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SEMEN CHARACTERISTICS OF TOBACCO USERS IN INDIA

A. BANERJEE, A. PAKRASHI, S. CHATTERJEE, S. GHOSH, and S. K. DUTTA

Qualitative analysis of semen samples have been compared between 79 different types of tobacco addicts (smokers, chewers, and multiple addicts) with 21 nonaddicts (never consumed any form of tobacco). The percentage of motile sperm and total sperm count of the tobacco chewers are significantly low ($p < .05$). The frequency of abnormal sperm is also significantly high ($p < .001$) for smoking and multiple addict groups. Differential effects of smoking and chewing tobacco on sperm characteristics are discussed.

Key Words: Tobacco addicts; Sperm motility; Sperm count.

INTRODUCTION

Tissue that turns over rapidly, including sperm produced by the testis, may be particularly sensitive to the mutagenic and carcinogenic materials found in cigarette smoke [22]. The principal criteria for alterations in sperm quality, such as density, motility, and morphological features, in response to cigarette smoking have been reported [11, 15, 17). Oldereid et al. [14] has found no significant difference in any aspect of sperm quality as affected by smoking. Dixit et al. [4] has also reported the quality of semen analysis between chewing and smoking tobacco and a nonaddict group which showed insignificant changes in sperm characteristics.

Cigarettes marketed in India have high tar (19–27 mg/cig) and high nicotine (1.00–1.4 mg/cig) yields. There is no difference in the amount of tar and nicotine delivered in filtered and nonfiltered cigarettes marketed in India [21]. In addition to cigarettes, 50% of the total population consume other popular forms of tobacco, both smoking and nonsmoking products. The end-products formed during the use of these tobacco products include polynuclear aromatic hydrocarbons from burning tobacco; chewing and other nonsmoking forms produce nicotine and nitrosamines in water-soluble fraction [9, 18]. The principal objective of this study is to analyze the quality and morphology of spermatozoa from men addicted to different forms of Indian tobacco, especially the chewing type.

From the Reproductive Biology Research Division, Indian Institute of Chemical Biology, 4 Raja S.C. Mullick Road, Calcutta 700 032, India; and the Department of Pathology, Post Graduate Institute for Basic Medicine, University of Calcutta, Calcutta, India.

Address correspondence to Dr. A Pakrashi, Reproductive Biology Research Division, Indian Institute of Chemical Biology, 4 Raja S.C. Mullick Road, Calcutta 700 032, India.

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MATERIALS AND METHODS

Semen samples were obtained over a 1 year period from 165 men aged 27–44 years receiving a seminopathological examination at the clinical laboratory in Calcutta. Each ejaculate was collected (after 3 days of abstinence) by masturbation in a sterile plastic container and was allowed to liquefy at room temperature. All men completed a questionnaire when they submitted samples for routine analysis of semen. Questions were asked about tobacco habits, use of alcohol and medicines, and history of diseases and operations.

All semen samples were interpreted and classified by the same person, who was unaware of the patients tobacco habits. Parameters included were ejaculate volume, sperm density, total sperm count, percentage of motile sperm, percentage of sperm with morphologically abnormal heads, midpieces, and tails, and total percentage of abnormal sperm. One hundred spermatozoa on each slide were classified for sperm morphology. Analysis of these data has been carried out following WHO protocol. Samples were excluded from further analysis if there was a history of systemic disorders, genital tract infections, operations, varicocele, drug or hormone treatment, exposure to radiation, heavy drinking, or sperm density below 1 million/mL. Altogether 65 samples were eliminated, leaving 100 men for final analysis. Of these, 21 were non-tobacco addicts, 29 were tobacco chewers (nonsmoking form), 40 were smokers, and 8 were addicted to multiple tobacco habits. We defined *nonaddicts* as individual healthy males who never smoked or consumed any form of tobacco. Nonaddicts were not occupationally exposed, had a good socioeconomic status, and did not have a history of diseases that may cause reproductive disability. The individuals with azoospermia and asthenozoospermia with known disease history were eliminated. *Smokers* were defined as those who had smoked only cigarettes of commercial brands for at least 10 years. *Chewers* were individuals who had chewed tobacco such as zarda for at least 10 years. *Multiple addicts* were those who consumed more than one form of tobacco, such as those having smoking + chewing + snuffing habits; this group normally consumed a very high quantity of tobacco.

