Genotoxic Effects of Tobacco Dust Exposure on Bidi Rollers

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***Abstract*—Bidi rolling is one of the largest cottage industries in India. Most of the rollers are women of low socio-economic status who are exposed to bidi tobacco dust while working. Hence, this study deals with the assessment of genotoxicity in bidi rollers occupationally exposed to bidi tobacco dust in Jabalpur.The study deals with the detection of the level of genotoxicity in the subjects in order to pre-warn the bidi rollers who showed more symptoms of genotoxicity so that they might take some precautions.The experimental data was collected and arranged according to age and exposure. The sample size was 34 for the bidi rollers and 30 for controls.The end points studied were chromosomal aberration assay using lymphocyte culture, comet assay with Trevigen's (USA) Comet Assay™ Silver Kit and urinary thioether estimation according to Ellman's spectrophotometric method. The Student's t test was used for stastistical analysis of the data. Bidi rollers exposed to bidi tobacco dust expressed significantly increased chromosome aberration (CA) % 3.0 ± 0.63 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 $P < 0.05$. Significantly higher excretion of urinary thioether (12.58 ± 2.17 µmol/mmolcreatinine) in > 30 year exposure group as compared to controls was found. The comet and tail length of bidi rollers also showed significant increase as compared to controls. Thus, the results indicated the elevation of genotoxicity in bidi rollers occupationally exposed to bidi tobacco dust as assessed by the above parameters.**

***Index Terms*—genotoxicity, tobacco dust, chromosome aberration, comet assay, urinary thioether.**

I. Introduction

Bidi, which is known as Indian cigarette is consumed world-wide. A great number of the consumers are found in India alone. According to a World Health Organization (WHO) report (1998), only 10-15% of the people in India are cigarette smokers while 80-90% consume tobacco in the form of bidis. Bidi is smoked by rural as well as urban males and females belonging to lower socio-economic groups (Yadav *et al*, 2001). Bidi rolling industry is one of the largest cottage industries in India. It gives occupation to 4.4 million bidi rollers and more than two lakh Tendu leaf (*Diospyrosmelonoxylon*) pluckers. Bidi rollers and Tendupluckers comprising women and children are among the most exploited working groups in the country earning bare minimum wages and unable to access healthcare facilities (Majumdar *et al*, 2010).On an average each roller makes 500-1000 bidis and handles 225-450 gram of tobacco / day (Bhisey *et al*, 1991).

Bidi rollers work in their homes which are ill ventilated and confined so they are exposed to massive chronic and involuntary exposure to tobacco purely as a consequence of their occupation. Occupational exposure to tobacco dust in bidi rollers has been shown to be associated with increased urinary cotinine (a metabolite of nicotine) level, urine mutagenicity (Bhisey *et al*, 1991), increased frequency of micro nuclei in buccal epithelial cells (Bagwe *et al*, 1993) and elevated urinary thioether excretion. It has been observed that tobacco dust increases salivary cotinine even higher than masher (dentifrice/nashmanjan) use (Mahimkar *et al*, 1995). Bidi rollers mostly women are exposed to tobacco constituents through the cutaneous route and inhalation of tobacco dust (Shukla *et al*, 2010), which may be having cumulative effects on their genetic material with the years of exposure. 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. Tobacco dust can also cause occupational asthma (Haber *et al*, 2004)and it has also been reported to cause respiratory tract diseases such as wheezing, dyspnea and rhinitis (Uitti *et al*, 1998&Bhisey *et al*, 1999). 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 dust (Shukla *et al*, 2010).The carcinogenetic potential of tobacco is well known (Mahimkar *et al*, 1995 & Umadevi *et al*, 2003).CA are abnormalities of chromosome number or structure visible in metaphase stage during cell division and are in general a result of breakage of a chromosome or breakage and rejoining within chromosome. Because of their strong association

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with cancer CA are viewed as indicator of increased cancer risk (Bonassi *et al*, 2004). Detection of DNA damage at the level of an individual eukaryotic cell warrants high significance in the field of toxicology, pharmaceuticals, genotoxicity testing, environmental /human biomonitoring, diagnosis of genetic disorders etc. Single cell gel electrophoresis (SCGE) or the comet assay is a versatile, sensitive yet simple and economical technique used to measure DNA damage and repair in individual cells. A higher than normal level of urinary thioether excretion indicates a higher exposure to electrophilic compounds. Exposure to tobacco dust may lead to cumulative effects on the genetic material of bidi rollers with the years of exposure. Hence this study has been done to study the extent of genotoxicity in bidi rollers of central India (M. P.).

II. Materials and Methods

The subjects for this study were female bidi rollers of different ages and exposures and matching controls.

A. Sample Collection and Blood Culture

Peripheral blood samples were collected for lymphocyte culture and alkaline comet assay from 34 healthy female 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. The peripheral blood samples were collected by brachial venipuncture in sterile heparinized vials and blood culture was done according to the method of Moorhead *et al.* (1960).

