

FREQUENCY OF SATELLITE ASSOCIATIONS IN SMOKERS AND NON SMOKERS- A CHROMOSOMAL STUDY

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ABSTRACT

Cigarette smoking exposes individuals to numerous carcinogenic and genotoxic substances that can induce DNA damage and chromosomal instability. The frequency of satellite associations (SA) among acrocentric chromosomes (D- and G-groups) serves as a cytogenetic biomarker for assessing tobacco-induced chromosomal damage. This comparative cytogenetic study evaluated peripheral-blood lymphocyte cultures from 30 adult male cigarette smokers and 30 matched non-smoker controls. Smokers were subdivided into three equal categories based on their smoking index (SI): Category I (SI ≤ 150), Category II (SI 150–300), and Category III (SI ≥ 300). Fifty well-spread metaphases per participant (totalling 500 per smoker category and 1,500 across controls) were examined to score total satellite associations (SA), including D–G (DG), G–G (GG), and D–D (DD) associations. Mean satellite-association frequencies increased progressively with higher smoking index categories, recording 14.0 in Category I, 24.0 in Category II, and 34.0 in Category III, compared to matched control means ranging between 7.0 and 7.5. Student's t-test confirmed statistically significant differences between smokers and controls across all categories ($p = 0.0005$ for Category I; $p < 0.0001$ for Categories II and III). DG associations were identified as the most frequent component in Categories I and II. A preliminary follow-up of two participants who ceased smoking for 1.5 years showed a descriptive decrease in SA frequency. Satellite association frequency is significantly higher in cigarette smokers than in non-smokers and exhibits a dose-dependent increase corresponding to the smoking index. These findings suggest that satellite association holds potential value as a cytogenetic biomonitoring parameter for evaluating tobacco-associated genomic effects, though larger longitudinal studies are warranted.

KEYWORDS: Cigarette smoking, Satellite associations, Cytogenetic Smoking index, Chromosomal damage.

1. INTRODUCTION

Tobacco, one of the greatest disease producing products is the creation of man that nurtured a bad habit in the form of cigarette smoking. Tobacco has been hailed as a gift from the gods, a miraculous cure for all life's physical ills, a solace to the soldier or sailor, but tobacco smoking has become the largest cause of preventable Death around the globe. The majority of tobacco products are consumed in the form of cigarette that is reported to contain more than 4000 added chemicals which are carcinogens and tobacco smoke itself is carcinogenic. Today about 25-33% of adult male and female smokes cigarettes^{1, 2}. According to the survey smoking kills 900,000 people per year in India, unless the government takes a stringent action and awareness among the smokers the number may further increase. WHO estimation showed that about 5 million people die prematurely due to smoking^{3,4}.

Cigarette smoking is the major cause of cancer, cardiovascular diseases, premature death, impotency, erectile dysfunction, yet people continue to smoke. Even passive smoking can cause some health problem and respirator disorder⁵. Young children exposed to smoking resulted respiratory disorders, in pregnant females are linked to miscarriages, low birth weight baby⁶. Cigarette smoke contains carcinogenic pyrolytic products that bind to the DNA and cause genetic mutation and may inhibit the programmed cell death resulting in cancer cell⁷. Around 19 chemical carcinogenic substances have been identified in cigarette smoke: Benzopyrene, Acroline, Nitrosamine, Nicotine etc. Humans are exposed to variety of chemicals, radiations, mutagens and carcinogens causing health hazard to the future generation. Genotoxic substances that are present in the cigarette may interact with and alter DNA molecule causing cytotoxicity⁸. An increased frequency of chromosome breaks has been demonstrated in the initial stage of carcinogenesis. Taking this hypothesis, the cytogenetic damage can be taken as a biomarker to identify the effect of exposure of chromosome damage due to cigarette smoking. Rapid development of genetic tests like Chromosome Aberration Analysis (CAA), Satellite Associations (SA), Micro nuclear Test (MN), Sister Chromatid Exchange (SCE), Fluorescent in situ hybridization (FISH), Flow Cytometry and Spectral Karyotyping (SKY) are the rapid tests available to assess chromosome damage by mutagenic and or carcinogenic chemicals. The frequency of areocentric chromosome associations can be applied as a biomarker to assess the chromosome damage due to cigarette smoking⁹.

