



## Original Research Article

## Study of semen analysis in tobacco users

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## ABSTRACT

Infertility is defined as the inability of a sexually active, non-contracepting couple to achieve pregnancy in one year. In a developing country, like ours, where most societies are organized, the inability to bear children is a tragedy for the families and the conflux of personal, social and religious expectations brings a sense of failure, exclusion and loss who are infertile. So it becomes important to know about various risk factors associated with infertility, as awareness of male infertility is low in India.

**Aim:** To study sperm characteristics in tobacco users and non users undergoing infertility evaluation in a tertiary care hospital.

**Settings and Design:** It was a cross sectional study, done for one and a half years from January 2016-December 2017 in a tertiary hospital.

**Methods and Material:** After taking consent and detailed history, semen analysis according to WHO guidelines were done. Male patients who came for semen analysis for evaluation of their infertility were included. Amongst these semen parameters of tobacco users were compared with nonusers.

**Statistical analysis used:** The data was analyzed using SPSS-22

**Results:** We observed 102 cases for semen analysis, amongst them 46% samples were tobacco users and 54% samples were non-tobacco users. An increase in grade of tobacco usage was associated with a decrease in normal sperm parameters.

**Conclusions:** Fertility counsellors, clinicians need to be more focused to control male infertility by spreading the awareness of this addiction to enhance fertility potential.

**Key Messages:** We advice men to stop tobacco usage to improve fertility and their quality of life.

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## 1. Introduction

Infertility is defined as the inability of a sexually active, non-contracepting couple to achieve pregnancy in one year.<sup>1</sup> In a developing country, like ours, where most societies are organized, the inability to bear children is a tragedy for the families and the conflux of personal, social and religious expectations brings a sense of failure, exclusion and loss who are infertile.<sup>2</sup> So it becomes important to know about various risk factors associated with infertility as awareness of male infertility is low in India.

With the transforming of the 20th century to 21st, a cultural shift in society is identified, more westernization has occurred and therefore many culprit substances like tobacco are identified as they may negatively contribute to male reproductive system. Tobacco, biologically titled, *Nicotiana tabacum*, is the only legal drug that kills. It is estimated that tobacco use both as smoking and smokeless is currently responsible for six million deaths across the world each year.<sup>3</sup> In 2010, WHO estimated 13 % of India's population smokes. Among them 24% men and 3% women smoked while smokeless tobacco use is 32.9 % in men.<sup>4</sup>

Tobacco is the greatest cause of preventable disease. Regardless of how it is consumed, adverse effects are many

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like it is a risk factor for hypertension, stroke, myocardial infarction, gum recession, causes cancers of oral cavity, Larynx, pharynx, esophagus and lungs etc.<sup>5</sup> Also tobacco is addictive, leading to its continuous use despite the adverse effects.

To know about the effect of tobacco on male reproductive health, primary investigation, Semen analysis is done, as it is simple, cost effective, non-invasive, accurate and best investigation.<sup>6</sup>

Many studies have suggested that chronic smoke exposure positively correlated with lower semen parameters,<sup>7-9</sup> and advised stopping tobacco usage can improve the male infertility as these are preventable and reversible, knowing about its association with male fertility and discouraging its usage can lead to problem solving. Hence, it became imperative to conduct a study.

## 2. Aim and objectives of the study

1. To study sperm characteristics in tobacco users and non users undergoing infertility evaluation in a tertiary care hospital.
2. To grade tobacco users as mild, moderate and severe.
3. To compare the sperm characteristics between tobacco smokers and tobacco chewers.
4. To find out the association between grade of tobacco usage and sperm characteristics.

It was a cross sectional study, done for one and a half years from January 2016- December 2017 at a tertiary hospital.

The ethical clearance was obtained from the home institution.

### 2.1. Inclusion criteria

All patients in the age group of 21 to 55 yrs referred to the Department of pathology for Semen analysis for infertility evaluation in the study period.

