

Original Research Article

Chromosomal aberrations: A tool for early diagnosis of cancer in smokers in a rural Pondicherry population, India

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A B S T R A C T

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Cytogenetic assay in peripheral blood lymphocytes have been done to assess the incidence of chromosomal aberrations among 30 smokers and 30 nonsmokers belonging to a small hamlet in Pondicherry. Smokers were divided into three groups based on their Smoking Index (SI) using the formula : $SI = \text{No. of cigarettes / bidi's / cigars per day} \times \text{Total duration in years}$. 30 smokers were sub stratified into THREE groups based on their smoking index (SI): 10 Smokers I - SI less than 150, 10 Smokers II- SI 150 to 300, 10 Smokers III- SI more than 300. The results showed that among the smokers , chromosomal aberrations were seen only in smokers of smoking index greater than 300. Such findings may provide scientific evidence to encourage national campaigns to prevent tobacco consumption and devise intervention strategies that might reduce the persisting morbidity and mortality from smoking related cancer.

Introduction

An increased risk of cancer in smokers with high levels of chromosomal aberrations (CAs) in peripheral blood lymphocytes has been described in recent epidemiological studies. A smoker is exposed to a variety of carcinogenic constituents present in cigarettes, making it necessary to analyze the cells at metaphase as these can be a health hazard to the future generations. Genotoxic carcinogens in cigarette interact

with and alter DNA molecule causing cytotoxicity and therefore cytogenetic damage seems to be an excellent biomarker for determining the effect of exposure to chromosome damaging agents in smoke. In support of this hypothesis, an increased frequency of chromosome breaks has been demonstrated to be an initial event in carcinogenesis, suggesting that these alterations may play a significant role in

assessing oncogenic risk. Lung cancer remains by far the most lethal of all cancers due to cigarette smoking. 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 among which chromosomal aberrations, appears to be one of the most suitable test to assess the effect of cigarette smoke on chromosomes. They reflect chromosome damage and may thus provide a biomarker of early-stage carcinogenesis.

Chromosomal Aberrations occur because of lesions in the DNA that lead to discontinuities in the DNA double helix. The lesions may be corrected to reconstitute the original base sequence or transformed to produce gene mutations and/or Chromosomal Aberrations. Exposure to physical or chemical agents, viruses and life style habits including smoking and alcohol consumption affect the genetic material of the individual and can be detected in the form of various types of chromosomal aberrations (Wolff, 1978; Evans, 1977). Tobacco smoke is known to contain thousands of potentially hazardous chemicals including radioactive agents (Falk 1977; Cohen, 1980), the formation of free radicals from radioactive and non-radioactive chemicals is probably one of the major pathways by which tobacco smoke causes genetic damage, chromosomal aberrations and cancer (Pryor, 1987).

Bender *et al.* (1988) observed more frequency of chromatid deletions and dicentric chromosomes in healthy individuals but no significant influence of smoking, age and sex on these aberrations. However, other studies showed the influence of age, sex, race and cigarette smoking on the frequency of chromosomal aberrations (Obe and Herha, 1978; Evans, 1979;

Lambert *et al.*, 1978; Krishna Murthy, 1979; Hopkin and Evans, 1980; Butler, 1981; Vijayalaxmi and Evans, 1982; Soper *et al.*, 1984; Margolin and Shelby, 1985; Galloway *et al.*, 1986; Wulf *et al.*, 1986; Yadav and Thakur, 2000a,b; Yadav *et al.*, 2001a). Van Diemen *et al.* (1995) observed no significant difference between smokers and nonsmokers in the frequencies of unstable and stable type of chromosomal aberrations. However, significantly higher frequency ($P \leq 0.05$) of hyperploidy was evident in smokers compared with nonsmokers. Bochkov *et al.* (2001) showed that smoking has been found to increase the frequency of chromosome aberrations in individuals with occupational hazards, but not in those who are not occupationally exposed to radiation or chemicals. Yadav *et al.* (2002), found tobacco smoke to cause chromosomal aberrations from cytogenetic investigations on a random sample of general population from Haryana.

