewers and smokers compared with those of tobacco non-chewers, non smokers. Since tobacco consumption is more prevalent in Saurashtra region and unavailability of more literature detailing such study in this region, more tobacco chewers included in study group.^[8] Thus, the present study aimed to conclude the deleterious effects of tobacco consumption on various semen parameters.

Materials and Methods

The study carried out in Clinical Pathology section of the department of Pathology after taking permission from IRB & ethical committee, Government Medical College & Sir T. Hospital, Bhavnagar, Gujarat. Male patients referred from the Gynecology department for infertility evaluation and volunteer subjects from the society are studied. We have included age group 25-35 years and with the history of tobacco consumption for more than one year duration are cases and those with no any history of substance abuse were considered as control group. We excluded subjects with history of urogenital diseases like Mumps orchitis, sexually transmitted infections/urethritis, groin/testicular trauma, torsion, prior inguinal or scrotal surgery; developmental anomalies like Klinefelter's syndrome, Cystic fibrosis; endocrine disorders like diabetes mellitus, metabolic syndrome, thyroid function; occupational exposure to toxic chemicals or higher temperature, alcohol abuse or other addictions, medications like herbal remedies for infertility, anabolic steroids.

Study groups were thoroughly interviewed about their chief complains, past and present occupation, disease and tobacco consumption history in detail, which includes mode, duration and frequency of addiction. Subjects then categorized according to the frequency of daily tobacco consumption. Tobacco chewers stratified into 3 categories: Group A - (Mild chewers <3times/day), Group B (moderate chewers 3-6times/day) and Group C (severe >6 times/day. Similarly smokers into 3 groups, Group A - (≥ 1 and ≤ 10 cigarettes/ day), Group B - (moderate, >10 and <20 cigarettes/ day) Group C- (severe, ≥ 20 cigarettes/ day).

Semen analyses of the studied subjects were conducted as per standard WHO guideline 2010, 5th edition. With prior 3

days abstinence, subjects advised to collect semen in sterile wide mouth, non-toxic plastic calibrated containers labeled with details information. Initially liquefaction time, volume evaluated and pH assessed by pH comparator strips. Sperm motility evaluated by wet preparation and viability by eosin exclusion test, usually done when progressive motility <40% but we have performed in all cases. Eosin exclusion test done by using 0.5% Eosin Y solution prepared with 0.9% NaCl Solution to maintain proper hyper osmolar environment for sperm to avoid errors. After proper mixing 5 μ l of semen and 5 μ l eosin solution on a slide, covered with cover slip and kept for 30 seconds and tally 200 spermatozoa, the number of stained (dead) and unstained (vital) cells with the aid of a laboratory counter under high power field examination in light microscope in 10-15 minutes. The procedure was performed twice and the mean value obtained which was reported in percentage. Sperm count measured with modified neubars chamber, for that semen is diluted with ready to use market prepared semen dilution fluid which contains formalin as fixative. Dilution decided during initial wet preparation in which we made dilution 1:20 for >100sperm/high power field, 1:5 for 15-100 sperm/high power field and 1:2 for less than 15sperm/high power field. We didn't found any cases with occasional sperm in multiple high power field in our studied cases as the exact sperm count couldn't obtained in such cases accurately although such cases could considered as very low count for evaluation and require repeated analysis at intervals advised as per clinician. After proper dilution, neubars chamber charged on two sides and sperm counted both side under high power field. Obtained average count multiplied with dilution factor and expressed per ml of semen volume. Total sperm count obtained by multiplying with the total volume of semen. Sperm smears were fixed with isopropyl alcohol and stained with rapid pap stain. Sperm morphology accessed first by scanning for even distribution of spermatozoa and then in oil emersion field, tallies 200 sperm, twice for abnormality in head, principal piece, tail and abnormal residual cytoplasm. Morphology reported as average of multiple results in percentage value.

The semen parameters analyzed statistically by comparing mean and SD value between various study group using ANOVA and unpaired-t test. P value <0.05 shows significant alteration between the parameters of different groups.

Result

Out of 157 subjects 25 are considered as control group, with no any habit of tobacco consumption, 87 are tobacco chewers, 32 are tobacco smokers, and 13 with both the

habits of consumption are considered as the case study populations. Further cases with tobacco consumption are stratified in to 3 groups (fig.-1).

