



Original Article

Evaluation of Chromosome Aberration and its Correlation with GSTM1 Gene Polymorphism in Women Bidi Rollers of Madhya Pradesh

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ABSTRACT

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Objectives: -The present study was conducted to investigate the extent of genotoxicity in women bidi rollers of Jabalpur, Madhya Pradesh, India. These bidi rollers were occupationally exposed to tobacco dust. **Experimental approach:** - Investigation of genotoxicity was done in 34 women bidi rollers and 30 age matched controls by assessing chromosome aberration % (CA%) in cultured peripheral blood lymphocytes & the correlation of human GSTM1 gene polymorphism with CA%. **Findings and discussion:** - Bidi rollers occupationally exposed to tobacco dust showed significantly increased CA%. It was found to be 3.0 ± 0.63 (Mean \pm SE) and 3.7 ± 0.39 in 30-35 years and 60-65 years age groups when compared to age matched controls (1.3 ± 0.32 and 1.8 ± 0.24 respectively) at $P < 0.05$. In exposure groups also the CA% was higher than that of controls. It was found 2.9 ± 0.36 & 4.1 ± 0.199 in >20 years & >50 years exposure groups respectively. The GSTM1 null controls expressed a slightly higher CA% (1.5 ± 0.2) than GSTM1 positives (1.2 ± 0.41). Similarly, the null rollers showed a higher CA% (2.8 ± 0.4) than the positives (2.55 ± 0.35) but the differences in both bidi rollers & controls were not significant. **Conclusion:** -The null genotype leads to increase CA% in rollers as well as in null controls.

Keywords: Women bidi rollers, Genotoxicity, GSTM1 gene polymorphism, chromosome aberration, Tobacco dust.

1. INTRODUCTION

Tobacco (*Nicotiana tabacum*; Family-Solanaceae) is a major cash crop grown widely in India and many farmers with small holdings work for as well as grow tobacco for wealthy multinational companies. Tobacco production provides employment and livelihood to above 6 million tobacco farmers, 4.4 million bidi rollers, and more than

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2 lakh Tendu leaf (*Diospyros melonoxylon*) pluckers in India. Bidi rollers and Tendu pluckers comprising women and children are among the most exploited working groups in the country earning bare minimum wages and unable to access healthcare facilities and they work in ill-ventilated and confined environments mostly from their homes. It is well-known that tobacco contains a variety of toxic substances such as nicotine, nitrosamines, polycyclic aromatic hydrocarbons, formaldehyde, hydrogen, etc., which are released into the ambient air during the processing of bidis. Nicotine released from the tobacco leaves can be absorbed in body tissues including skin, respiratory epithelium, and mucous membrane of the mouth. The carcinogenic potential of tobacco is well-known¹. Umadevi *et al* (2003)² evaluated the cytogenetic effects of exposure to tobacco dust in male tobacco factory workers and age and sex-matched controls by evaluating chromosomal aberrations (CA) in peripheral blood lymphocytes. A statistically significant increase was observed in the frequency of CA in non smoking and smoking groups of workers when compared to their respective controls. An increase in the frequency of CA was also observed with increase in years of service in the exposed group. Tobacco dust can also cause occupational asthma³ and it has also been reported to cause respiratory tract diseases such as wheezing, dyspnea, and rhinitis^{4,5}. It may also cause nausea, dizziness, and vomiting. Thus, bidi rollers (which are mostly women) are exposed to tobacco constituents through the cutaneous route or through inhalation of tobacco dust⁶. On an average each roller makes 500-1,000 bidis and handles 225-450 g of tobacco/day⁷, so they are exposed to a good amount of tobacco dust which may be having cumulative effects on their genetic material with the years of exposure.

The GST super family represents a major group of detoxification enzymes. There are four main classes of GST iso-enzymes in humans (α , μ , π and θ), with partially overlapping substrate specificities⁸. The GSTM1 (GSTM1 glutathione S-transferase mu 1) enzyme belongs to the μ class and the GSTT1 enzyme to the θ class. Genes coding for GSTM1 and GSTT1 proteins can have null genotypes in humans due to the deletions of both paternal and maternal alleles, resulting in lack of active protein^{9,10}. Inherited homozygous deletions of the GSTT1 or GSTM1 genes lead to an absence of enzymatic activity.

Hence, this study was conducted to Assess GSTM1 polymorphism and its correlation with chromosome aberration in women bidi rollers of Madhya Pradesh.

