Effect of Tobacco Consumption On Sperm Morphology and Semen Quality

Verma Rahul[*], Tripathi Shambhavi [*], Gaur Dushyant S [^], Kusum Anuradha[^], Harsh Meena [#]

[*]Post Graduate Resident,
[^]Professor,
[#] Professor & Head of Department.
Department of Pathology, Himalayan Institute of Medical Sciences, SRH University,
Swami Ram Nagar, Jolly Grant, Dehradun 248140, Uttarakhand

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***Abstract*- Introduction:**

Male fertility is affected by a variety of lifestyle habits that include tobacco use. Tobacco consumption, both by chewing and smoking, has been recognized as an important lifestyle factor that contributes to abnormalities in sperm morphology (teratozoospermia).

Objective:

1) To analyze semen samples as per WHO guidelines.
2) To study the pattern of Teratozoospermia amongst Tobacco smoker and tobacco-chewer males

Material and Methods:

Out of 220 males whose semen samples were analyzed, 30 controls (strict non-tobacco users), 30 pure smokers (non tobacco chewers), and 30 pure tobacco tobacco-chewers (as per non smokers) were selected, using strict exclusion criteria. Semen samples were analyzed WHO guidelines. Sperm morphology was studied in papanicolaou-stained smears. Defects of Head, Mid-piece, tail and Cytoplasmic droplet were observed in 200 spermatozoa for each case.

Results

In comparison to control group, Teratozoospermia and Asthenozoospermia were the most common findings in semen of males addicted to tobacco smoking and tobacco tobacco-chewing. Morphological defects were observed in 70.5% spermatozoa of Tobacco-smokers and 71.4% spermatozoa of Tobacco-chewers. Furthermore, head defects were most common morphological abnormality seen in both tobacco-smokers (63%) and tobacco-chewers (69.5%); while tail defects (28.5%) were more common in tobacco-chewers, where most common tail defect observed was in the form of coiled tail. Data was statistically analyzed.

Conclusion:

Lifestyle factors like tobacco smoking and tobacco tobacco-chewing show significant negative impact on sperm morphology as shown by statistically significant result analysis

***Index Terms*-** Head defect; coiled tail defect; asthenozoospermia; teratozoospermia; sperm deformity index; tobacco consumption.

I. INTRODUCTION

The quest for solutions to infertility is ages old. The social and biological importance of being a parent has been acknowledged by every culture in every historical period [1]. Infertility occurs in approximately 30% of all human couples; 30% of these have a predominant male factor, while another 20% have both male and female factors. While it is estimated that roughly 15% of men are infertile, the number of males who are sub fertile could be much greater [2].Tobacco consumption, both by chewing and smoking, has been recognized as an important lifestyle factor that contributes to abnormalities in sperm morphology (teratozoospermia). In India, a manufactured smokeless tobacco product, 'gutka', has been targeted towards youth and has become extremely popular. The situation is further aggravated when these habits of tobacco chewing and smoking co-exist [3]. The detection of nicotine, and its major metabolite cotinine, in the seminal plasma showed that the tobacco compounds cross the blood-testis barrier and create a toxic environment for the

spermatozoa. Toxic components in the cigarette smoke can disrupt the testicular microcirculation and cause DNA or chromosomal damage in germinal cells. The Tobacco components and combustion products also reduce semen volume, sperm concentration, motility and normal morphology [4].The present study was undertaken to establish the effect of tobacco consumption on sperm morphology of male partners of infertile couples seeking treatment in our Institute.

II. MATERIALS AND METHODS

An analytical cross-sectional study was carried out in Department of Pathology, Himalayan Institute of Medical Sciences, Dehradun, where, over a period of twelve months, 220 males were referred for semen analysis. A careful detailed history of smoking, alcohol, tobacco chewing, occupation, any chronic illnesses, long term medications and environment was taken, and physical examination was done. Each sample was collected in the Andrology laboratory by masturbation, in a clean, wide mouthed plastic container, non-toxic to the spermatozoa, from cases with strict abstinence of 2 - 7 days. Universal precautions were taken in handling the specimen. All semen samples were analyzed manually following strictly the criteria given by WHO, immediately after liquefaction or within one hour of ejaculation. An Objective Scoring was done for all cases, from which Control and Study Groups were selected.