### 2.2. Exclusion criteria

1. Cases who were not willing to give consent.
2. Cases with male genital anatomical anomalies.
3. Cases with systemic diseases eg. Varicocele, Hydrocoele, undescended testis, diabetes,
4. Hypertension, etc. affecting the fertility
5. Cases with a history of alcohol intake or any other drug usage.
6. Men below 21 years and over 55 years of age.

A detailed history of tobacco usage was entered in the proforma. An informed written and bilingual consent was obtained from the patients before including them in the study. The patients were explained about the procedure including its limitations and complications. Need of research and the role of their participation is explained to the patient.

## 2.3. Methodology

Male Patients who came for semen analysis for evaluation of their infertility were included. Need of research and the role of their participation was explained to the patient.

Written consent was taken. Questions were asked as per the questionnaire provided. Detailed history was asked. Detailed clinical information and period of abstinence was verified. Sample collection was done following the standard protocol. Clear instructions concerning the collection of the semen sample were given to patients emphasizing that the semen sample needed to be complete and that he should tell any loss of any fraction of the sample. The importance of sperm rich first part of semen and that any loss of it can lead to altered results, was explained to the patient. The sample obtained by masturbation was collected in the labelled, pre weighed, non toxic, sterile container.

After receiving the sample specimen is kept at 37 °C, room temperature, to avoid large changes in temperature that may affect the spermatozoa. Time of collection was noted. Thorough semen analysis was done as per the WHO guidelines.

The tobacco smokers were divided into mild, moderate and severe grades as follow-

Mild- 1 to 9 Cigarettes per day

Moderate-10 to 19 Cigarettes per day

Severe-More than 20 Cigarettes per day

The tobacco chewers were divided into mild, moderate and severe grade as follow

Mild- Tobacco chewing < 3 times/day

Moderate- 3 to 6 times/day

Severe- > 6 times/day

The data collected were statistically analyzed and observation tables were prepared.

Statistical analysis was done by using Microsoft word, excel and statistical package, SPSS-22. To analyze qualitative data, proportion and percentages, Z-test (Standard Error of Difference between two proportions) was applied, whereas to analyse quantitative, data, mean and standard deviations were obtained and Z-test (Standard Error of Difference between two means), ANOVA were applied.

## 3. Observation and Results

**Table 1:** Showing the distribution of total cases

Total cases	Tobacco users	Tobacco non users
102	47	55

Table 1 We observed a total 102 cases for semen analysis, and we found that out of 102 samples tobacco users were 46 % whereas tobacco non users were 54%. Amongst 46%, who were tobacco users we found that 55% of the people

used tobacco as smoking via any medium like cigarette, bidi, cigar or hookah, whereas 45% of the people used tobacco as chewing by means like gutka, pan masala, khaini etc.

**Table 2:** Showing Non Tobacco users with affected parameters

Parameters Affected of Non users	No. of person with affected parameters
Sperm count	1
motility	7
morphology	0
vitality	1

Table 2 Possible causes of affected parameters in non tobacco users could be any, other than tobacco usage.

**Table 3:** Showing grade wise distribution of Tobacco smokers and chewers

Tobacco smokers (21)			Tobacco chewers (26)		
Mild (18)	Moderate (3)	Severe (0)	Mild (16)	Moderate (8)	Severe (2)

Table 3 In smokers, no men were in the severe group.

**Table 4:** Grade wise affected and non-affected men in smokers and chewers

Semen Parameters	Tobacco Smokers (21)			Tobacco Chewers (26)		
	Mild (18)	Moderate (3)	Severe (0)	Mild (16)	Moderate (8)	Severe (2)
Affected	7	2	0	4	5	1
Not affected	11	1	0	12	3	1

Table 4 On grade wise distribution of smokers, men in the moderate smokers group were more affected than those in the mild smokers group. The p value was not significant in sperm count, motility and vitality, but significant in morphology.

On grade wise distribution of the Chewers, the values of parameters were lowest in the severe group, than in moderate and high in the mild group. This showed that with increased grade of chewing, the values of sperm parameters decreased.