2. MATERIALS AND METHODS

A). Culture and processing of blood for chromosome preparation

Sample Collection

Peripheral blood samples were collected by brachial veni puncture in EDTA vacutainer for GSTM1 Polymorphism study and in sterile heparinized vials for lymphocyte culture from 34 healthy women bidi rollers and 30 healthy volunteers as controls with their written informed pre consent. The subjects and controls included in this study were neither smokers nor tobacco chewers. All the subjects filled in a questionnaire in which the information about their age, exposure (duration of work), addictions, medications and illness, if any was documented. The institutional ethical committee approved of this study.

Chromosome Aberrations (CA%)

Blood culture was done according to the method of Moorhead *et al* (1960)¹¹. It was done in TC199 (Hi Media) medium supplemented with foetal calf serum (Hi Media), taking PHA (10 μ g/ml) as a mitogen. Slides were prepared for the analysis of CA% according to the standard air drying/hypotonic/Giemsa technique after arresting metaphase with colchicine (10 μ g/ml). The study of CA % was done by scoring hundred well spread metaphase plates for each sample (Fig. 1, 2 & 3). 'Student-t' test was used for statistical data analysis.

B). GSTM1 gene polymorphism:-

Isolation of human genomic DNA

Human genomic DNA from the blood samples of bidi rollers (34) and controls (30) was isolated using Genei Pure™ Blood genomic DNA purification Kit. In brief, Fresh blood was collected in EDTA vacutainer after venipuncture. Blood (200 μ l) was taken in separate microfuge tubes for each donor. Proteinase K (50 μ l) was added to the tube. Then, 200 μ l of lysis buffer was added. Solution was vortexed vigorously. It was incubated at 70 °c for 20 min. Solutions were vortexed after each 10 min for 20 sec. Colour of the solution turned black. Then, 4 μ l of RNase was added to each tube. The tubes were vortexed for 20 sec. Incubation with RNase was given for 10 min at room temperature (RT), then 210 μ l of ethanol was added to each tube and vortexing was done. The mixture was then poured into genei pure columns (provided in the kit). These columns were put in 2ml collection tubes and centrifuged at 15000 rpm for 2 min at 4 °c. Two washes were given using wash buffer-I and Wash buffer- II provided in the kit by centrifugation at 12000 rpm for 2-2 min separately. 70 μ l of pre-warmed elution buffer at 70 °c (2 min) was added to each tube separately, incubated for 1 min at RT. Centrifugation at 12000 rpm for 1 min was done, Eluted buffer was kept in fresh microfuge tubes. The tubes were labeled and stored at -20 °C for further use.

Agarose Gel electrophoresis was done to locate isolated human genomic DNA on 0.8% agarose gel (SIGMA ALDRICH) using Tris Boric acid EDTA buffer (TBE) buffer (GENETIX) at 50 Volt for 30 min. Ethidium bromide staining was performed to visualize DNA under UV light.

DNA quantification was done using Nano drop equipment. The concentration of genomic DNA ranged from 16.1 ng/ μ l to 75.1 ng/ μ l. OD at 260/280 ratio was found to be between 1.7 to 1.9.

PCR amplification

The genetic polymorphism analysis for the GSTM1 gene was done using the polymerase chain reaction (PCR) procedure of Arbag *et al.* (2006)¹². Isolated DNA (50-100ng) was amplified in a 25- μ l reaction mixture using 2X PCR Master mix (GENETIX). The primers used were: -

(a) Human *GSTM1* gene

Forward primer - 5'- GAA CTC CCT GAA AAG CTA AAG C-3'

Reverse Primer - 5'- GTT GGG CTC AAA TAT ACG GTG G-3'

(b) Human β -globin gene as an internal control

Forward primer - 5'-CAACTTCATCCACGTTCCACC-3'

Reverse Primer - 5'-GAAGAGCCAAGGACAGGTAC- 3'

The above mentioned primers were supplied by IDT (integrated DNA technologies). Concentration of the primers used in PCR was 0.1 μ M. After a 4-min incubation at 95°C, a first round of 12 cycles was performed (30s at 94°C, 1 min at 59°C, and 1 min at 72°C), followed by a second round of 25 cycles (30 s at 94°C, 30 s at 57°C, and 1 min s at 72°C.) with final extension 72°C for 10 min. Presence or absence of the β -globin gene band was used to determine failed PCR. The products of the multiplex PCR (215 bp for *GSTM1*, and 268 bp for β -globin) were separated by electrophoresis through a 2.2 % agarose gel, stained with ethidium bromide and visualized under UV light (Fig 4).

3. RESULTS

A). Chromosome aberration %

A scan of the metaphase plates (Fig 1, 2 & 3) of the subjects and the controls indicated that there was a general trend of increase in CA% with age both in the non-exposed (control groups) and the exposed groups (bidi rollers) (Table 1). Some of the aberrations such as aneuploidy, extra acentric fragment, dicentric chromosome, end to end association, haploid metaphase plate and centromeric separation were found in this study. The CA% increased uniformly with age in the control group. In general the bidi rollers had a greater percentage of CA than the controls of the same age groups. In each group the increase in CA % in bidi rollers was significant ($P<0.05$) when compared to the CA % of the controls of the same group. The CA % was also related to the duration of exposure (Table 2).

