Original Article

Smoking-induced satellite associations in a rural population of south India: An *in vitro* study

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Abstract

Background: Genotoxic carcinogens in cigarette smoke interact with DNA, causing cytotoxicity. Cytogenetic damage therefore seems to be an excellent biomarker for determining the effect of exposure to chromosome-damaging agents in cigarette smoke. **Purpose:** To study the utility of measurement of frequency of satellite associations (SA) as a biomarker for chromosomal damage using cytogenetic assay in peripheral blood lymphocytes. **Materials and Methods:** This study was conducted on 30 smokers and 30 nonsmokers drawn from a rural population of South India. Smokers were divided into three groups of ten each based on their smoking index (SI) (group I: SI < 150, group II: SI 150–300, and group III: SI > 300) and the frequency of SAs was studied. **Results:** The frequency of SAs was significantly greater in smokers than in nonsmokers and the frequency of SAs among the smokers was also seen to increase with increase in SI. **Conclusion:** The results of this study indicate that the genotoxic effect of cigarette smoke on chromosomes increases with smoking intensity. These findings can be used to support smoking cessation interventions.

Key words: Chromosome damage, genotoxicity, satellite associations, smoking index

Introduction

Tobacco use is one of the greatest public health threats the world has faced. Smoking causes a variety of disabilities in man. It kills more than five million people a year – an average of one person every 6 seconds – and is responsible for one in ten adult deaths. More than 80% of the one billion smokers worldwide live in low- and middle-income countries, where the burden of tobacco-related illness and death is heaviest.[1] In India, smoking kills 900000 people every year and unless corrective action is taken that number will soon increase to more than 1 million, indicating that urgent measures are needed to address the problem of tobacco abuse.

The smoker is exposed to a wide variety of genotoxic carcinogens present in cigarette smoke, making it necessary to analyze the cells at metaphase as these can be a health hazard to the future generations.[2] Genotoxic carcinogens in cigarette smoke interact with and alter the DNA molecule, causing cytotoxicity. Cytogenetic damage therefore seems to be an excellent biomarker for determining the effect of exposure to the chromosome-damaging agents in smoke.[3] In support of this hypothesis, an increased frequency of chromosome breaks has been demonstrated to be an initial event in carcinogenesis, suggesting that measurement of these alterations may be useful for assessing oncogenic risk.

The last three decades have witnessed the introduction of a number of relatively rapid genetic tests for detecting the activity of mutagenic and/or carcinogenic chemicals. Satellite chromosomes in associations, appears to be one of the most suitable test to assess the effect of cigarette smoke on chromosomes. Satellite associations (SAs) reflect chromosomal damage and may thus provide a biomarker of early-stage carcinogenesis.

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The phenomena of SA, where satellite chromosomes assume a specific position with their satellites directed towards each other, was first observed in mitotic human chromosomes[4,5] and was later also found in meiotic chromosomes. All 'D' and 'G' group chromosomes (except 'Y' chromosomes) have satellites in their 'p' arm. The formation of SA has often been attributed to the involvement of satellite chromosomes in nucleolar formation. The sticky nucleolar material has a tendency to hold the associated chromosomes together through mitosis.[6] The fusion of two or more nucleoli tends to stretch the nucleolar-forming chromosome segment mechanically, with obvious risk of breakage. Breaks may occur in more than one of the chromosomes involved, and proximity of the broken ends would predispose to translocations and the SA would thus be active also in the origin of translocation between satellite chromosomes.

A high incidence of SA has often been considered to predispose to an increased tendency toward nondisjunction in satellite chromosomes and to lead to the induction of D (trisomy 13 or Patau syndrome) and G (trisomy 21 or Down syndrome) trisomies. Several workers have reported evidence of an increased acrocentric association in the mothers of children with Down syndrome.[7] Higher frequency of SA was seen in test mothers using oral contraceptives than in control mothers, among couples who had had an abortion, and in XXY Klinefelter and XO Turner syndrome,[8] suggesting that drug intake and chromosomal anomalies predispose to satellite associations. An increased incidence of SA, as well as decreased mitotic index and chromosomal aberrations, have been reported in smokers.[2,9,10] Smokers engaged in occupations like farming and workers in industries exposed to variety of chemicals have been shown to have chromosomal damage in somatic cells.[11–13] Chromosomal damage has also been reported in *bidi* and *hookah* smokers.[14,15] The addition of antioxidants to the diet of the smokers was found to minimize the genotoxicity of the mutagenic agents present in tobacco smoke.[16]

The present *in vitro* cytogenetic study was conducted to investigate the frequency of SA in smokers and nonsmokers. All subjects were from a rural area and had an agricultural background, all were males, all had rice as their staple diet, and all were from the lower socioeconomic strata.