The phenomenon of Satellite Association (SA) involving specific position of the satellite chromosomes (D and G group chromosomes except Y chromosome) with their satellites attracted towards each other was first observed in mitotic chromosomes and later in meiotic chromosomes. The formation of SA has been attributed to the involvement of satellite chromosome in nucleolar formation¹⁰. The sticky nucleolar material has a tendency to hold the associated chromosomes

together through mitosis. The fusion of two or more nucleoli would tend to stretch mechanically the nucleolar forming chromosome segment with obvious risk of breakage. If breaks occur in more than one of the chromosomes involved, the closeness of the broken ends would predispose to translocation and thus SA would be active in the origin of translocation between satellite chromosomes¹¹.

A high incidence of satellite associations has been considered as predisposing to an increased tendency of nondisjunction in satellite chromosomes and thus to the induction of D and G trisomies. Higher frequency of satellite associations has been reported in Down's syndrome children and mothers using contraceptive pills and among the couples with recurrent abortion. This shows there is some kind of influence on the satellite chromosome to associate in the event of toxic substance intake⁹. The present cytogenetic study is designed to investigate frequency of satellite association in smokers and non-smokers and assess the possible chromosomal damage due to cigarette smoking.

MATERIALS AND METHODS

Study design and setting

This was a comparative cytogenetic study designed to evaluate the frequency of satellite associations (SA) in peripheral-blood lymphocytes of cigarette smokers and non-smoker controls. The study population comprised 30 adult male smokers attending the Medicine/TBCD Department of Mahatma Gandhi Medical College & Research Institute. An equal number of non-smoker controls were selected and matched with respect to age, sex and social status. The primary comparison was the frequency and pattern of acrocentric chromosome satellite associations across increasing levels of smoking exposure. The study methodology was structured to permit comparison of three smoking-index categories and corresponding matched controls. The study protocol states that 50 metaphases were examined for each participant and that associations involving D- and G-group acrocentric chromosomes were scored.

Eligibility criteria

Table 1. Eligibility criteria

Criteria	Smokers	Controls
Inclusion	Active cigarette smokers; age >18 years; smoking exposure classified by SI	Non-smokers; age >18 years
Exclusion	Alcohol or other substance abuse; ex-smoker considered as control; known chromosomal anomaly	Not applicable in the original protocol

Study population and smoking-index classification

Thirty smokers were subdivided into three categories of 10 participants each according to smoking index (SI). The smoking index was calculated using the formula: SI = number of cigarettes, bidis or cigars smoked per day × number of years of smoking. Category I comprised participants with SI ≤150; Category II included SI ≥150 to ≤300; and Category III included SI ≥300. An equal number of controls were matched for age, sex and social status.

Table 2. Distribution of study participants according to smoking-index category.

Category	Smoking index	Smokers, n	Matched controls, n	Total participants
I	≤150	10	10	20
II	≥150 to ≤300	10	10	20
III	≥300	10	10	20
Total	—	30	30	60

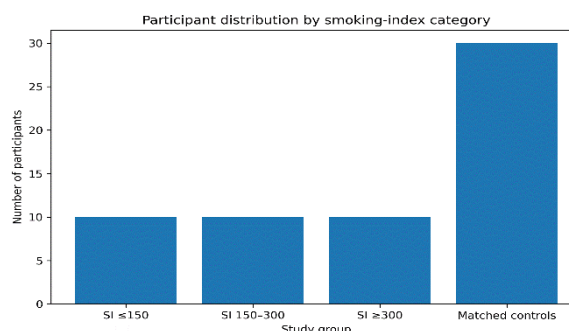


Figure 1. Distribution of participants across smoking-index categories and the matched control group. The study included 10 smokers in each SI category and 30 matched controls.

Blood collection and lymphocyte culture

Following informed consent, 2 mL of peripheral venous blood was collected aseptically into a heparinized sterile container. Lymphocyte cultures were established using the Hungerford method with minor modifications. The source

protocol describes inoculation of approximately 0.5 mL blood into complete culture medium followed by incubation for approximately 69–70 hours at 37°C. Cultures were handled under sterile conditions and mixed periodically¹².