**Table 5:** Showing grade wise distribution of smokers with affected parameters

Parameter Affected of Smokers	Mild	Moderate
Count	3	1
Motility	5	1
Morphology	0	0
Vitality	1	0

Table 5 We found 3 men with affected sperm count in the mild group and 1 in the moderate group. Similarly, we found

5 men in the mild group and 1 in the moderate group with affected sperm motility. Also, we found 1 man with affected vitality in mild group

However, in the present study, we did not find any men in a severe group as per the laid criteria.

**Table 6:** Showing grade wise distribution of chewers with affected parameters

Parameter Affected in chewers	Mild	Moderate	Severe
Count	2	5	1
Motility	3	1	1
Morphology	0	0	0
Vitality	2	1	0

Table 6 We did not find any men in who the sperm morphology was affected.

**Table 7:** Showing Sperm parameters affected in number of tobacco smokers and chewers

Sperm Parameters Affected	No. of Affected Tobacco smokers	No. of Affected Tobacco chewers
Count ( $\geq 15$ millions/ ml)	4	8
Motility ( $\geq 32\%$ )	7	5
Morphology ( $\geq 4\%$ )	0	0
Vitality ( $\geq 58\%$ )	3	2

Table 7 Study was performed relating to affected parameters of tobacco users distributed as smokers and chewers. Parameters studied were sperm count, motility, morphology and vitality.

Among 14 tobacco smokers,

1. 4 men had a count  $\geq 15$  millions/ml.
2. 7 men had a motility  $\geq 32\%$ .
3. 3 men had a vitality  $\geq 58\%$ .

Among 15 tobacco chewers,

1. 8 men had a sperm count  $\geq 15$  millions/ml.
2. 5 men had motility  $\geq 32\%$ .
3. 2 men had vitality  $\geq 58\%$ .

Morphology was not affected in either group (i.e, smokers and chewers).

Table 8 On comparing the values of sperm concentrations amongst tobacco users and non users, more tobacco users were affected. Tobacco users are affected more in prospect of sperm progressive motility.

Sperm morphology was found to be within normal limits in both users and nonusers.

On comparing tobacco users and non users, more tobacco users were affected with low vitality percentage, and only

**Table 8:** Showing distribution of affected sperm parameters amongst Tobacco users and Non users.

Sperm Parameters	Groups of users and non users		Total
	Non users (n) %	Users (n) %	
Sperm count (<15million/ml)	(01)7.7%	(12)92.3%	(13)100%
Motility (<32%)	(8)40.00%	(12)60%	(20)100%
Morphology ( $\geq 4\%$ )	(55)53.9%	(47)46.10%	(102)100%
Vitality (<58 %)	(4)33.3%	(8)66.8%	(12)100%

few tobacco users had normal vitality. Though, the results were not significant.

**Table 9:** Showing distribution of affected sperm parameters amongst tobacco smokers and chewers

Sperm parameters	Groups of smokers and chewers		Total
	Smokers (n) %	Chewers (n) %	
Sperm count (<15million/ml)	(4)33.3%	(8)66.7%	(12)100%
Motility (<32%)	(7)58.3%	(5)41.70%	(12)100%
Morphology ( $\geq 4\%$ )	(21)44.7%	(26)55.3%	(47)100%
Vitality (<58 %)	(3)37.5%	(5)62.5%	(8)100%

Table 9 Amongst smokers, 33.30% had a decreased sperm concentration, while 48.6% had a normal sperm concentration. Amongst chewers, 66.70% had a decreased sperm concentration, while 51.4% had a normal sperm concentration. Clearly tobacco chewing had affected sperm concentration values than smoking.

We observed that sperm motility was affected in 58.30% of smokers and 41.70% of chewers. Though this shows that smokers were more affected than chewers, the p-value 0.27 was not significant.

Amongst 39 men with normal vitality, 21 (53.80%) were chewers, and 18 (46.20%) were smokers, chewers were seen to be more affected than smokers, but the difference was not significant.