Studied semen parameters were liquefaction time (minutes), Volume (ml), pH, Sperm concentration (10⁶/ml), Vitality, Rapid progressive motility, Total motility (Rapid progressive motility+ Non-progressive motility) Normal morphology (%).

In Table-1 there is comparison of smokers group according to quantity of consumption, between Group- A, Group- B, and Group- C in which numbers of subjects are 25, 23, and 2 respectively. In our study it shows significant reduced mean value in important parameters like sperm concentration, rapid progressive motility (PR motility), total motility and normal morphology. Rest of other parameters has not much significant difference in the mean value (fig.2)

Table -2 shows comparison of semen parameters of 87 subjects with the habit of tobacco chewing are further studied according to daily frequency of consumption divided in group A, B, C comprise 20, 60, 7 subjects respectively. Sperm concentration, PR motility, total motility and normal morphology shows significant decreased mean (fig.3) between control group and within the chewers groups.

In Table-3 semen parameters of 87 tobacco chewers are compared with the 32 tobacco smokers groups. There is no significant difference in p value it shows there is no any significance with relation to mode of consumption.

Oligozoospermia is the main abnormality observed in the all groups with 12% (3/25) in control group, 11.4% (10/87) in chewers group, 15.6 % (5/32) in smokers group, and 23.07% (3/13) in subjects with both habits.

Table 1: Comparison between control group and tobacco smokers according to amount of consumption:-

Semen parameters	Control (n=25)	Group-A (n=23)	Group-B (n=7)	Group-C (n=2)	p- Value
Liq. Time	39.80 ±4.20	36.52± 3.17	38.57± 2.44	40.00± 0.0	0.126
Volume	3.92 ±0.46	3.87±0.50	3.86± 0.56	3.75 ±0.35	0.951
pH	7.50± 0.16	7.50± 0.18	7.54± 0.05	7.55± 0.21	0.7895
Sperm conc.	51.12± 22.76	40.88 ±13.88	26.94± 11.93	26.90± 6.08	0.041
Vitality	79.86± 4.11	78.0± 5.37	80.29± 4.27	73.50±12.02	0.3061
PR motility	57.72 ±12.54	57.83± 13.21	45.71± 11.70	40.0± 7.07	0.0365
Total motility	71.92 ±11.27	69.13 ±12.03	55.71± 10.97	55.00± 0.00	0.0209
Morphology	33.12± 7.69	29.30± 7.05	23.14± 5.90	20.00± 2.83	0.0423

*p-value <0.05 is significant between the mean comparison of groups

Table 2: Comparison between control group and tobacco chewers according to amount of consumption:-