Materials and Methods

This observational study included 30 male smokers attending a tertiary care hospital; all were from a rural area in South India. The 30 subjects were divided into three groups of 10 each based on their smoking index (SI), as follows: group I: SI < 150, group II: SI 150–300, and group III: SI > 300. SI was calculated using the formula given by Srinivasan *et al*.[17]:

SI = Numbers of cigarettes/*bidis*/cigars per day × total duration of smoking in years

An equal number of age-, sex-, diet- and social status–matched nonsmokers formed the control group for the study. None of the individuals were habituated to alcohol or drugs. Ex-smokers and smokers with chromosomal anomalies were excluded from the study. Informed written consent was taken from all participants. The study was designed in accordance with the Helsinki II declaration and approved by the Institutional Human Ethical Committee.

Lymphocyte cultures were set up from heparinized blood, with minor modification of the method of Hungerford,[18] by the addition of chilled fixative at the end of hypotonic treatment. For each person, 50 well-spread metaphase plates, stained with 4% buffered Giemsa, were analyzed for SAs. SAs involve a specific arrangement of acrocentric chromosomes of 'D' group (chromosomes 13, 14, and 15) and 'G' group (chromosomes 21 and 22) with their satellites directed towards each other [Figure 1]. For identifying SAs, the criteria used by Hansson were applied;[7] i.e., the satellite ends of the associating chromosomes had to be directed towards each other with their longitudinal axes meeting between their short arms, and the distance between the centromeres of associated chromosomes should not exceed the total length of one 'G' chromosome after excluding its satellite. Odds ratios and the Student's *t*-test were used for statistical analysis.

Results

The mean age of the subjects was 38.25, 40, 40.75, and 39.67 years in smoker groups I, II, and III, and in nonsmokers, respectively. The mean frequency of SAs in nonsmokers, calculated using the SI formula, was 29.83 ± 2.88 [Figure 1];

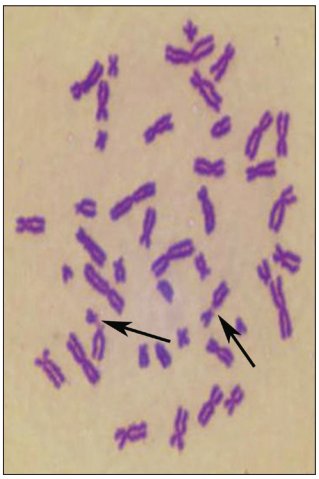

Figure 1: Photomicrograph showing satellite associations between the 'DG' group chromosomes in nonsmokers

whereas the mean frequency of SAs in smokers belonging to group I, II, and III was 44.25 ± 3.30, 53 ± 3.46, and 67 ± 6.22, respectively [Figures 2–4]. The frequency of SAs in smoker groups I, II, and III was higher than in the nonsmokers and, moreover, SA was seen to increase with increase in the SI [Table 1].

The odds for SAs was greater in smokers as compared to nonsmokers and, among the three smoker groups, the odds increased with increase in SI. The Student's *t*-test also showed that the differences in SAs between the nonsmoker group and the three smoker groups were highly significant ($P<.001$) [Table 2].

Discussion

Tobacco-related cancer is a common and lethal malignancy. The role of tobacco smoking in the etiology of cancer disease has been known for many decades, and any approach aimed at expediting the detection of population subgroups at increased risk should be assigned high priority. It may be possible to use genotoxicity assays to identify those subgroups of smokers that are more susceptible to the DNA-damaging effect of cigarette smoke and/or to determine the level of smoking that produces significant increases in mutation rates over baseline. Many of the substances contained in cigarette smoke are genotoxic and therefore cytogenetic damage seems to be an excellent biomarker for determining the effect of exposure to chromosome-damaging agents in smoke.

Smokers engaged in different occupations (like farming and industry) with exposure to a variety of chemicals have shown a higher frequency of chromosomal damage in somatic cells than nonsmoking controls working in the same occupations.[11–13] Increases in the frequencies SAs have been reported with exposure to cigarette smoke,[9,10] *bidi* smoke,[14] and *hookah* smoke.[15] The present report confirms these findings.

The analysis of SAs has gained popularity as an *in vitro* genotoxicity test and a biomarker assay in humans for genotoxic exposure and effect, as the scoring of SA is relatively simple, requires only a short training, and is not very time consuming. A number of studies have been designed to evaluate the potential influence of factors such as gender, age, or smoking habit on SA frequency. Many of these studies suffer from a poor assessment of exposure, with subjects being often roughly classified as smokers *vs* nonsmokers, without consideration of the levels of cigarette consumption. The status of those who have stopped smoking has been even more confusing and 'former smokers' are sometimes included along with 'current smokers' and sometimes with 'nonsmokers.' Proper planning of the study to elicit high-quality, reliable, information regarding the individual's smoking habit and possible confounders such as occupational exposures is essential to understand the value of SAs as a marker of exposure/effect on chromosomes.