Table 10 When the sperm parameters were compared according to the grade of tobacco usage a significant difference was found. In the mild group 34 men (33.3%) were there, in moderate group 11 (10.8 %) men and in severe group 2 (2%) men were included. When sperm concentration was observed in mild, moderate and severe groups, a significant grade based relationship was found. In mild group mean  $\pm$  SD was  $49.61 \pm 34.44$ , in moderate  $18.63 \pm 11.59$  and in severe group values of mean  $\pm$  SD was  $9 \pm 9.89$ . P value was 0.008 which was significant. Hence we found a decrease in mean  $\pm$  SD from severe group to mild group thus showing a significant grade based relationship.

**Table 10:** Mean, Standard deviations and p value of semen parameters in Tobacco Smokers and Chewers

Group Statistics	Groups of smoker and chewer	No.	Mean	Standard Deviation	p value
Count	Smoker	21	43.7143	31.17073	0.569
	Chewer	26	38.1538	35.14165	
Vitality	Smoker	21	70.8571	19.07691	0.253
	Chewer	26	64.4231	18.77908	
Motility	Smoker	21	50.4762	25.24405	0.293
	Chewer	26	43.6538	16.40474	
Morphology	Smoker	21	89.0476	10.48559	0.344
	Chewer	26	85.5385	14.61296	

When mean  $\pm$  SD was compared for sperm progressive motility. It was found that in the mild group it was  $51.02 \pm 21.45$ ,  $37.27 \pm 13.48$  in moderate and  $25 \pm 21.21$  in the severe group. P value was 0.049 hence showing a significant association in grade of tobacco usage and decrease of progressive sperm motility, More the grade, lesser was the sperm progressive motility. Similarly, when the percentage of normal morphology of spermatozoa was compared in mild, moderate and severe groups, we found mean  $\pm$  SD values as  $91.61 \pm 8.45$  in the mild group,  $79 \pm 11.80$  in moderate and  $55 \pm 21$  in the severe group. So we observed that the percentage of normal sperm morphology was highest in the mild group, higher in the moderate group than in the severe group. P value is 0.000. When the vitality was compared in all the three groups, the mean  $\pm$  SD was  $70.97 \pm 18.16$  in the mild group,  $61.81 \pm 12.50$  in the moderate group and  $35 \pm 35.35$  in the severe group. P value was 0.015. Thus we observed that there is a significant relationship between the grade and sperm vitality. Higher the grade lesser was the percentage of sperm vitality.

Concluding, we observed a significant difference in sperm parameters between tobacco users and non users. Users were more affected.

Tobacco chewers were more affected than smokers.

And there was an inverse relationship between grade of tobacco usage and sperm parameters.

#### 4. Discussion

Male infertility plays a key role in conception difficulties of upto 40 % infertile couples. Although the effect of cigarette smoking on sperm function was noticed a long time ago, the mechanism by which tobacco affects sperms remains poorly understood. And thus, the impact of tobacco use on male infertility and sperm characteristics remains a highly controversial issue. Many studies have examined the effect of tobacco usage on male fertility and showed a negative effect on various sperm characteristics.<sup>7,9</sup>

#### 4.1. Tobacco Smokers discussion

In our present study of differences between semen parameters of smokers (n =21, 20.6 %) and non smokers (n =55; 53.9 %). We found that the mean  $\pm$  SD of sperm count in smokers was  $43.71 \pm 31.17$ , of progressive sperm motility was  $50.48 \pm 25.24$ . We observed that sperm concentration and sperm motility were low in smokers.

Our findings were similar to Ansari et al and Lingappa et al. They refereed the values of semen parameters as per WHO manual, 2010 for the examination and processing of human semen. They observed that both sperm count and motility were decreased in cigarette smokers, as seen in our study.

In our study, along with sperm concentration and sperm motility, normal morphology was also found to be lower in smokers than non-smokers, though it was within the normal standard range of 4% as per WHO, 2010, Mean and SD of morphology was  $89.05 \pm 10.49$ .