Exclusion Criteria

Following males were not considered while selecting cases for the Control and Study Groups:

- Azoospermics
- Alcoholics
- Ex-alcoholics, ex-smokers, ex-tobacco chewers
- Cases with history of prolonged medication
- Cases with history of intake of herbal medicines / herbal tonics for infertility
- Cases with history of occupational exposure to various chemicals or extremes of temperature
- Cases with history of injury to testes, varicocele, hydrocele, undescended testes, vasectomy surgeries
- Cases with history of pyospermia, hemospermia, chronic urinary tract infection
- Cases with history of Secondary Infertility
- Males above 45 years of age

Control and Study Groups:

Group A (n=30) **= Controls** who were Strict non tobacco chewer or smokers with Normozoospermia

Study Groups:

Group B (n=30) = **Tobacco Smokers** who were strict non tobacco chewers

Group C (n=30) = **Tobacco chewers** who were strict non tobaacco smokers

Sample Rejection

Samples were rejected in following cases:

1. Coital abstinence of more or less than 2-7 days
2. Incomplete collection of sample
3. Samples exposed to extremes of temperature
4. Sample collected at home or elsewhere
5. Use of condom for collection of sample

Semen Analysis as per WHO guidelines:

Each semen sample was observed for Liquefaction time, Volume, Viscosity, Particulate matter and Sperm agglutination.

Initial Microscopic Examination

A wet smear was prepared after complete liquefaction, by putting a drop between glass slide and coverslip, to get a rough estimate of the concentration, motility, presence of agglutination of spermatozoa and presence of cellular elements other than spermatozoa.

Assessment of Sperm Motility

A minimum of 200 spermatozoa were studied. Their motility was categorized under four grades according to WHO. Specimen showing less than 25% Grade A spermatozoa was diagnosed as Asthenozoospermia (A).

Observation	Interpretation
Grade (A)	Rapid, Linear, Progressive
Grade (B)	Sluggish, Linear, Progressive
Grade (C)	Non Progressive
Grade (D)	Immotile

Assessment of sperm concentration

The concentration of spermatozoa was determined using an improved Neubauer's chamber. A 1:20 dilution of liquefied semen with semen dilution fluid (containing Sodium bicarbonate and aqueous gentian violet) was prepared and charged in improved Neubauer's chamber for counting spermatozoa. Calculation: N × V × D × 1000 = million sperms / ml. (N = Number of sperm in 1sq. mm area; V = Volume - 1^2 ×1/10 mm; D = Dilution factor -20). Sperm concentration below 20 million/ml was diagnosed as Oligozoospermia (O).

Assessment of sperm morphology

Smears were stained with Papanicolau's stain and mounted with DPX and glass coverslip; studied with a 100X oil immersion bright field objective and 10X eyepiece; counting 200 consecutive spermatozoa. Specimen with less than 35% normal sperm morphology was diagnosed as Teratozoospermia (T).

Agglutination

Agglutination of spermatozoa can be head to head, tail to tail, or head to tail. The presence of agglutination was taken as suggestive of an immunological cause of infertility. The adherence of immotile spermatozoa to each other, motile spermatozoa to mucous threads, cells other than spermatozoa or debris was considered to be nonspecific aggregation.

Sperm vitality

Sperm vitality was observed after a 1:1 mix of liquefied semen and aqueous 2% Eosin stain were mixed and one drop was placed on glass slide under coverslip. Percentage of spermatozoa that took up stain were considered 'dead' while colorless were recorded as viable.

Objective Scoring System

Semen sample was studied for various semen variables and an Objective scoring was done. A score of 0, 1 and 2 was given as follows:

Table1:

Score	**2**	**1**	**0**
Liquefaction (minutes)	20 to 30	35 to 45	>45
Volume (ml)	1.5 to 4.5	4.5 to 5	>5
Viscosity	Normal	Moderate	High
Particulate matter	Nil to mild	Moderate	High
Agglutination	Nil	5 to 10%	> 10%
Motility (Rapid Progressive motion – GRADE A)	>25%	10 to 20%	<10%
Vitality (%)	>60	40 to 60	< 40
Sperm count ($x10^6$/ml)	>20	10 to 20	<10
Normal Morphology (%)	>35	30 to 35	<30
Headless (%)	<20	15 to 20	>20

A total of individual scores was done and interpreted as follows:

Score : 15-20 = Fertile; 10-14 = Subfertile; <10 = Infertile (Score 19-20 = Normozoospermia)