Types of tobacco were classified as follows: *Cigarette* tobacco of urbanized common type prepared from commercially roasted tobacco contains 1 g tobacco/cigarette. *Bidi* consists of 0.5 g sundried and cured tobacco flakes hand rolled into a rectangular piece of dried leaf of *Diospyros* sp. This traditional form of smoking is practiced mainly in rural parts of India among men and women. Commercial forms are also available in urban areas and are more popular among the low income group. Bidis have been shown to deliver high tar (more than 23 mg) and nicotine levels (1.7–3 mg). *Zarda* is the common chewing form of tobacco available in rural and urban areas. These are bits of tobacco leaves sundried and combined with different flavors and additives which are used with betel leaf. An average of 5 mg of zarda flakes is consumed per dose (mean value obtained by individual survey).

Statistical analysis was performed by one-way analysis of variance (ANOVA). A probability level of $p < .05$ was considered statistically different in case of ANOVA test. All statistical calculations were done using a statistical package, Epistat (Epistat Services, 2011 Caprock Circle, Richardson, TX 75080-3417).

RESULTS

All semen samples showed similar volume, pH, and liquefaction time. In the three tobacco addict groups, the density and total count of spermatozoa in ejaculates were significantly lower among chewers than nonaddicts and other tobacco users. Comparison of tobacco addicts with nonaddicts showed that there was a significant difference between sperm density, motility, and count (Table 1).

The chewing group had a more significant change in the sperm density and total count than all other groups ($p < .05$). Motility was also lowered ($p < .05$). Among the tobacco smokers the density and motility of the sperm was not significantly lower than that of the control group.

TABLE 1 Average Semen Characteristics of Tobacco Users and Controls

	Tobacco Users D means ± (SE)	Controls D means ± (SE)
Number of patients	79	21
Age (in years)	34.30 ± 1.12	36.18 ± 3.56
Semen volume (mL)	3.03 ± 0.38 (0.043)[b]	3.2 ± 0.22 (0.05)[a]
Sperm density ($\times 10^6$/mL)	34.16 ± 7.45 (0.84)[b]	48.43 ± 15.93 (3.47)[a]
Total sperm count ($\times 10^6$)	110.55 ± 23.19 (2.61)[b]	155 ± 50.00 (11.12)[a]
Motility*	53.84 ± 10.68 (1.20)[b]	62.36 ± 9.17 (2.00)[a]
Abnormality	7.0 ± 2.63 (0.30)[b]	2.57 ± 0.54 (0.12)[a]

*Examined 1 h after ejaculation.
[a,b]Values with different superscripts differ significantly at the 5% level.
Values in parentheses indicate SE.

Total sperm count, however was altered to a significant level. The degree of abnormal sperm was higher ($p < .001$) in this group compared to the control group and the chewing group (Fig. 1). The total % of morphological abnormalities were mainly dose dependent. Since the multiple addiction group had the highest consumption dose (25 g/day), the % of abnormality was highest in this group. Count and sperm density was also low in this group (Table 2).

The abnormalities were mainly of the pyriform head (60%). Small head, pin head, and large head types of sperm were also found in a low frequency. Only one chewing case showed tail aberration in the sperm. Midpiece aberration, cytoplasmic droplet, and double head were very few in number. In general, types of aberration were not related to the type of tobacco habit.

DISCUSSION

Tobacco contains many hazardous chemicals in both smoking and nonsmoking forms. These compounds may show much stronger mutagenic action on male gonads than on egg cells because the spermatogenesis continues over the whole male reproductive period [17]. These effects are manifested with alteration in the count, motility, and morphology in different degrees in different addicted groups. Heavy smoking reduces plasma testosterone concentrations, but levels rise if smoking is stopped [2]. The reduced biosynthesis of testosterone is due to the inhibition of Leydig cell microsomal hydroxylase by carbon monoxide present in cigarette smoke. Karagounis et al. [10] and Mann and Lutwak-Mann [13] suggested a similar pathway for microsomal inhibition of cytochrome P450 by carbon monoxide. A reduction in testosterone concentration may result in disturbed spermatogenesis, but the level must be severely lowered before appreciable effects on sperm production occur [3]. Tissues including sperm, may be particularly sensitive to the mutagenic and carcinogenic materials found in cigarette smoke. Smokers have increased numbers of sister chromatid exchanges in peripheral lymphocytes [12, 16] and increased numbers of covalent DNA adducts in placentas [6] compared with nonsmokers.