Similar findings were obtained by Davar et al.<sup>11</sup> Their values of mean and SD of sperm concentration of smokers were  $86.7 \pm 41.06$ , mean motility values were  $72.3 \pm 71.4$  and morphology values were  $47.4 \pm 15.6$ . They concluded that sperm concentration, sperm motility and normal sperm morphology were lower in smokers, similar to our study. He also concluded that sperm concentration, sperm motility and normal sperm morphology were lower in smokers.

In our study the fourth parameter sperm vitality was also low in smokers along with sperm concentration, sperm motility and morphology. Mean  $\pm$  SD of sperm vitality was  $70.86 \pm 19.08$  in smokers. Similar findings were seen in a study of Kunzle et al<sup>15</sup>. For the semen variables values they referred to WHO1992. Mean and SD values for vitality in smokers was  $43.3 \pm 19.8$ . Also values for sperm density was  $67.7 \pm 65.9$ , Progressive motility  $37.1 \pm 18.6$ , normal morphology forms  $21.2 \pm 14.6$ . They found that all four sperm parameters were low in smokers as seen in the findings of our study.

In our study, we also found that sperm parameters were affected more with increase in grade of tobacco. Out of 21 smokers, 18 were included in the mild group and 3 were included in the moderate smoking group. However, we did not find any smoker who fulfilled the criteria of a severe smoking group in our study.

In a study by Collodel et al,<sup>13</sup> the sperm concentration showed not much significant differences between the groups unlike in our study. However, sperm progressive motility was significantly affected in smokers in their study similar to our findings in our study. Also, they found sperm concentration was low in heavy smokers than in the group of mild and moderate smokers, thus consistent with our findings. In our study we also found low sperm concentration in moderate groups than mild smokers.

Gaur et al,<sup>7</sup> categorized smokers on the grade basis. Total count and active sperms were significantly lower in smokers

similar to observations in our study. As well as, like in our study with increase in grade of smoking there was decrease in normal values of sperm parameters.

Trummer et al<sup>14</sup> He observed sperm concentration and motility was lower in smokers as found in our study. However he did not find much difference in morphology unlike in our study. Significant differences were found in his study when grades of tobacco were compared similar to our study.

Renu Jain et al,<sup>16</sup> Meri et al,<sup>17</sup> divided the smokers into heavy and non heavy smokers. They found an inverse relationship of grade and values of sperm parameters like ours.

Two studies, Aghamohammadi et al,<sup>18</sup> and Chohan R et al,<sup>19</sup> revealed that there were no differences in the semen parameters amongst smokers and non-smokers.

#### 4.2. Tobacco Chewers discussion

In a study by Phatale et al,<sup>20</sup> when sperm count and sperm motility were compared amongst the groups, they found that as compared to controls, smokers and chewers were affected, however they found smokers were more affected than chewers. However their findings were not significant. In our study we found that chewers were more affected. The possible reason could be less sample size in our study.

Ketan et al,<sup>22</sup> and Parmar et al,<sup>21</sup> found an inverse dose related relationship with increase in grade of tobacco, decrease in sperm parameters occurred. We obtained similar results in our study. In the study by Ketan et al,<sup>22</sup> the mean and SD values of sperm characteristics in their study of sperm concentration  $49.96 \pm 11.08$ , sperm motility  $56.48 \pm 9.57$  and sperm viability  $57.45 \pm 10.90$ . In our study values of sperm concentration  $38.15 \pm 35.14$ , sperm motility  $43.65 \pm 16.40$  and sperm vitality  $64.42 \pm 18.77$ .

Parmar et al,<sup>21</sup> found mean and SD values for sperm concentration, sperm motility and sperm viability as  $45 \pm 7.03$ ,  $4.86 \pm 10.69$  and  $52.64 \pm 7.85$  respectively. Similarly, in our study mean values were  $38.15 \pm 35.14$  for sperm concentration,  $43.65 \pm 16.40$  for sperm motility and  $64.42 \pm 18.77$  for vitality.