Classification of sperm morphology

Spermatozoon was considered normal when: Sperm Head, Midpiece, and Tail were intact; Head was oval; acrosomal region comprised of 40-70% of the head; Midpiece was less than 1 micrometer in width, and attached axially to the head; Tail was straight, uniform, and thinner than the midpiece, uncoiled and approximately 45 micrometer long; no cytoplasmic droplet seen. The following categories of defects were noted:

Head defects: a) Pyriform head, b) Large head**,** c) Small head, d) Pin head**,** e) Amorphous head**,** f) Vacuolated head g) Small acrosome h) No acrosome

Midpiece defects: a) Bent neck**,** b) Asymmetrical insertion into the head**,** c) Thick midpiece**,** d) Thin midpiece

Tail defects: a) Coiled tail**,** b) Short tail**,** c) Multiple tails**,** d) Bent tail

Cytoplasmic droplet

Diagnosis

A diagnosis of Oligozoospermia (O), Asthenozoospermia (A) and Teratozoospermia (T) was assigned to each semen sample, depending on the values of three major sperm variables: Sperm Motility, Sperm Count and Sperm Morphology below the recommended WHO range.

Calculation of Sperm deformity index (SDI):

$$SDI = \frac{\text{Total defect (head + midpiece + tail defect + cytoplasmic droplet)}}{\text{Total no. of sperm counted (200)}}$$

Data Management and Statistical Analysis:

Observed results were interpreted and analyzed using SPSS software (Statistical Package for Social Services) version 17. *P* value was calculated using paired-t test.

III. RESULTS

Of the 90 cases analyzed, maximum number of cases were in the age group of 26-35 years (64.5%) followed by 16-25 years (21%).Frequency of three semen variables: Oligozoospermia, Asthenozoospermia and Teratozoospermia was measured amongst Group B and C, excluding Group A (Controls).(Figure 1).

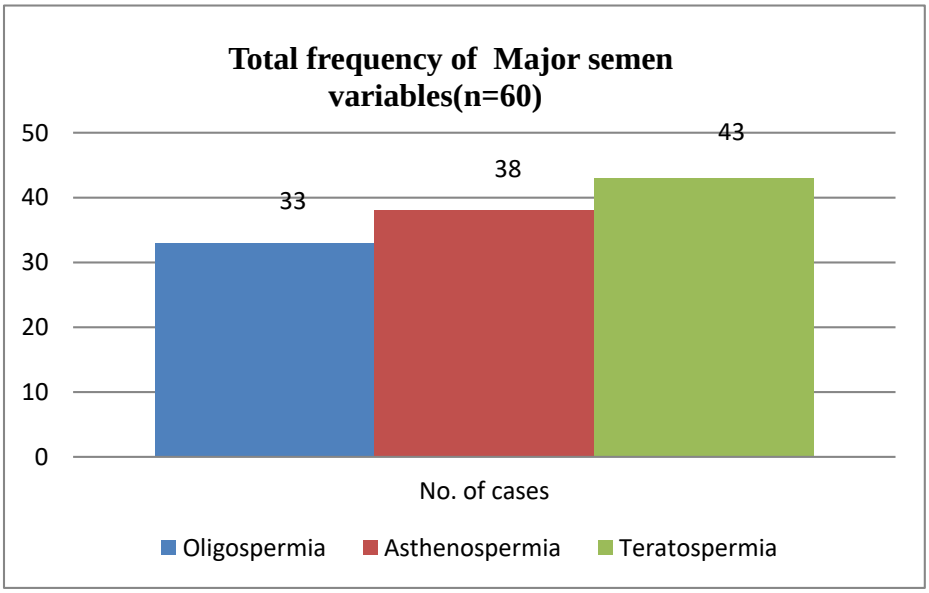


Figure: 1

*Figure 1 shows the total frequency of three major semen variables. Teratozoospermia was found to be the most common abnormality (n=43/60, 72.22 %), followed by Asthenozoospermia (n=38/60, 63.8 %), and Oligozoospermia (n=33/60, 55.5%). The normozoospermic controls (Group A, n=30/90) were not considered.

Semen variables were compared in Group B (Tobacco smokers) and Group C (Tobacco chewers) (Figure 2). We observed that in Group B Teratozoospermia was the most frequent finding (n=19/30) and in Group C Asthenozoospermia was most frequent (n=21/30).