Table 3. Peripheral-blood lymphocyte culture and metaphase preparation protocol¹³.

Step	Procedure	Key condition
1	Collect peripheral venous blood	1–2 mL; heparinized sterile syringe
2	Inoculate blood into complete culture medium	Approximately 0.5 mL
3	Lymphocyte culture	69–70 h at 37°C
4	Mitotic arrest	0.01% colchicine; approximately 25 min at 37°C
5	Hypotonic treatment	0.075 M KCl; approximately 7 min at 37°C
6	Fixation	Methanol: glacial acetic acid, 3:1
7	Centrifugation	Approximately 1000 rpm for 5 min during processing
8	Slide preparation	Fixed cell suspension used to prepare metaphase slides

At the end of the culture period, colchicine was added to arrest dividing lymphocytes at metaphase. The cells were then subjected to hypotonic treatment with 0.075 M potassium chloride followed by repeated fixation using cold methanol: glacial acetic acid (3:1). The source protocol describes centrifugation at approximately 1000 rpm for 5 min during processing, followed by preparation of metaphase slides¹⁴.

Fifty well-spread metaphases were examined for each participant. Satellite associations were scored using the criteria attributed to Hansson. The analysis focused on the acrocentric D-group chromosomes (13, 14 and 15) and G-group chromosomes (21 and 22), including D–G (DG), G–G (GG) and D–D (DD) associations. The original protocol also describes microscopic documentation using an Olympus BX51M microscope and photography under an oil-immersion objective¹⁵.

Table 4. Operational definitions used for cytogenetic scoring¹⁶.

Variable	Operational definition
SA	Total number of satellite associations observed among 50 metaphases
DG	Association involving a D-group and a G-group acrocentric chromosome
GG	Association involving two G-group acrocentric chromosomes
DD	Association involving two D-group acrocentric chromosomes
SA%	Satellite associations expressed as a percentage of the 50 metaphases examined
D group	Chromosomes 13, 14 and 15
G group	Chromosomes 21 and 22

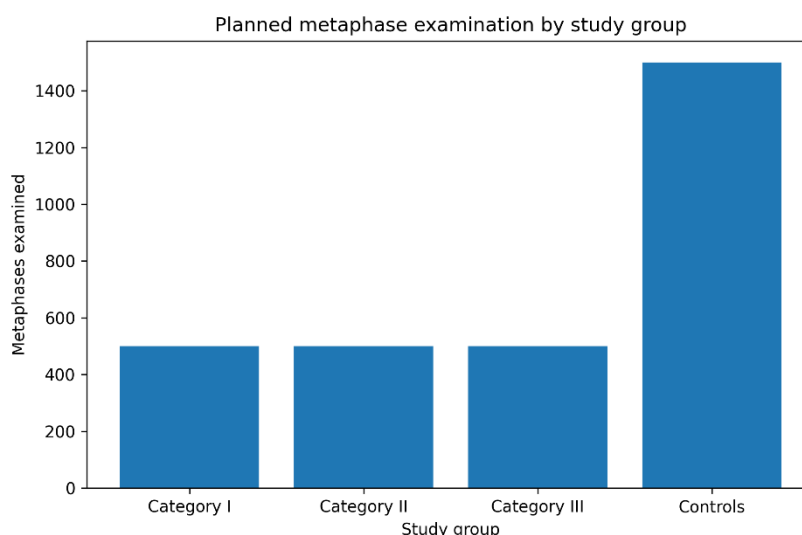


Figure 2. Number of metaphases evaluated in each study group. Fifty metaphases were examined per participant, corresponding to 500 metaphases for each smoker category and 1,500 metaphases across the 30 matched controls.

Satellite-association scoring criteria

A metaphase was considered to show a satellite association when the satellite-bearing ends of the relevant acrocentric chromosomes were directed toward each other and their longitudinal axes met between the short arms. The source methodology further specifies that the distance between the centromeres of associated chromosomes should not exceed

approximately the total length of one G-group chromosome excluding its satellite; when a connecting thread was visible, a somewhat greater distance could be accepted provided it remained below the specified chromosome-length criterion¹⁷.