Sierra *et al.* (2004) showed that the frequency was significantly higher in smokers than in non-smokers showing the highest number of Chromosomal Aberrations (CA) among heavy smokers (>20 pack-years). In addition, a significant positive correlation was found between the frequency of CA and the intensity of smoking in pack-years.

The present invitro cytogenetic study has been used to investigate the Chromosomal Aberrations in Smokers and Non Smokers in a rural population in Pondicherry and to correlate the findings to detect early stages of carcinogenesis in them.

Materials and Methods

The study comprised of 30 smokers, all males, attending the tertiary care hospital at a small hamlet in Pondicherry, divided into

three groups based on their smoking index, calculated using formula viz:

Smoking index (SI) = No. of cigarettes / beedi's / cigars per day x Total duration in years

Subdivision of smokers:

- 10 Smokers I - SI less than 150
- 10 Smokers II - SI 150 to 300
- 10 Smokers III - SI more than 300

An equal number of control individuals (30 nonsmokers) matched with respect to age, sex and social status. They all belonged to the lower strata of the society with agricultural background. None of the individuals consumed alcohol or took any drugs. Ex-Smokers and Smokers with chromosomal anomaly were also excluded from the study. Lymphocyte cultures were set up from heparinised blood according to the method of Hungerford (1965) with minor modifications. For each person 50 well spread metaphase plates, stained with 4% buffered Giemsa were analyzed for Chromosomal Aberrations (CA).

Results and Discussion

It was of interest to observe that while scoring 50 metaphases for chromosomal aberrations in the leukocyte culture of the 3 groups of Smokers there was an increase evidence of chromosomal aberrations in smoker group III only. Chromosome aberration in smokers with smoking index III showed 23% aberrant metaphases (Table 1) with minutes, breaks and fragments (Fig. 1).

Tobacco related cancer is the most common malignancy, and one of the most lethal. The role of tobacco smoking in the etiology of cancer disease has been well known for many decades, and any approach aimed at expediting the detection of population sub-

groups at increased risk should be considered a high priority task. It may be possible to use genotoxicity assays to identify which sub-groups of smokers are more susceptible to the DNA-damaging effect of cigarette smoke and/or which level of smoking produces significant increases in mutation over base-line. Many of the substances contained in cigarette smoke are genotoxic causing carcinogenesis in man and therefore cytogenetic damage seems to be an excellent biomarker for determining the effect of exposure to chromosome-damaging agents in smoke.

Anderson *et al.*, (1988) found no influence of smoking on chromosome aberrations. However, many reports confirm significant effect of smoking on chromosome aberrations (Obe and Herha 1978; Lambert *et al.*, 1978; Vijaylaxmi and Evans, 1982; Yadav and Thakur, 2000b; Yadav *et al.*, 2001b,c). Smokers engaged in different occupations like farming and workers in industries are exposed to variety of chemicals. Most of chemicals used by industry workers and pesticide sprayers have produced chromosomal damage in human somatic cells (Van Bao *et al.*, 1974; Fredga *et al.*, 1982; Rita *et al.*, 1987; Sharma and Sobti 1988; Rupa *et al.*, 1989; Yadav and Kaushik 1996; Yadav and Seth 1998a,b; Yadav *et al.* 2001a,b,c).

The analysis of chromosomal aberrations has gained increasing popularity as an *in vitro* genotoxicity test and an assay for human genotoxic exposure and effect. The main reasons for this development are obvious. The scoring of the Giemsa stained Chromosomal aberrations is simpler, requires shorter training and is less time consuming.

Sierra-Torres *et al.* (2004) showed that the frequency was significantly higher in

smokers than in non-smokers showing the highest number of Chromosomal Aberrations (CA) among heavy smokers (>20 pack-years). In addition, a significant positive correlation was found between the frequency of CA and the intensity of smoking in pack-years. The present report confirms these findings. Two studies on chromosomal aberrations in leukocyte cultures, smokers (Uma *et al.*, 2011) and tobacco related oral cancer (Uma *et al.*, 2014a) and two studies on the micronucleus assay in the exfoliative buccal cells of tobacco related oral cancer conducted by Uma *et al.* (2014b,c) on the Pondicherry population showed a significant elevation of chromosomal damage indicating the population to be at a high risk for cancer.