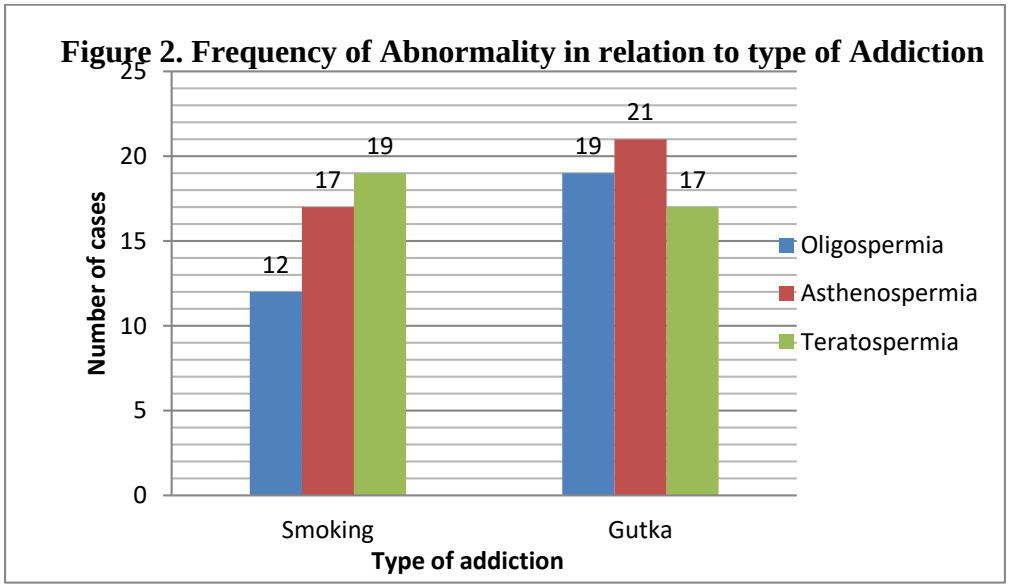


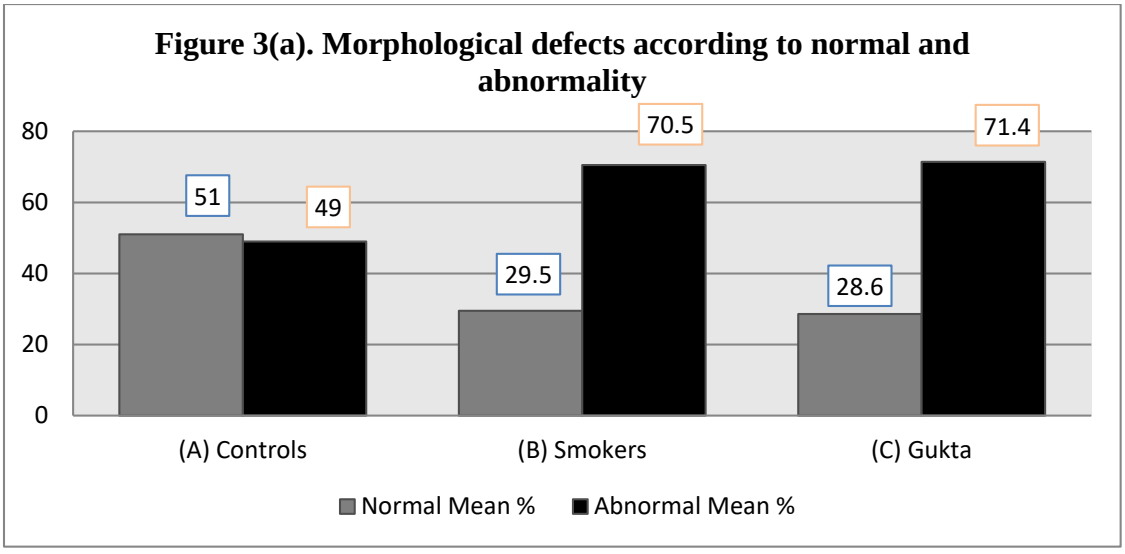
*Figure 2 shows the frequency of Semen variables in Group B and Group C. Asthenozoospermia was the most predominant finding in case with tobacco chewers (n=21/30) while Teratozoospermia was more predominant in tobacco smokers (n=19/30). Controls (n=30) were not included in the analysis.