Spermatogenesis may be also directly affected by the toxic effect of nicotine on testicular tissue [10]. Our results with chewing tobacco addicts also confirm these effects. Since nicotine and aromatic compounds are water soluble at room temperature, the consumption of nonsmok-

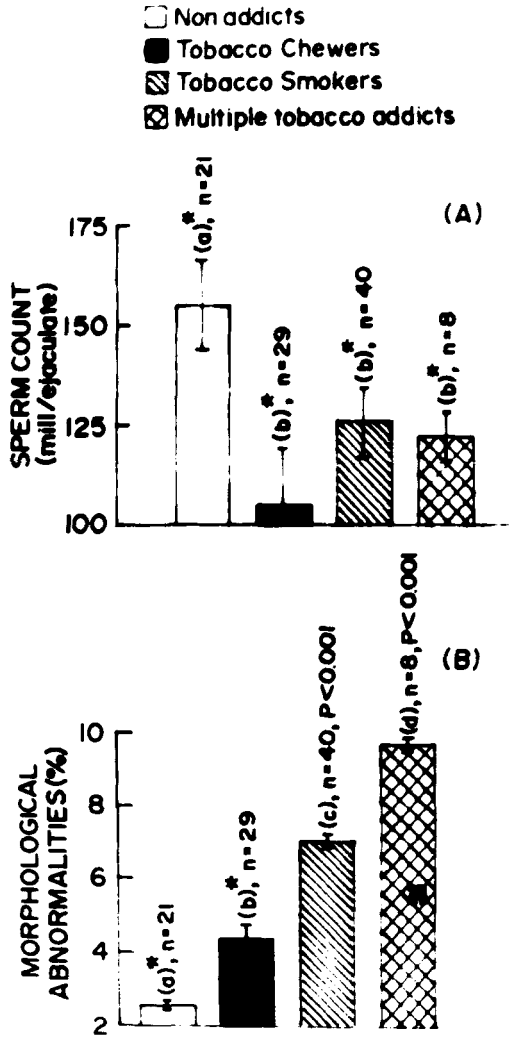

FIGURE 1 The total sperm count (A) and morphological abnormalities (B) in nonaddicts and addicts in relation to different types of tobacco habits. *Bar with different superscripts within parenthesis differ significantly at the 5% level. *n* = number of cases studied.

ing forms of tobacco results in the intake of nicotine, and a group of nitrosoamine compounds known to be mutagenic [8] directly affects spermatogenesis. Low density and count of spermatozoa due to the effect of nicotine may cause mutagenic spermatogonial alteration or genetic damage [5].

In our multiple-addict group, high quality of tobacco consumption with more than 25 years of heavy smoking may have an adverse effect on spermatogenesis. Both qualitative and quantitative changes (count and motility) of the sperm in this group may be due to the primary

TABLE 2 Semen Quality, Sperm Count, Motility, and Morphology in Nonaddicts and Addicts in Relation to Different Types of Tobacco Habits

Types	*n*	Dose Range* (g)	Duration Range (yr)	Volume (mL) (mean ± SE)	Sperm Density** ($\times 10^6$/mL) (mean ± SE)	Sperm Count ($\times 10^6$/ ejaculate) (mean ± SE)	Motility (%) (mean ± SE)	Total Morphological Abnormalities (%) (mean ± SE)
Nonaddicts	21	—	—	3.07 ± 0.08	48.43 ± 3.47	155 ± 11.12^a	62.36 ± 2.00^a	2.57 ± 0.12^a
Tobacco chewers	29	0.2–1	5–20	3.27 ± 0.11	41.16 ± 4.32	105 ± 14.20^b	40.57 ± 5.46^b	4.35 ± 0.42^b
Tobacco smokers	40	2–25	4–15	3.34 ± 0.21	46.11 ± 2.29	125.39 ± 18.65^b	61.73 ± 1.96^a	7.03 ± 0.18^c
Multiple tobacco addicts	8	5–25	10–25	3.13 ± 0.10	40.21 ± 6.63	121.83 ± 6.00^b	63.42 ± 2.60^a	9.62 ± 0.18^d

*As calculated from actual human consumption.

**Examined 1 h after ejaculation.

^{a,b,c,d}Values with different superscripts differ significantly at the 5% level.

disturbances in the spermatogenesis and sperm maturation caused by hemodynamic changes in the testis and epididymis as a result of heavy smoking [7]. The mutagenic alterations of the spermatogonial cells due to genetic damage caused by toxic chemicals of tobacco [5] is also another cause associated with these changes.

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