Smoking-cessation follow-up

All participants received counseling regarding smoking cessation. Two participants from the highest smoking-index category subsequently returned after approximately 1.5 years of smoking cessation. A repeat 2-mL heparinized venous blood sample was collected and analyzed using the same lymphocyte-culture approach. Satellite-association frequencies were compared descriptively with their pre-cessation observations. The source document reports the two follow-up participants and their before/after SA values¹⁸.

Table 5. Smoking-cessation follow-up methodology.

Follow-up component	Method
Participants	Two participants previously classified in SI ≥ 300 category
Follow-up interval	Approximately 1.5 years after smoking cessation
Sample	2 mL heparinized venous blood
Laboratory method	Hungerford lymphocyte-culture method
Outcome	DG, GG, DD and total SA frequency
Analysis	Descriptive before-versus-after comparison

Statistical analysis: Satellite-association frequencies were summarized for each smoking-index category and compared with the corresponding matched controls. The study used Student's t-test to evaluate differences between smokers and controls, with statistical significance assessed from the reported p values.

RESULTS:

Genotoxicity associated with cigarette smoking was assessed by determining the frequency of satellite associations among acrocentric chromosomes. Satellite associations were evaluated involving the D-group chromosomes (13, 14 and 15) and G-group chromosomes (21 and 22). Thirty male smokers were classified into three categories according to the smoking index (SI): Category I, SI ≤ 150 ; Category II, SI 150–300; and Category III, SI ≥ 300 . Fifty metaphases were screened for each participant using the satellite-association criteria described in the study protocol.

The participant-level observations demonstrated progressively higher satellite-association frequencies with increasing smoking index. In Category I, individual smoker values ranged from 8 to 22 associations, compared with 6–8 among matched controls. In Category II, smoker values ranged from 18 to 30, compared with 5–10 in controls. In Category III, smoker values ranged from 23 to 44, whereas controls showed 5–10 associations.

Table 6. Descriptive characteristics and satellite-association components among smokers.

Smoking index	n	Mean age (y)	Mean SI	Mean SA	DG	GG	DD
≤ 150	10	34	78.6	14.0	7.1	4.7	2.1
150–300	10	36	218.5	24.0	10.5	9.3	4.2
≥ 300	10	44	389.6	34.0	12.6	11.0	10.3

Mean satellite-association frequency increased from 14.0 in Category I to 24.0 in Category II and 34.0 in Category III. The corresponding mean frequencies of DG, GG and DD associations also increased across the smoking-index categories, with DG remaining the most frequent component in Categories I and II.

Table 7. Comparison of satellite-association frequency between smokers and matched controls.

Smoking index	Controls, mean $\pm$ SD	Smokers, mean $\pm$ SD	t value	p value
≤ 150	7.0 $\pm$ 0.8165	14.0 $\pm$ 5.1640	4.234	0.0005
150–300	7.0 $\pm$ 1.6330	24.0 $\pm$ 3.7417	13.1681	<0.0001
≥ 300	7.5 $\pm$ 1.5092	34.0 $\pm$ 7.1647	11.4451	<0.0001

Student's t-test showed a statistically significant difference in satellite-association frequency between smokers and controls in all three categories. The reported p values were 0.0005 for Category I and <0.0001 for Categories II and III, indicating that the smoker groups had substantially higher satellite-association frequencies than their corresponding controls.

Table 8. Reported percentage of satellite association in smokers and non-smokers.

Smoking index	Non-smokers (%)	Smokers (%)
≤ 150	14	24
150–300	14	48
≥ 300	15	68

The reported percentage data showed an increasing pattern of satellite association among smokers as the smoking index increased: 24% in Category I, 48% in Category II and 68% in Category III. The corresponding reported values in controls were 14%, 14% and 15%, respectively. Thus, the difference between smokers and controls became progressively larger across the smoking-index categories.

Table 9. Reported odds-ratio analysis for increasing smoking-index categories.