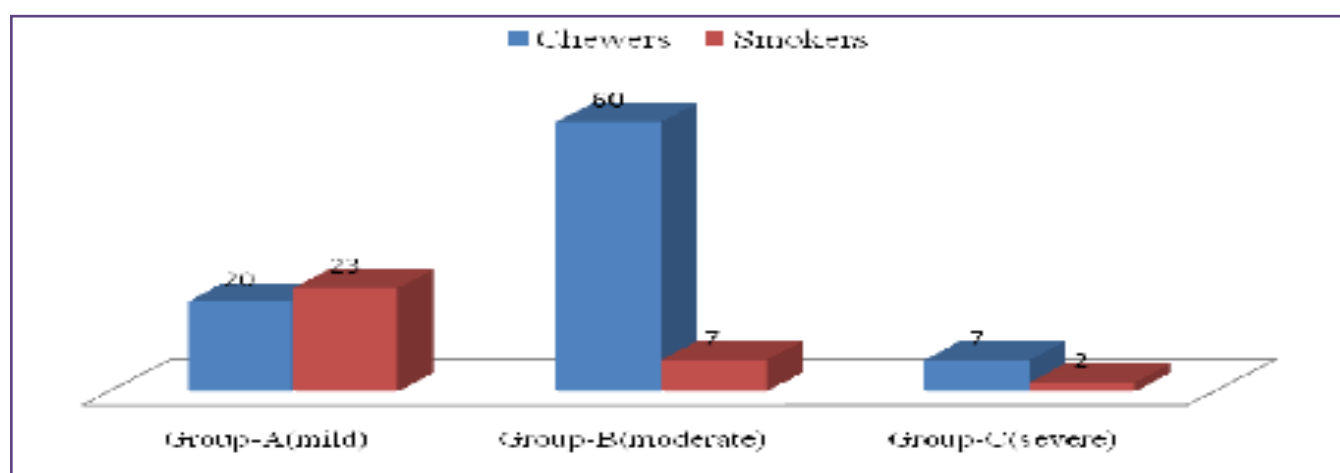
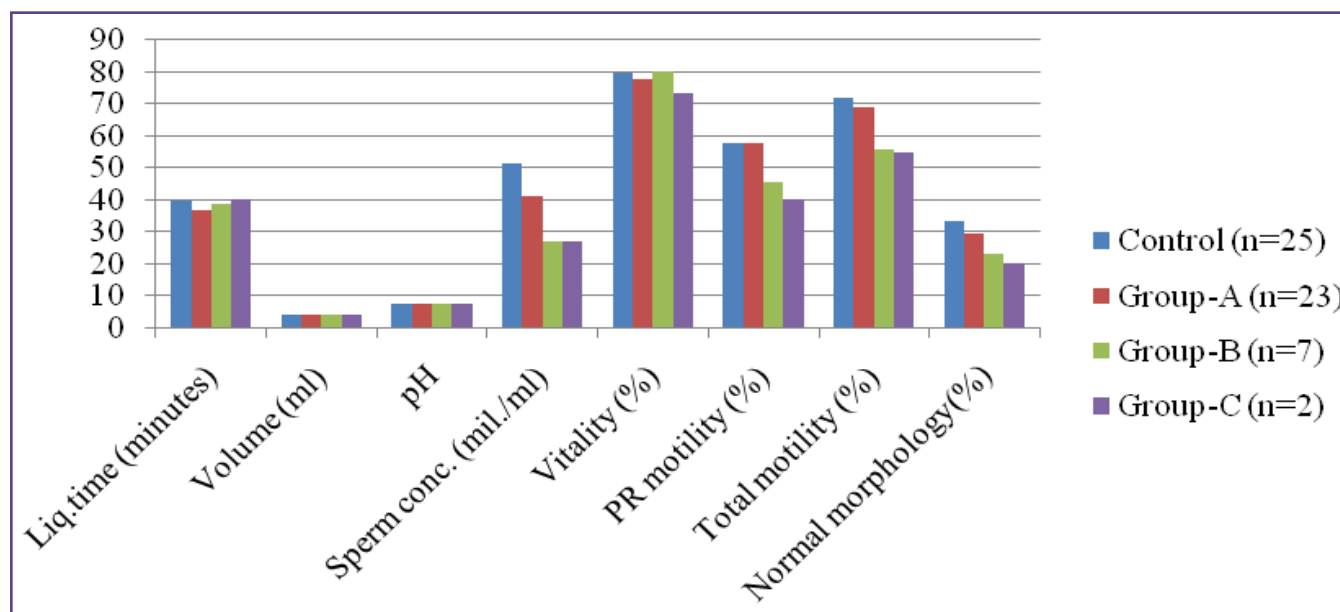
Semen parameters	Control (n=25)	Group-A (n=20)	Group-B (n=60)	Group-C (n=7)	p- Value
Liq. Time	39.80 ±4.20	37.75± 4.99	38.83± 3.95	35.71± 5.23	0.159
Volume	3.92 ±0.46	3.88± 0.43	3.78± 0.69	3.57± 0.89	0.568
pH	7.50± 0.16	7.47 ±0.13	7.46± 0.16	7.51 ±0.11	0.681
Sperm conc.	51.12± 22.76	43.35 ±12.98	37.34± 12.53	29.17±13.86	0.031
Vitality	79.86± 4.11	77.30± 6.13	76.52± 5.22	77.8± 5.9	0.746
PR motility	57.72 ±12.54	60.25± 10.32	51.42± 11.42	40.71 ±6.73	0.0002
Total motility	71.92 ±11.27	74.50 ±10.37	65.67 ±11.84	52.14± 5.67	0.0001
Morphology	33.12± 7.69	32.50± 7.31	31.02 ±5.87	26.50 ±6.27	0.0303