B). GSTM1 gene Polymorphism Study

The present study demonstrated that 5 out of 30 (16.66%) control samples showed a homozygous null genotype of GSTM1 whereas, in the samples of bidi rollers 11 out of 34 (32.35%) were of the null type (Fig 4). 83.33% of control

samples were found to be GSTM1 positive whereas only 67.64% samples of rollers were GSTM1 positive (Table 3).

The associations between the genotype frequencies of GSTM1 with the control, and bidi roller groups was assessed by Odds Ratios (OR) and Confidence Intervals (95% CI) using online available free statistical calculator (MedCalC). The odds ratio was 2.39 and 95% confidence intervals - from 0.72 to 7.93.

Correlation of CA% with GSTM1 gene

The GSTM1 null controls showed a slightly higher CA% (1.5 \pm 0.2) than GSTM1 positives (1.2 \pm 0.41) but the increase was not significant. Similarly the 52 null rollers showed a higher CA% (2.8 \pm 0.4) than the positives (2.55 \pm 0.35) but the difference was not significant. Thus apparently the null genotype leads to increase CA% in rollers as well as in null controls. (Table 4)

4. DISCUSSION

A). CA%

In the present study, the frequency of the average CA% (Table 1) of all the bidi rollers examined was found to be 3.3% as compared to 1.5% in controls in all the age groups. Similar findings were also reported by Mahimkar and Bhisey (1995)¹³ who found a significant increase in CA in bidi tobacco processors due to occupational exposure. Yadav and Thakur (2000)¹⁴ worked on the genotoxic effects of bidi smoking and found a significant increase in the CA% as compared to that in the nonsmokers. Increase in CA% of smokers was also found by a Nordic study group (1990)¹⁵. Increased CA% in peripheral blood lymphocytes of smokers as compared to those of nonsmokers was found by Kopjar *et al* (2006)¹⁶. However, Battershill *et al* (2008)¹⁷ expresses the view that a significant increase is found in the biomarkers of genotoxicity only in people smoking excess of 20-30 cigarettes/day. Harmful effects on chromosome integrity have been found due to tobacco chewing by a number of workers. Patel *et al* (2009)¹⁸ found a significant increase in the CA% of chewers over controls.

Cytogenetical damage in petrol pump workers was studied by Yadav and Seth (2001)¹⁹. They found the frequency of total cells with chromosome aberrations in exposed workers was 2.54% whereas the background frequency was 0.72% and the difference was statistically significant ($P<0.05$).

B). GSTM1 polymorphism

Based on the study by Monteiro *et al* (2012)²⁰ GSTM1 null genotype and GSTT1 do not appear to play a major role in hepatic injury induced by anti-tuberculosis drugs in Brazil, although there has been evidence that GSTM1 is associated to toxicity intensity.

Sharma *et al* (2012)²¹ worked on genetic polymorphisms of GSTM1 and GSTT1 genes in Delhi and comparison with other Indian and global populations. They found 33.6% of the population had a GSTM1 null genotype in a sample size of 500, whereas, in the present study null genotype of

controls was 16.66% (Table 3). This difference may be due to a small sample size.

Correlation of CA% with GSTM1 gene polymorphism

Gulgunet al (2006)²² studied the Individual sensitivity to cytogenetic effects of benzo[a] pyrene in cultured human lymphocytes. They studied the influence of glutathione S-transferase M1 genotype on 31 Turkish individuals. They also found that there was no significant difference in the CA% of GSTM1null and GSTM1 positive genotypes. Rossi et al (2009)²³ found that the association between frequency of chromosomal aberrations and cancer risk is not influenced by genetic polymorphisms in GSTM1 and GSTT1. The study was carried out in three laboratories in Italy, Norway, and Denmark. However, in the present study it was found that the GSTM1 null controls showed a slightly higher CA% (1.5±0.2) than GSTM1 positives (1.2±0.41) but the increase was not significant. Similarly the null rollers showed a higher CA% (2.8±0.4) than the positives (2.55±0.35) but the difference was not significant. (Table 4)