Smoking index	Control mean	Smoker mean	Odds ratio	CI lower	CI upper
≤150	7.0	14.0	1.00	—	—
150–300	7.0	24.0	1.7143	0.4972	5.9107
≥300	7.5	34.0	2.2667	0.6803	7.5521

The reported odds ratio increased from 1.7143 in Category II to 2.2667 in Category III, with the source describing a gradual increase in odds ratio with increasing smoking index. The confidence intervals reported in the source were 0.4972–5.9107 for Category II and 0.6803–7.5521 for Category III

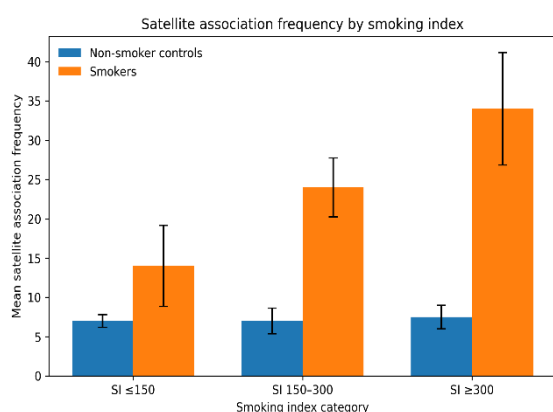


Figure 3. Mean satellite-association frequency in smokers and non-smoker controls across smoking-index categories. Error bars represent the reported standard deviations

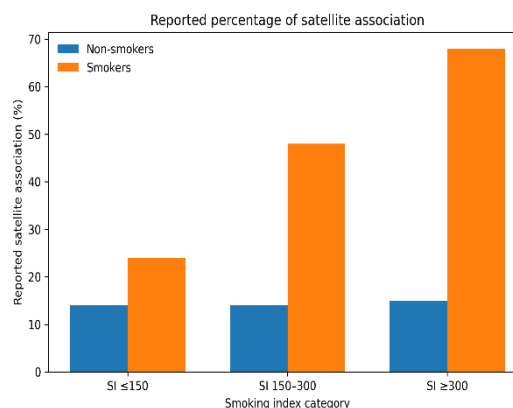


Figure 4. Reported percentage of satellite association in smokers and non-smokers across smoking-index categories.

Smoking cessation follow-up

Two participants from Category III returned for follow-up after reported smoking cessation for one and a half years. Satellite-association frequency decreased in both participants. Subject 47 decreased from 44 associations before cessation to 38 after cessation, while Subject 49 decreased from 42 to 34.

Table 10. Satellite-association frequency before and after smoking cessation.

Participant	Before cessation	After 1.5 years	Absolute change	Percentage-point change
47	44 (88%)	38 (76%)	–6	–12 percentage points
49	42 (84%)	34 (72%)	–8	–12 percentage points

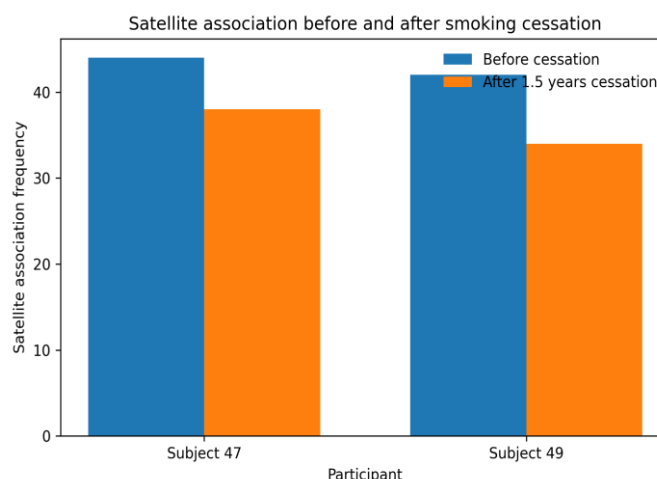


Figure 5. Individual satellite-association frequencies before and after 1.5 years of smoking cessation in two Category III participants.

Both participants showed a reduction in satellite-association frequency after smoking cessation. Because only two participants were available for follow-up, these observations are descriptive and should not be interpreted as evidence of a statistically tested cessation effect.