p-value <0.05 is significant between the mean comparison of groups

Table 3: Comparison between tobacco chewers and tobacco smokers:-

Semen parameters	Chewers (n=87)	Smokers (n=32)	p- Value
Liq. Time	38.33± 4.36	37.19± 3.09	0.110
Volume	3.78± 0.65	3.86 ±0.50	0.479
pH	7.47± 0.15	7.51± 0.16	0.220
Sperm conc.	38.06± 13.10	36.96± 14.35	0.757
Vitality	76.80± 5.44	78.22± 5.57	0.221
PR motility	52.59± 9.20	51.40± 6.58	0.437
Total motility	66.61± 9.56	65.31± 8.52	0.478
Morphology	32.11± 6.51	27.38± 7.25	0.241

p-value <0.05 is significant between the mean comparison of groups

**Fig. 1: Frequency according to amount of consumption within the smokers and chewers group:****Fig. 2: Comparison between control group and tobacco smokers according to amount of consumption:-**

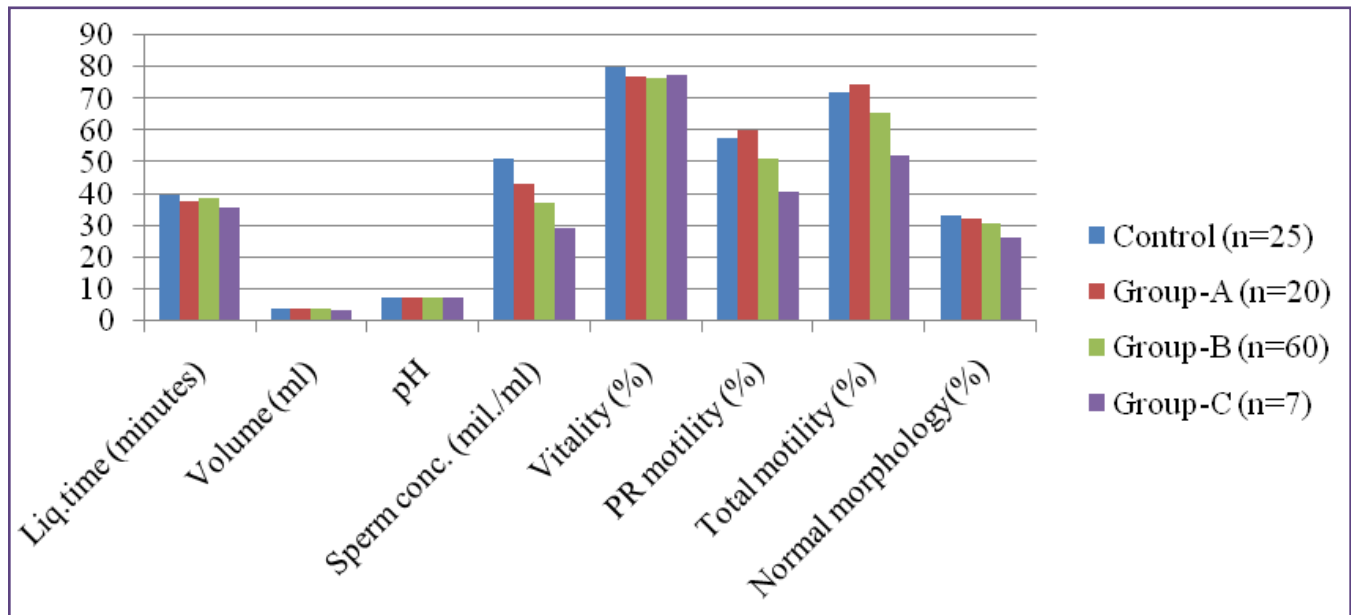


Fig. 3: Comparison between control group and tobacco chewers according to amount of consumption:-

Discussion

Apart from other well known health hazards tobacco consumption by any mode may have deleterious impacts on reproductive function too.^[8] We have studied the possible effects of tobacco consumption on male fertility by evaluating semen parameters. Tobacco consumption as smoking or smokeless form may have direct relation when its amount of consumption and impacts on semen parameters studied.^[8] In our study we have found significant reduced vital semen parameters like sperm count, sperm motility and normal morphology that may leads to sub-fertility or infertility in male partners of infertile couple.

We obtained mean and standard deviation of various parameters like semen volume, pH, liquefaction time, sperm concentration, % of sperm motility and normal sperm morphology and compared with the different groups. We found significantly reduced quality in semen parameters (sperm concentration, motility and morphology) in tobacco users. We found abnormality significantly correlate with quantity of tobacco consumption.