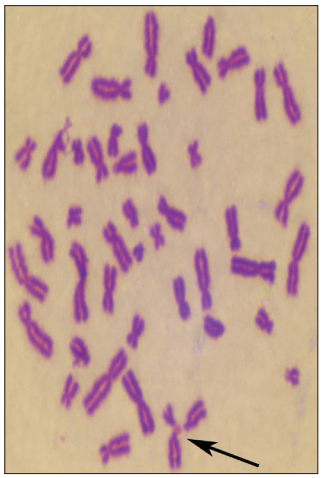

Figure 2: Photomicrograph showing satellite associations between the 'DDG' group chromosomes in smoker group I

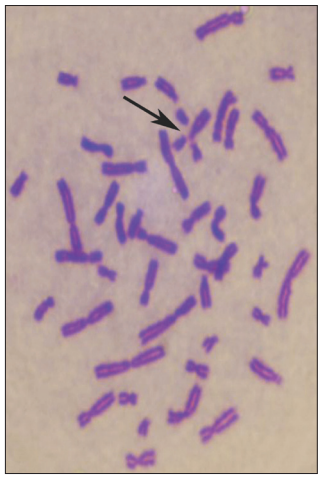

Figure 3: Photomicrograph showing satellite associations between the 'DGGG' group chromosomes in smoker group II

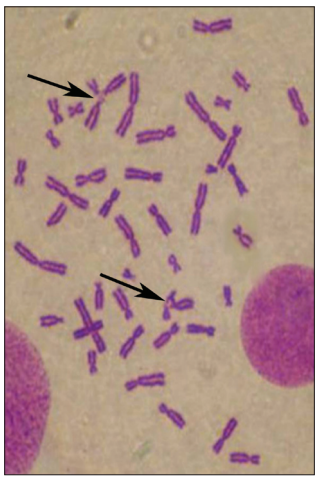

Figure 4: Photomicrograph showing satellite associations between the 'DDG' and 'DGG' group chromosomes in smoker group III

Table 1: Satellite associations in smokers and nonsmokers

Group	Mean age (in years)	Mean number of smoking years	Mean number of cigarettes smoked	SI	SA (mean ± SD)
Group I	38.25	16	8.75	135.75	44.25 ± 3.30
Group II	40	17.25	16.5	224	53 ± 3.46
Group III	40.75	20	23.75	460	67 ± 6.22
Nonsmokers	39.67	-	-	-	29.83 ± 2.88

SI: Smoking index; SA: Satellite associations

Table 2: Odds ratios and Student's 't' test

Group	SA in control (mean ± SD)	SA in smokers (mean ± SD)	Odds ratio (95% CI)	*t* test	*P* value
Group I	29.50 (29.5 ± 1.00)	45 (44.25 ± 3.30)	1 (0.52 to 1.93)	8.54	<.001
Group II	29 (29 ± 4.16)	53 (53 ± 3.46)	1.12 (0.63 to 2.29)	8.86	<.001
Group III	31 (31 ± 2.58)	67 (67 ± 6.22)	1.42 (0.75 to 2.66)	10.69	<.001

SA: Satellite associations; CI: Confidence interval

Keeping the above criteria in mind, in the present study, all the subjects – smokers and nonsmokers – were selected from among the population of a small village; the subjects had the same occupational background, were of the same sex and around same age (40 years), were all from a low socioeconomic strata; we also ensured that the smokers were all exclusively active smokers. In this study there was a higher frequency of SAs in smokers than in the control (nonsmoker) group. Among the smokers, the frequency of SAs increased with increase in SI, indicating clearly that the chromosomal changes are correlated to the smoking intensity. These results are similar to the findings of Yadav *et al*.[13]

A dose-response effect was also evident, with a steady increase in the odds ratio with increase in SI among the three smoker groups; this clearly demonstrates that there is an increase in the susceptibility to DNA-damaging effect with increase in SI. The '*P*' values of the three smoking groups were highly significant compared to their nonsmoking counterpart, indicating that smokers are exposed to more ill health outcome from tobacco smoking.

All the smokers and nonsmokers in our study were agricultural laborers and were exposed to a variety of chemicals, e.g., fertilizers and pesticide sprays. It has been shown that, among subjects exposed to the same occupational hazards, chromosomal damage is more in smokers than in nonsmokers.[12] This synergism also probably contributed to the increased frequency of SAs in the smokers in our study.

In conclusion, our study clearly indicates that all smokers should be considered to be at high risk of developing neoplastic disease. This study indicates that the genotoxic effects demonstrated in the lymphocytes of smokers are most likely caused by cigarette smoke constituents. This scientific evidence can be used in support of national campaigns to prevent tobacco consumption and for devising intervention strategies to reduce the morbidity and mortality from smoking-related cancer.

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