Chromosomal observations

Representative microphotographs in the source document illustrate metaphases without satellite association, satellite associations in controls, and associations observed in smokers across the three smoking-index categories. Arrows identify D–G, D–D and other satellite associations as described in the figure legends

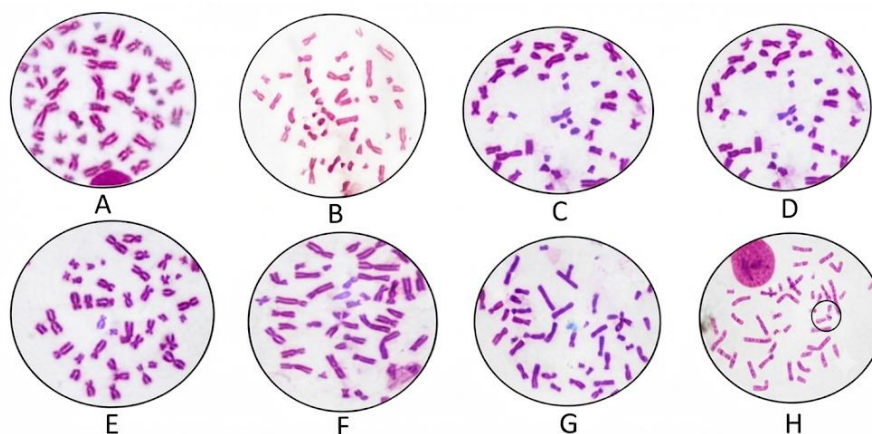


Figure 6. A. metaphase without satellite association, B. satellite association in control group, C. Satellite association between D and G group with $SI \leq 150$, D. Satellite Associations with $SI \leq 150$, E. Satellite association between D and G group with $SI \leq 300$, F. Satellite Associations Seen in $SI \geq 150 \leq 300$, G. Satellite Association with $SI \geq 300$ H. Associations Seen After Smoking Cessation

DISCUSSIONS:

The present study demonstrated an increase in satellite association (SA) among cigarette smokers with increasing smoking index (SI). The mean SA increased from 14.0 in smokers with $SI \leq 150$ to 24.0 in those with $SI 150-300$ and 34.0 in those with $SI \geq 300$, whereas the corresponding control groups showed much lower values (7.0–7.5). The differences between smokers and controls were statistically significant, indicating an association between cigarette exposure and increased satellite association. A progressive increase was also observed in DG, GG and DD associations, with DG being the predominant association type. These findings suggest that increasing tobacco exposure may be associated with increased chromosomal interaction involving the acrocentric chromosomes.

The findings are consistent with earlier cytogenetic studies of tobacco exposure. Yadav and Thakur reported increased satellite association among bidi smokers and hookah smokers and observed that satellite association increased with greater smoking duration and number of cigarettes smoked per day¹⁹. Their observation of a predominance of DG associations is also comparable with the present findings. Other studies of cigarette smokers have reported increased chromosomal abnormalities, sister-chromatid exchanges and other cytogenetic damage compared with non-smokers, supporting the biological effect of tobacco exposure on peripheral blood lymphocytes²⁰. Studies by Vijayalaxmi and Evans and by Obe et al. similarly demonstrated increased chromosome damage in smokers, although these studies evaluated different cytogenetic endpoints. Thus, the present findings extend previous observations by demonstrating a dose-related pattern specifically for satellite association⁹.

The biological basis for these findings may involve the complex mixture of tobacco smoke constituents, including reactive oxygen species and DNA-damaging compounds²¹. Chronic exposure can produce oxidative stress, DNA damage and chromosomal instability, which may influence nuclear organization and chromosome behaviour. However, satellite association should be interpreted as a cytogenetic biomonitoring endpoint rather than direct evidence of mutation or cancer development. The predominance of DG associations in the present study may reflect the particular behaviour of acrocentric chromosomes during nucleolar organization and cell division²².

A small follow-up observation was also available for two smokers who discontinued smoking for approximately 1.5 years. Their SA frequencies decreased from 44 to 38 and from 42 to 34, respectively, corresponding to reductions of 12 percentage points in both individuals. This finding is consistent with previous evidence showing that some biomarkers of tobacco-related DNA damage can decline after smoking cessation. However, because only two individuals were followed, this observation should be considered preliminary and cannot establish a statistically confirmed reversal of satellite association.

LIMITATIONS:

The study include the relatively small sample size, inclusion of only male smokers, cross-sectional assessment, possible influence of other environmental or lifestyle factors, and the absence of biochemical verification of tobacco exposure. Nevertheless, the consistent increase in SA across smoking-index categories and the significant differences from controls support the potential usefulness of satellite association as a cytogenetic biomonitoring parameter in tobacco-exposed individuals. Larger longitudinal studies incorporating biochemical exposure markers and additional cytogenetic or

molecular endpoints are required to confirm these findings and clarify the relationship between smoking intensity, chromosomal changes and their possible biological consequences.