Table 2: Morphological changes according to Addiction

Groups	Normal Mean %	Abnormal Mean %	Morphological Defects			
			Head %	Mid piece %	Tail %	Cyto %
(A) Controls	51	49	41	21	16	2
(B) Smokers	29.5	70.5	63	31.5	23	3.3
(C) Gukta	28.6	71.4	69.5	36.5	28.5	4.5

*Table 2 shows that in Group A (controls), the mean of normal spermatozoa was 51% while mean of morphologically abnormal was 49%, morphological abnormalities being in form of head defect, mid piece defect, tail defect and cytoplasmic droplet. In group B (smokers), the mean morphologically abnormal sperm were 70.5%, followed by group C (tobacco) with morphological defect in 71.4% cases [Figure 3(a)].

Amongst Group B and C, the head defects was much more (63-69.5%) compared to control group A (41%). Individually, highest tail defects (28.5%) were seen in tobacco chewers. [Figure 3(b)]



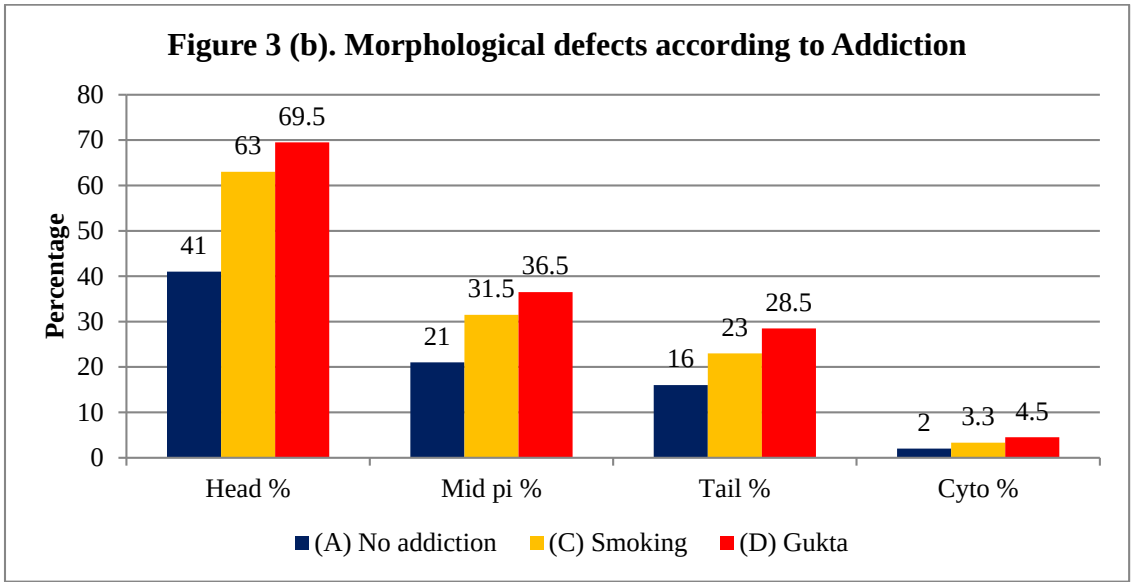


Table 3: Comparison of Control group (A) with Tobacco smokers (B)

Morphological Defects	A (n=30) (Mean ±SD)	B (n=30) (Mean ±SD)	*P* value	Statistical Significance
Head defects				
Pyriform	9.53±5.606	14.27±9.634	0.394	
Round	10.73±6.389	14.57±9.258	0.839	
Large	3.6±2.634	5.5±4.622	0.218	
Small	21.6±9.357	35.87±16.414	**0.002**	**Significant**
Pin	5.13±4.232	9.2±7.078	0.255	
Amorphous	13.8±8.364	20.07±12.454	0.275	
Vacuolated	10.63±5.75	15±7.991	0.336	
Small acrosome	5.5±3.214	9.4±5.992	**0.031**	**Significant**
No acrosome	1.8±1.827	2.57±2.473	1	
Mid-piece defects				
Bent	10.83±5.038	16.43±8.386	**0.021**	**Significant**
Asymmetrical	8.47±4.265	12.97±9.076	0.82	
Thick	19.47±9.878	28.83±10.151	**0.05**	**Significant**
Thin	3.43±3.148	5.1±3.854	1	
Tail defects				
Coiled	11.37±5.404	18.13±9.111	0.591	
Short	4.47±3.014	6.17±4.893	1	
Multiple	4.43±3.875	4.7±3.098	1	
Bent	12.5±6.252	17.53±9.659	1.18	
Cytoplasmic Droplet	4.43±3.692	6.37±4.303	1	

***P* value <0.05 = Significant**

Table 3 shows Comparison of Control group (A) with Tobacco smokers (B). Considering individual sperm morphological defects, head defects like small head and small acrosome, thick mid piece defects and bent mid piece defects were found to be statistically significant in Group B.