Sunanda et al,<sup>23</sup> also divided the tobacco chewers according to the intensity of tobacco chewing. Sperm parameters were evaluated according to WHO 1999 and they found a slight lowering of sperm count, motility and vitality in chewers, however morphology was significantly affected in their study. In our study sperm count, sperm motility and vitality were significantly affected but there was a slight decrease in normal morphological forms but was not significant.

Said et al,<sup>24</sup> divided the chewers as mild, moderate and severe. He found that when values of sperm parameters were significantly lower in a severe group and as the grade of usage decreased, sperm parameter values increased, as seen

**Table 11:**

Study	Smokers	Controls (non-smokers)	Main outcome
Our Study	21	55	Decreased sperm count, motility, vitality and morphology unaffected.
Jaldahaq M et al <sup>10</sup>	51	54	Decreased total sperm count and active sperm count.
Ansari et al <sup>9</sup>	117	28	Decreased sperm motility and sperm count.
Davar et al <sup>11</sup>	53	98	No significant decrease in sperm parameters.
Lingappa et al <sup>12</sup>	103	20	Decreased sperm count and motility
Gaur et al <sup>7</sup>	100	100	Decreased total sperm count and active sperm count.
Collodel et al <sup>13</sup>	118	153	Decreased sperm motility and morphology. Sperm concentration is significantly low in heavy smokers.
Trummer et al <sup>14</sup>	478	517	Decreased sperm count and motility.
Kunzle et al <sup>15</sup>	655	1131	Decreased sperm count, motility, vitality and morphology.
Ramlau et al <sup>8</sup>	1052	1490	Decreased sperm concentration and motility.

**Table 12:**

Study	Tobacco chewers	Control (non-tobacco users)	Main outcomes
Our study	26	55	Decreased sperm count, motility, vitality and morphology was unaffected.
Phatale et al <sup>20</sup>	25	25	Decreased sperm count and motility.
Parmar et al <sup>21</sup>	50	50	Decreased sperm concentration, motility and vitality.
Ketan et al <sup>22</sup>	95	50	Decreased sperm concentration, motility and vitality.
Sunanda et al <sup>23</sup>	194	448	Morphology was significantly affected but the sperm count and motility were unaffected.

in our study.

In our study we did not include the duration of tobacco usage and status of ex-smokers, due to the limited time period for this study. However, this limitation can be overcome by including them in further study and longer duration of study.

## 5. Conclusion

Most men with no proven cause for infertility are tobacco users. Seminal parameters are decreased affecting sperm count, sperm motility, sperm vitality and sperm morphology. Further, with increase in grade of tobacco usage, more decrease in normal sperm parameters occurs. In this regard, fertility counsellors, clinicians need to be more focused to control male infertility by spreading the awareness of this addiction to enhance fertility potential.

Therefore, we advice men to stop tobacco usage to improve fertility and their quality of life.

## 6. Summary

We conducted a study of semen parameters to find out the association of tobacco usage by men and infertility in them. Both smokers and chewers were included in the study. Total 102 men were enrolled in the study, among them 26 were chewers, 21 were smokers and 55 were non tobacco users. Further, we divided tobacco users on a grade basis in mild, moderate and heavy tobacco usage groups. We studied semen parameters like Sperm count, Progressive sperm motility ,sperm vitality and sperm morphology. Latest WHO 2010 was used for reference for semen analysis .We found that sperm parameters like sperm count, motility and vitality were affected more in tobacco users than non users. However, sperm morphology was within normal limits in both groups as per WHO semen examination. Similarly, when chewers and smokers were compared, we found sperm parameters were affected more in chewers than smokers. We also found that in mild tobacco usage groups, sperm parameters were affected less when compared to moderate and severe groups and with increase in grade more decrease in values of sperm parameters was noted. So, we found a

definitive relationship between tobacco usage and infertility. Also, an inverse grade based relationship was appreciated. Therefore we advise men to stop tobacco usage in any form to maintain optimal fertility.

## 7. Source of Funding

None.

## 8. Conflict of Interest

None.

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