CONCLUSION:

The present study demonstrates that satellite association frequency is higher among cigarette smokers than among non-smokers and increases progressively with increasing smoking index. The predominance of DG associations observed in the present study agrees with previous investigations of cigarette, bidi and hookah smokers. The findings are also supported by broader cytogenetic evidence demonstrating increased chromosome damage among tobacco-exposed individuals. The reduction in SA observed after smoking cessation is encouraging but remains preliminary because of the very small follow-up sample. Taken together, the findings suggest that satellite association may have value as a cytogenetic biomonitoring parameter for studying tobacco-associated genomic effects. However, further large-scale, longitudinal studies incorporating biochemical exposure markers and complementary endpoints such as chromosome aberrations, micronucleus frequency, comet assay parameters, DNA adducts and molecular cytogenetic analysis are necessary to determine the reproducibility, biological significance and potential clinical utility of satellite association in tobacco-exposed populations.

REFERENCES

1. Sakthisankaran, S. M., Sakthipriya, D., & Swamivelmanickam, M. (2024). Health Risks Associated with Tobacco Consumption in Humans: An Overview. *Journal of Drug Delivery and Therapeutics*, 14(5), 163-173. <https://doi.org/10.22270/jddt.v14i5.6523>.
2. Sadeghi R, Masoudi M, Khanjani N (2020). A Systematic Review about Educational Campaigns on Smoking Cessation. *Open Public Health J*, 13, 748-755. <http://dx.doi.org/10.2174/1874944502013010748>.
3. Jha, P., & Peto, R. (2014). Global effects of smoking, of quitting, and of taxing tobacco. *The New England journal of medicine*, 370(1), 60–68. <https://doi.org/10.1056/NEJMr1308383>.
4. Mishra, G. A., Pimple, S. A., & Shastri, S. S. (2012). An overview of the tobacco problem in India. *Indian journal of medical and paediatric oncology : official journal of Indian Society of Medical & Paediatric Oncology*, 33(3), 139–145. <https://doi.org/10.4103/0971-5851.103139>.
5. Mohanty, D. P., Rout, T., Shankar, K. V., Nag, R., Agrawala, S., & Mishra, S. N. (2023). Impact of health education and cognitive behavioural therapy intervention during tobacco cessation session for smokers: A comparative study in Eastern India. *Journal of Associated Medical Sciences*, 56(2), 29–38. retrieved from <https://he01.tci-thaijo.org/index.php/bulletinAMS/article/view/258576>.
6. Gupta, A., Bansal, A., Dixit, P., & Kumar, K. A. (2024). The crossroads of work and home: linkages between smoke-free policies at work and household environments. *BMC public health*, 24(1), 1127. <https://doi.org/10.1186/s12889-024-18658-9>.
7. Vlachou, M., Kyrkou, G., Georgakopoulou, V. E., Kapetanaki, A., Vivilaki, V., Spandidos, D. A., & Diamanti, A. (2025). Smoke signals in the genome: Epigenetic consequences of parental tobacco exposure (Review). *Biomedical reports*, 23(3), 146. <https://doi.org/10.3892/br.2025.2024>.
8. Siqueira Borges, M., Garcia, A. L. H., Serpa, E. T., Martins, B. A. A., Dihl, R. R., da Silva, F. R., & da Silva, J. (2025). Evaluation of cytotoxic and genotoxic effects of nicotine, tobacco-specific nitrosamines, and dried tobacco leaf extract on HepG2. *Journal of toxicology and environmental health. Part A*, 88(22), 920–934. <https://doi.org/10.1080/15287394.2025.2516815>.
9. Uma, A., Pajanivel, R., Raj, S., & Lokeshmaran (2011). Smoking-induced satellite associations in a rural population of south India: An in vitro study. *International journal of applied & basic medical research*, 1(2), 75–79. <https://doi.org/10.4103/2229-516X.91148>.
10. Tapia-Pastrana, F., & Tapia-Aguirre, F. (2021). Cytogenetics Analysis in Somatic Cells of *Dermatophyllum secundiflorum* (Mescal Bean) Reveals Satellite Association on Subtelocentric Chromosomes. *CYTOLOGIA*, 86 (1), 79–83. <https://doi.org/10.1508/cytologia.86.79>.
11. Ferguson-Smith M. A. (2021). Human cytogenetics at Johns Hopkins Hospital, 1959-1962. *American journal of medical genetics. Part A*, 185(11), 3236–3241. <https://doi.org/10.1002/ajmg.a.62366>.
12. Alsbeih, G. A., Al-Hadyan, K. S., Al-Harbi, N. M., Bin Judia, S. S., & Moftah, B. A. (2020). Establishing a Reference Dose-Response Calibration Curve for Dicentric Chromosome Aberrations to Assess Accidental Radiation Exposure in Saudi Arabia. *Frontiers in public health*, 8, 599194. <https://doi.org/10.3389/fpubh.2020.599194>.
13. Sedlák, V., Bujňák, A., Gajdoš, A., Gajdošová, D., Poráčová, J., Konečná, M., ... Mydlárová Blaščáková, M. (2023). Cytogenetic analysis of coke oven workers in Eastern Slovakia. *Central European Journal of Public Health*, 31(Suppl 1), S95-100. doi: 10.21101/cejph.a7845.
14. Bhartiya, A., Robinson, I., Yusuf, M., & Botchway, S. W. (2021). Combining Multicolor FISH with Fluorescence Lifetime Imaging for Chromosomal Identification and Chromosomal Sub Structure Investigation. *Frontiers in molecular biosciences*, 8, 631774. <https://doi.org/10.3389/fmolb.2021.631774>.
15. Akiyama, M., Tominaga, T., Takashima, Y., Ishii, K., & Suto, Y. (2024). Assessing the applicability of a modified replication banding protocol for the analysis of radiation-induced chromosomal aberrations in cultured human lymphocytes. *CYTOLOGIA*. 89(1), 39-46. <http://dx.doi.org/10.1508/cytologia.89.39>.
16. Yang, M., Vesterlund, M., Siavelis, I., Moura-Castro, L. H., Castor, A., Fioretos, T., Jafari, R., Lilljebjörn, H., Odom, D. T., Olsson, L., Ravi, N., Woodward, E. L., Harewood, L., Lehtiö, J., & Paulsson, K. (2019). Proteogenomics and Hi-C reveal transcriptional dysregulation in high hyperdiploid childhood acute lymphoblastic leukemia. *Nature communications*, 10(1), 1519. <https://doi.org/10.1038/s41467-019-09469-3>.