Table 4: Comparison of Control group (A) with Tobacco Chewers (C)

Morphological Defects	A (n=30) (Mean ±SD)	C (n=30) (Mean ±SD)	*P* value	Statistical Significance
Head defects				
Pyriform	9.53±5.606	15.87±9.573	**0.046**	**Significant**
Round	10.73±6.389	17.5±6.585	**0.012**	**Significant**
Large	3.6±2.634	6±3.62	**0.032**	**Significant**

Small	21.6±9.357	36.63±15.307	**0.001**	**Significant**
Pin	5.13±4.232	11.03±5.804	**0.009**	**Significant**
Amorphous	13.8±8.364	25.17±12.242	**0.0001**	**Significant**
Vacuolated	10.63±5.75	14.23±6.611	0.88	
Small acrosome	5.5±3.214	9.3±4.276	**0.04**	**Significant**
No acrosome	1.8±1.827	3.17±1.949	0.192	
Mid-piece defects				
Bent	10.83±5.038	20.28±8.643	**0.0001**	**Significant**
Asymmetrical	8.47±4.265	14.77±5.532	**0.002**	**Significant**
Thick	19.47±9.878	32.77±13.723	**0.001**	**Significant**
Thin	3.43±3.148	6.03±4.013	0.211	
Tail defects				
Coiled	11.37±5.404	22.03±18.635	**0.019**	**Significant**
Short	4.47±3.014	8.03±4.03	**0.02**	**Significant**
Multiple	4.43±3.875	6.37±3.996	0.811	
Bent	12.5±6.252	19.9±7.136	**0.004**	**Significant**
Cytoplasmic Droplet	4.43±3.692	8.47±3.821	**0.005**	**Significant**

***P* value <0.05 = Significant**

Table 4 shows Comparison of Control group (A) with Tobacco chewers (C). Considering individual sperm morphological defects, all the above mentioned defects were statistically significant other then vacuolated head defect, no acrosome, thin mid piece and multiple tail defects in Group C.

Table 5: Sperm deformity index (SDI) in Controls (A) and Study Groups (B & C)

Group	Number of cases	Min	Max	(Mean ±SD)	*P* –value in comparison to group A
A	30	0.3	2.1	0.837±0.3810	-
B	30	0.4	2.2	1.21±0.4708	0.03
C	30	0.7	2.2	1.38±0.3854	0.0001

**P* value <0.05 is statistically significant
†SDI = Total sperm defects / Total number of sperms.
Cut off range of SDI= 1.6

Table 5 show SDI in different groups (A, B and C). It was observed that the mean value of SDI was higher in both the groups (B and C) in comparison to Control group (A) and was statistically significant for both.

IV. Discussion

Infertility is one of the most tragic of all marital problems. Despite recent advances in the treatment of infertility, the problem could not be satisfactorily tackled so far, for varied reasons. Unfortunately, infertility is a social stigma in our society. Environment, coupled with a person's lifestyle, produces a major impact on the physical and mental health, including his or her reproductive health. The impact of these factors on an individual varies with the person's age and inherent qualities inherited genetically and other acquired factors. Of the various lifestyle patterns, three voluntary habits that have been shown to be detrimental to human reproductive health are tobacco smoking, tobacco chewing (gutka) and alcohol intake. In the present study we tried to correlate the impact of these two life style factors (Cigarette smoking and tobacco) on sperm morphology.

Out of total 220 males Group A of 30 Normozoospermics was taken as Control, with which Group B (n=30) of Tobacco smokers and Group C (n=30) were compared. Majority of cases in these three groups were in the age group of 26-35 years (n=58/90, 64.5%) followed by those in 16-25 years age group (19/90, 21%). Our observations were in accordance with the global trend as reported by various other studies [5].