B. Chromosome Aberrations (CA)

Blood culture was done in TC199 (Hi Media) medium supplemented with foetal calf serum (Hi Media), taking PHA (10 µg/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 µg/ml). For each sample hundred well spread metaphase plates were scored for the study of CA (Fig. 1 and Fig. 2). The data was statistically analyzed by the application of "t" test.

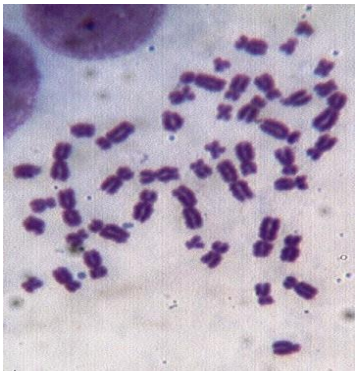

Figure 1. Normal metaphase plate in a control.

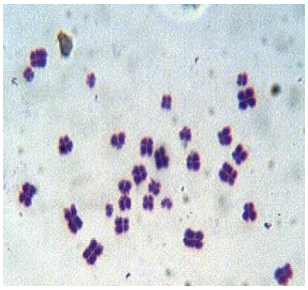

Figure 2. Aneuploidy in a bidi roller (29 chromosomes).

In brief, blood was combined with LM agarose in a ratio of 1:10 and 75 µl of this mixture was spread over sample area on the comet slide. The slides were immersed in pre-cooled lysis solution for 40 min. After subjecting the slides to freshly prepared alkaline unwinding solution, the electrophoresis was run for the slides for 30 min. The slides were stained by silver staining method. Comet Score 15 software was used to score comet length and tail length from 50 cells of each sample (Fig. 3 and Fig. 4). Comet length and Comet tail length were compared with the controls.

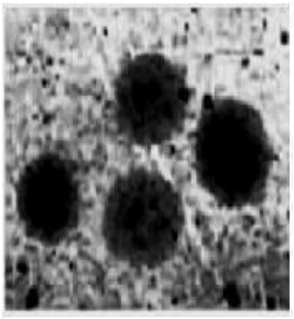

Figure 3. Comet of a control.

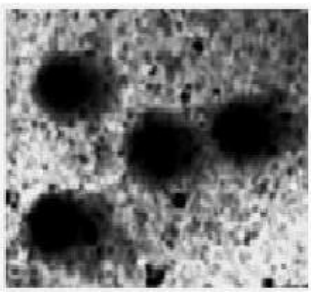

Figure 4. Comet of a bidi roller.

C. Urinary Thioether Determination

Early morning urine from the subjects after the working day was collected and thioether determination was done according to Ellman's (1959) spectrophotometric method as specified by Vainio *et al* (1978). Ascorbic acid was mixed with the urine samples to provide a reducing atmosphere for the removal of proteins from urine. Further treatment was carried out for hydrolysis for the determination of SH groups as specified by Vainio *et al* (1978).The SH groups were quantified by spectrophotometric molar absorbtivity of Ellman's reagent. The urinary creatinine from the same samples was determined in our lab by alkaline picrate

method by commercial kit. The thioether level was expressed in µmol/mmol of creatinine.

Statistical analysis was done using Student t-test.

III. Results and Discussion

A. CA%

Bidi rollers exposed to bidi tobacco dust expressed significantly increased ($P < 0.05$) chromosome aberration in all age groups than that of age matched controls (Table I). The CA% indicated a trend of increase with the exposure time of the subjects (Table II). It was minimum in the > 20 years exposure group (which was higher than the unexposed controls) and in the subjects of > 50 years exposure, it shows the maximum value.

B. Comet Assay

Effect of age and exposure on comet length and tail length –In all the four categories of age (ranging from 30 to 65 years) the comet length in the bidi rollers was found to be significantly greater than that of controls whereas a significant increase in comet tail length was found only in the 20-35 years age group (Table III).

The maximum tail length occurred in the comets of bidi rollers in the 20-35 years exposure group which is an indication of DNA damage in the lymphocytes of this group (Table IV).

TABLE I. Effect of Age on Chromosome Aberrations (CA%) in Bidi Rollers

S/N	Age Groups	CA% (Mean ±SE)	
		Controls (n=30)	Bidi rollers (n = 34)
1	30-35	1.3 ±0.32	3.0 ±0.63*(n = 8)
2	45-50	1.4 ±0.15	3.2 ±0.47*(n = 11)
3	50-55	1.6 ±0.25	3.3 ±0.45*(n = 6)
4	60-65	1.8 ±0.24	3.7 ±0.39*(n = 9)
*Significant at $P < 0.05$			

TABLE II. Effect of Exposure to Tobacco Dust on CA% in Bidi Rollers

S/N	Exposure (Years)	CA% (Mean ±SE) (n=34)
1.	> 20	2.9 ±0.36 (n = 8)
2.	> 30	3.1 ±0.34 (n = 10)
3.	> 40	3.5 ±0.256 (n = 8)
4.	> 50	4.1 ±0.199 (n = 8)