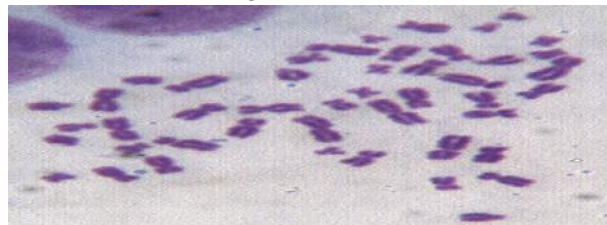


Fig 1: Normal Metaphase Plate in a control

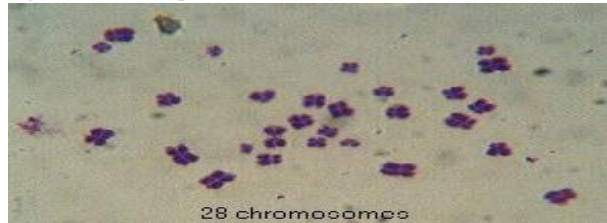


Fig 2: Aneuploidy in a bidi roller (28 chromosomes)

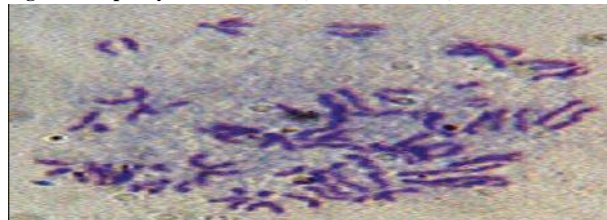


Fig 3: Centromeric separation and fragmentation

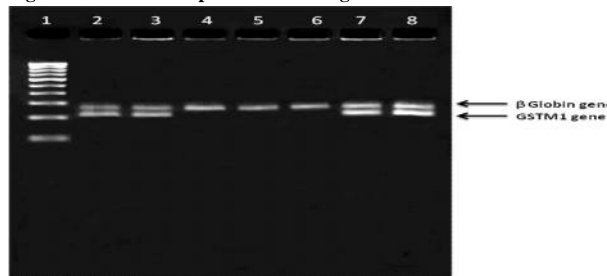


Fig 4: Multiplex PCR patterns for GSTM1 gene. The β -globin gene was used as an internal control.

Lane 1: - Ladder (100 bp)

Lanes 2, 3, 7 and 8: - Show presence of GSTM1 gene (215bp)

Lane 4, 5, 6:- Show absence of GSTM1 gene (null genotypes)

Lane 2-8:- Show the presence of β -globin (268bp) gene (Control)

Table 1: Effect of age on chromosome aberrations (CA %) in bidi rollers and controls.

S/N	Age Groups (Year)	CA% (Mean \pm SE)	
		Controls (n=30)	Bidi rollers (n=34)
1	30-35	1.3 \pm 0.32	3.0 \pm 0.63*(n=8)
2	45-50	1.4 \pm 0.15	3.2 \pm 0.47*(n=11)
3	50-55	1.6 \pm 0.25	3.3 \pm 0.45*(n=6)
4	60-65	1.8 \pm 0.24	3.7 \pm 0.39*(n=9)

*Significant at P<0.05

Table 2: Effect of exposure to tobacco dust on CA% in bidi rollers.

S/N	Exposure (Years)	CA% (Mean \pm SE) (n=34)
1.	Control (non exposed)	1.5 \pm 1.22(n=30)
2.	> 20	2.9 \pm 0.36(n=8)
3.	>30	3.1 \pm 0.34(n=10)
4.	>40	3.5 \pm 0.256(n=8)
5.	>50	4.1 \pm 0.199(n=8)

Table 3: GSTM1 % of null and positive genotypes found in bidi rollers and controls

S/No	GSTM1 genotype	Controls (n=30)	Controls %	Bidi Rollers (n=34)	Bidi Rollers %
1	Null	5	16.66%	11	32.35%
2	GSTM1 (+)	25	83.33%	23	67.64%

Table 4: -Effect of GSTM1 gene on chromosome aberration %

S/No	GSTM1 genotype	CA% in Bidi Rollers (Mean \pm SE)	CA% Controls (Mean \pm SE)
1	GSTM1 Null	2.8 \pm 0.4	1.5 \pm 0.2
2	GSTM1 (+)	2.55 \pm 0.35	1.2 \pm 0.41

5. CONCLUSION

- 1) In view of the above findings it can be said that genotoxic effects in terms of CA% analyzed begin to appear in the bidi rollers when they have been exposed to tobacco dust and handling tobacco during bidi rolling for 10 years (4 to 5 hours /day) and after 20 years effects become very marked.
- 2) It was found that the polymorphism of GSTM1 gene (regarding the null and positive genotype) was not found to be linked to the CA% considering their exposure or non-exposure to tobacco dust.
- 3) Bidi rollers must be advised to wear masks to prevent themselves from the exposure of the bidi tobacco dust to diminish the chance of genotoxicity, which may lead to cancer and other health risks at later stage of their lives.

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