Of the diagnosis of three semen variables Oligozoospermia, Teratozoospermia and Asthenozoospermia (sperm count, morphology and motility), sperm morphology can be the most informative semen parameter in differentiating between fertile and infertile semen samples. However, none of these measures, alone or in combination can be considered diagnostic of infertility. Biological evidence of male infertility is only present in cases of azoospermia or globozoospermia or in the presence of a complete lack of sperm motility with underlying genetic deficiencies such as Kartagener's syndrome [6].In the Control group (A) all selected cases showed Normozoospermia i.e where all three semen variables O, A and T were within normal limits as per WHO guidelines. We tried to summate the overall

frequency of these variables in study groups (Figure 1). Accordingly, teratoozoospermia (T) was the most frequently found variable (n=43/60, 72.22 %); Asthenozoospermia (A) was the second most frequent semen variable (n=38/60, 63.8 %) while oligozoospermia (O) was present in 55.5 % cases (n=33/60). As such, oligozoospermia has been shown to have variable cutoff values ranging from the one proposed by WHO (20 million/ml) to even as low as less than 5 million/ml by various researchers, hence losing its edge so far as its value as a predictor of fertility in concerned [6].

On correlating the three major semen variables (O, A and T) with the type of addiction, we found that asthenozoospermia was most common finding in subject addicted to tobacco chewing (n=21/30) and teratozoospermia was more predominant in tobacoo smokers (n=19/30).The toxins in cigarette reach male reproductive organ, interact with seminal fluid components and the accessory glands leading to increased viscosity, reduced seminal volume and delay the liquefaction time, and hence reducing forward linear progression of spermatozoa, manifesting as asthenozoospermia. Exposure of spermatozoa to the toxins in cigarette smoke probably tilts the delicate balance of reactive oxygen species (ROS) that are produced by spermatozoa for their special functions like decapitation. Increased quantities of ROS have been shown to be detrimental to the DNA of spermatozoa, thus producing a negative effect on the viability and morphology of spermatozoa.[7] In tobacco chewers, nicotine is absorbed through oral mucosa three to four times faster than smoking and residual chemicals remain in circulation for a prolong period than in smokers. Concentration of nicotine and other substance in tobacco in epididymis disturbs its normal functioning particularly the activity of alpha-1, 4 glycosidase enzyme. This inhibits secondary maturation of spermatozoa contributing to teratozoospermia. More over nicotine initiates huge ROS production leading to oxidative stress which causes DNA damage and contributes to teratozoospermia and oligozoospermia [8]. Experiments using murine models have documented severe inflammatory reaction and marked ultrastructural changes in the testes of animals exposed to nicotine. In addition, levels of nicotine and its major metabolites cotinine and trans-3=-hydroxycotinine in human seminal plasma negatively correlated with sperm parameters reported by Said TM et al [3].

We further studied the morphological changes associated with the type of addiction (Smoking and Tobacco) in detail (Table 1, Figure 3a, 3b). We observed that the mean value of normal and abnormal spermatozoa in group A (controls), were 51% and 49% respectively. Both group B (Tobacco smokers) and group C (tobacco chewer) showed abnormal morphology in much higher percentage of cases (70.5% to 71.4%) in comparison to Controls (A). Similar observations were made by several other observers.(3,9,10)

In Table 1, we further observed that in group A (controls), 41% defects were seen in head of sperm, 21% in mid piece, 16% defects in tail and 2% in cytoplasmic droplet. By contrast in group B (smokers), 63% defects are seen in head, mid piece defect was seen in 31.5% of sperms, 23% defects were in tail and 3.3% in form of cytoplasmic droplet. Similarly group C (tobacco) showed 69.5% sperms with head defects and very high tail defect (28.5%).

We further compared the various morphological defects of head, mid piece and tail in both group B and C with control group A in order to further analyze our observation (Table 2,3). Group B (smokers) when compared with group A control (Table 2) showed that head defect, namely small head and small acrosome, thick mid piece defects and bent mid piece defects were found to be statistically significant. Although group B (smokers) showed higher number of teratozoospermic spermatozoa, the number of defects per spermatozoon was much more in tobacco chewers (C). Further, Group C (Tobacco Chewer) showed very severe teratozoospermia covering the whole spectrum of morphological defect of head, mid piece and tail, most of which were statistically significant. (Table 3)Tobacco chewers have been found to have teratozoospermia as well as reduced sperm count and motility in several studies (11). Nicotine and other carcinogenic compounds in tobacoo have been correlated with acrosomal defect, bent mid piece and coiled tail, all of which were found to be in high number in our study particularly coiled tail, which was highest amongst all groups with *p* value of 0.019. This has been attributed to abnormal development of Golgi pro-acrosome vesicles and their improper attachment to sperm nuclei and other less understood processes [8].