TABLE III. Effect of Age on Comet Length and Tail Length

Age	Bidi roller (n = 34)		Control (n = 30)	
	Comet Length (µm)	Tail length (µm)	Comet Length (µm)	Tail length (µm)
20-35	73.80 ±0.71**	14.30 ±0.68**	51.3 ± 0.98	5.2 ± 0.64
36-50	74.78 ±0.98*	15.73 ±1.75	59.3 ± 0.54	7.72 ± 0.5
51-65	76.99 ±0.81	14.02 ±1.10	63.52 ± 1.27	11.58 ± 0.61
*- $P > 0.05$ and **-$P > 0.01$				

TABLE IV. Effect of Exposure on Comet Length and Tail Length of Bidi Rollers (N = 34)

Exposure	Mean ±SE (n = 34)	
	Comet Length (µm)	Tail length (µm)
10-20	74.00 ±0.78	14.33 ±0.77
21-30	75.88 ±1.02	17.28 ±1.62
31-40	74.65 ±0.85	11.81 ±0.84
41-50	78.35 ±1.13	14.94 ±1.97

C. Urinary Thioether

The average value of excretion of urinary thioether in < 30 year exposure group was found to be 12.58 ± 2.17 µmol/mmol creatinine, while in > 30 years exposed group, it was found to be 22.48 ± 4.92 µmol/mmol creatinine. In the controls it was found to be 6.65 ± 1.07 µmol/mmol creatinine. The data of exposed groups showed significant increase when compared to the control group at $P > 0.05$(Fig. 5).

The CA%, urinary thioether excretion levels and comet length were found to be greater in bidi rollers than in matching controls. These parameters showed an increase with time of exposure to tobacco dust in bidi rollers. Thus, genotoxicity of occupational exposure to tobacco dust is evident in the bidi rollers studied with reference to the parameters taken up.

Increase in CA of bidi tobacco processors due to occupational exposure to tobacco dust was reported by Mahimkar & Bhisey (1995). Cytogenetical damage in petrol pump workers was studied by Yadav and Seth (2001). 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).

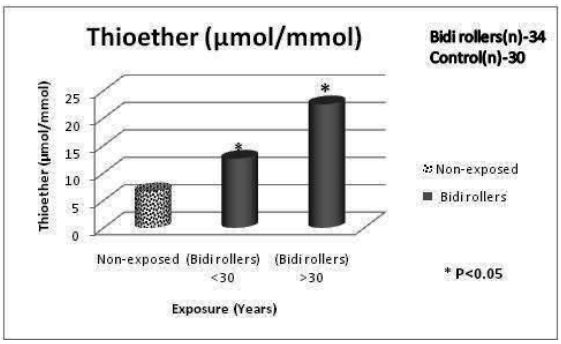

Figure 5. Effect of exposure to tobacco dust on Urinary thioether

Kopjar *et al.* (2006) assessed the degree of DNA damage by comet assay in healthy smokers and compared it to non-smokers. They found that the smokers had a significantly increased comet length, tail moment and larger number of long tailed nuclei than the non-smokers. Blasiak & Trzeciak (1998) found the comet length in treated lymphocytes (with 200 μM of Isomelathion) 56.1 ± 2.19 μm after one hour incubation at 37^0C. This is a 1.7 times increase as compared to the comet lengths of the controls. Our findings displayed 63.52 ± 1.27 μm comet length in non-exposed group. In the 51-65 year exposure group the mean comet length was 76.99 ± 0.81μm which is a 1.21 times increase as compared to the control values.

Garaj Vrhovac *et al.* (2009) found the control tail length to be 13.5 μm. They found the tail length in the exposed group of cigarette factory workers with an average exposure of 19.5 years was 14.34 ± 0.77 μm. This is similar to our finding of the average tail length of 14.33 ± 0.77 in the 10-20 year exposed group. The comet length and tail length data reveal that both these parameters (which are a measure of DNA damage) increase with duration of exposure as well as the age.

Truchon *et al.* (1998) found that the end-of-shift global thioether varied from 1.2-14 μmol/mmol of creatinine in his study of urinary excretion of thioether related to styrene exposure but according to these workers there is an effect of diet also on this parameter. Kilpikari (1981) while working on co relation of urinary thioethers with chemical exposure of a rubber plant found that the mean urinary thioether excretion was 47 ± 1.8 μmol/mmol of creatinine in exposed cases as compared to 37±1.6 of controls. Our data (Fig. 5) also expressed significantly increased thioether levels at $P > 0.05$ in the exposed group 12.58 ± 2.17 μmol/mmol creatinine in < 30 years exposure and 22.48 ± 4.92 μmol/mmol creatinine in > 30 years exposure group than that of non-exposed group (6.65 ± 1.07 μmol/mmol creatinine).

IV. Conclusion

On the basis of the above parameters taken by us for the study of genotoxicity in occupationally exposed bidi rollers, it seemed that the subjects are facing the genotoxic effects of bidi tobacco dust during their work. Hence, they 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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