Semen parameters from the present study of tobacco smokers compared to with Patel K et al. 2013^[17], in which the results are consistent with reduced parameters like sperm concentration and motility. However, in our study parameters like volume, sperm viability is less affected.

In study like of Kunzle et al.^[6] 2003 with smokers and nonsmokers, they found significant reduction in sperm concentration, sperm motility and normal morphology, while Ozgur K et al.^[19] and Osser et al.^[20] did not find much

statistically significant difference between the control group and smokers in terms of various semen parameters.

Said TM et al 2003^[21], showed significant decrease in semen quality (sperm count, motility, morphology) associated with a chewing tobacco habit in men undergoing infertility evaluation. Parmar et al 2015^[22] and Sunanda et al 2014^[23] are consistent with significantly reduced semen quality in tobacco chewers. In our study sperm viability is not affected significantly. However study conducted by Dikshit et al. 1987^[25] didn't found any remarkable reduced semen parameters like sperm concentration, motility and normal morphology.

The results of our study showed that the tobacco consumption adversely affects semen parameters. In this study, we observed a statistically relationship between quantity of tobacco consumption and several semen characteristics. The sperm concentration, the percentage of rapid progressive and total motile sperm, normal morphology spermatozoa dropped with increased severity of smoking and tobacco chewing.

Although various studies have demonstrated that cigarette smoking is associated with the abnormal semen parameters^[6,10,16] in heavy (frequent) smokers had lower sperm concentration and a lower total sperm count, motility, semen volume than non-smokers, the toxic effects of smoking on semen parameters are not fully understood. Distraction in endocrine system or mild hypoxia caused by the disruption of the testicular microcirculation are possible explanations, still direct toxic effect of the many chemical

components in the cigarette smoke on the germinative epithelium is a more likely explanation^[24]. Oxidants in cigarette smoke are thought responsible to damage sperm DNA, and tobacco smokers have more oxidative DNA damage in their sperm than do non-smokers.^[24]

With respect to male infertility, nicotine is absorbed in substantial quantities as a result of tobacco chewing and could contribute to the adverse consequences. Demonstration by murine models have documented virulent inflammatory reaction and marked ultrastructural changes in the testes of animals exposed to nicotine.^[11,26] Furthermore, nicotine concentration and its major metabolites cotinine and trans-3 hydroxycotinine in human seminal plasma negatively correlated with sperm parameters.^[27] However, further studies are needed to describe the exact impact of nicotine on spermatogenesis.^[27]

Despite the various harmful effects of smoking on male fertility, most male smokers are still fertile but have a higher risk of sub-fertility or infertility.^[28] Studies of natural conception in couples with a smoking male partner fail to demonstrate a significant reduction in fertility.^[29] While other studies indicated that smoking is associated with lower fertility rates, a higher risk in IVF results and adverse reproductive outcomes.^[30] The knowledge currently available indicates that the balance of Reactive oxygen species (ROS) and antioxidants in semen fluid, sperm and testes have vital role in maintaining proper sperm function of spermatozoa because it is highly susceptibility to oxidative damage. Increased ROS level will lead to DNA fragmentation and apoptosis causes the impairment of sperm function leading to lower male fertility.^[28] Nicotine and its metabolite cotinine have complicated effects in male reproduction system.

Conclusion

Tobacco consumption in young male population is noticeable health hazards. Significant reduced semen parameters in the subjects with habits of tobacco consumption are sperm concentration, sperm motility and sperm morphology. Amount of tobacco consumption has significant role in reduced semen parameters in tobacco smokers as well as in tobacco chewers. There is no significant difference with mode of consumption, so tobacco by any means may impacts on semen parameters and thus on male infertility and sub-fertility. Male infertility is conveniently evaluated with the careful semen analysis as per the latest WHO guidelines 2010, 5th edition. However there is insufficient insight into the underlying mechanisms of the observed deleterious effects. Further studies desirable to know whether tobacco effects on fertility is inherited to offspring or not by knowing epigenetic modification with molecular

studies to affirm the trans-generational effects of tobacco consumption. All modes of tobacco consumption are possibly effects on fertility and Public awareness should be made to limit the users in especially in young population.

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