V. CONCLUSION

Lifestyle factors like cigarette smoking and tobacco chewing do produce significant negative impact on semen quality. Individuals being treated for infertility should abstain from these largely avoidable addictive habits – Tobacco smoking and tobacco chewing, as they have significant negative impact on sperm morphology, as shown by statistical analysis. Head defects were most commonly seen in males addicted to both tobacco smoke and chewing, while higher tail defect in the form of coiled tail were more frequent in tobacco chewers. Sperm deformity index (SDI), a useful tool to detect the prevalence of teratozoospermia and sperm DNA damage amongst sub fertile men, was also found to be higher amongst Tobacco chewers in comparison to Tobacco smokers.

Abbreviations:

WHO World Health Organization
DNA Deoxyribonucleic acid
O Oligozoospermia
A Asthenozoospermia
T Teratozoospermia

SDI Sperm deformity index

The Authors declare no conflicts of interest.

REFERENCES

[1] Gaur DS, Talekar MS, Pathak V. Alcohol intake and cigarette smoking: Impact of two major lifestyle factors on male fertility. Indian J PatholMicrobiol. 2010; 53:35-40.

[2] Jequier AM. Male infertility: A guide for the clinician. Oxford; Blackwell Science; 2002.

[3] Tamer M. Said, Ranga G, Agarwal A. Relationship between semen quality and tobacco chewing in men undergoing infertility evaluation. Fertility and Sterility. 2005; 84(3): 649-53.

[4] Chohan KR, Badawy SZA. Cigarette smoking impairs Sperm bioenergetics. International Braz J Urol. 2010; 36(1): 60-5.

[5] Grace M. Centola, Kenneth A. Ginsburg. Evaluation and Treatment of the Infertile Male. Cambridge: Cambridge University Press; 1996.

[6] Ombelet W, Bosmans E, Janssen M, Cox A, Vlasselaer J, Gyselaers W et al. Semen parameters in a fertile versus subfertile population: a need for change in the interpretation of semen testing. Hum Reprod. 1997; 12(5):987-93.

[7] Elizabeth AA, Kalaiselvi VS,Prabu K,Krishna P, PrasanaVenkatash E. Impact of Cigarette Smoking and Alcohol Intake on Male Infertility. International Journal Of Pharma and Bioscience. 2013; 4 (4):513-8.

[8] Sunanda P, Panda B, Dash C, Ray PK, Padhy RN, Routray P. Prevalence of abnormal spermatozoa in tobacco chewing sub-fertile males. J Hum Reprod Sci. 2014; 7:136-42.

[9] Guo H, Zhang HG, Xue BG, Sha YW, Liu Y, Liu RZ. Effects of cigarette, alcohol consumption and sauna on sperm morphology. Zhonghua Nan KeXue. 2006; 12(3): 215-7.

[10] Kumar R, Gautam G. Tobacco chewing and male infertility. Indian J Urol. 2006; 22: 161-2.

[11] El-Melegy NT, Ali M. Apoptotic markers in semen of infertile men: Association with cigarette smoking. IntBraz J Urol. 2011; 37: 495-506

AUTHORS

First Author – Dr.Rahul Verma, Post Graduate Resident, Department of Pathology, Himalayan Institute of Medical Sciences, SRH University, Swami Ram Nagar, Jolly Grant, Dehradun 248140, Uttarakhand,Mobile: 9971831328, E- mail: rahulsrhu@gmail.com
Second Author – Tripathi Shambhavi , Post Graduate Resident, Department of Pathology, Himalayan Institute of Medical Sciences, SRH University, Swami Ram Nagar, Jolly Grant, Dehradun 248140, Uttarakhand
Third Author – Gaur Dushyant S, Professor, Department of Pathology, Himalayan Institute of Medical Sciences, SRH University, Swami Ram Nagar, Jolly Grant, Dehradun 248140, Uttarakhand
Fourth Author – Kusum Anuradha, Professor, Department of Pathology, Himalayan Institute of Medical Sciences, SRH University, Swami Ram Nagar, Jolly Grant, Dehradun 248140, Uttarakhand
Fifth Author – Harsh Meena, Professor & Head of Department, Department of Pathology, Himalayan Institute of Medical Sciences, SRH University, Swami Ram Nagar, Jolly Grant, Dehradun 248140, Uttarakhand