17. Cohen, M.M. and Shaw, M.W. (1967), The association of acrocentric chromosomes in 1000 normal human male metaphase cells. *Annals of Human Genetics*, 31: 129-140. <https://doi.org/10.1111/j.1469-1809.1967.tb00544.x>
18. Wulf, H. C., Husum, B., & Niebuhr, E. (1985). Cessation of smoking enhances sister chromatid exchanges in lymphocytes. *Hereditas*, 102(2), 195–198. <https://doi.org/10.1111/j.1601-5223.1985.tb00615.x>.
19. Yadav, J. S., & Thakur, S. (2000). Genetic risk assessment in hookah smokers. *Cytobios*, 101(397), 101–113. <https://pubmed.ncbi.nlm.nih.gov/10756983>.
20. Haveric, Anja & Haveric, Sanin & Ibrulj, Slavka. (2016). Chromosome aberrations frequency in peripheral blood lymphocytes in young tobacco smoking and non-smoking people. *Journal of Health Sciences*. 6. 121-127. [10.17532/jhsci.2016.368](https://doi.org/10.17532/jhsci.2016.368).
21. Caliri, A. W., Tommasi, S., & Besaratinia, A. (2021). Relationships among smoking, oxidative stress, inflammation, macromolecular damage, and cancer. *Mutation research. Reviews in mutation research*, 787, 108365. <https://doi.org/10.1016/j.mrrev.2021.108365>.
22. Galperin H. (1969). Number of each type of acrocentric association and probability of its occurrence in human male and female metaphase cells. *Cytogenetics*, 8(6), 439–446. <https://doi.org/10.1159/000130055>.