A number of studies have been designed to evaluate the potential influence of background factors such as gender, age, or smoking habit on Chromosomal aberrations frequency. Many of these studies suffer from a poor assessment of exposure, and subjects are often roughly classified as smokers versus non-smokers, without considering the levels of cigarette consumption. The evaluation of smoking cessation is even more difficult, because former smokers sometimes are included in the group of current smokers, and sometimes with non-smokers. The planning

and high-quality information regarding smoking habit is the best approach to understand the possible use of Chromosomal aberrations as a marker of exposure/effect on the chromosomes of tobacco smokers.

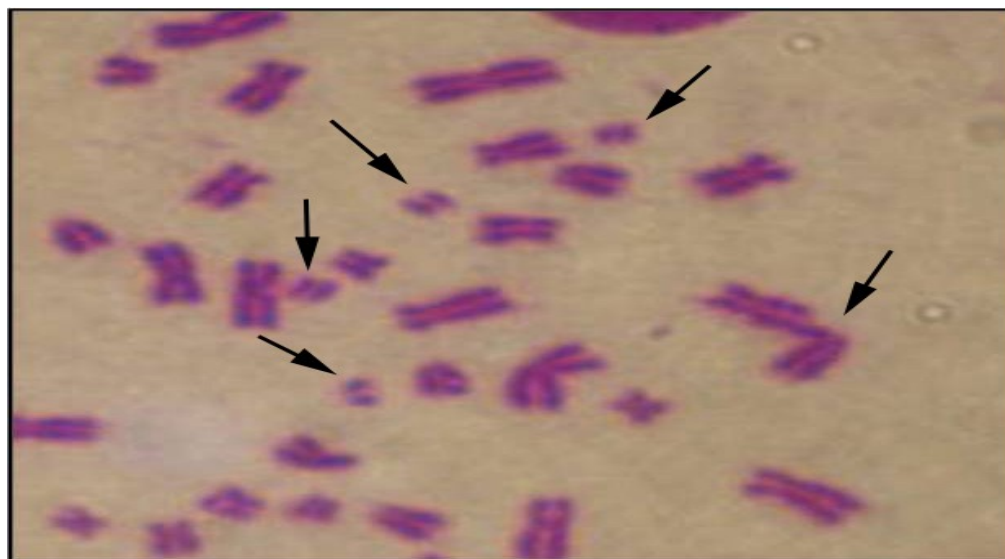
Keeping the above criteria in mind, in the present study all the samples (Smoker and Non Smokers) was taken from a small village population with agricultural background, low social strata, with a mean age of 40 years, same sex and smoking habits (exclusively active smokers only) based on their smoking index.

The results showed that among the smokers there were aberrant chromosomes with breaks and fragments in smokers of smoking index greater than 300 (Table 2) indicating that smoker who smoked more than 20 cigarettes per day suffer greater genotoxic effects in their lymphocytes caused by cigarette smoke constituents than their other nonsmoking and smoking counterparts (smoker I and II) and further indicating a high risk of carcinogenic activity in them. Thus, there is a significant positive correlation in the frequency of Chromosome aberration and the intensity of smoking in heavy smokers (smoker group III) (Sierra-Torres *et al.*, 2004) making this group of smokers more prone to cancer in immediate future.

Table.1 Chromosomal aberrations in Smokers and Non Smokers

Group	Mean Age	SMOKING INDEX (SI)			Aberrant metaphases (%)
		Mean No. of smoking years	Mean No. of cigarettes smoked	Mean Smoking index (SI)	
10 Smokers-Group -I	38.25	16	8.75	135.75	Nil
10 Smoker-Group -I	40	17.25	16.5	224	Nil
10Smoker-Group -III	40.75	20	23.75	460	23
30 Non smokers	39.67	-	-	-	Nil

Fig.1 Chromosomal aberrations in Smoker III group showing minutes, breaks and fragments



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