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EVALUATION OF GENOTOXICITY IN WOMEN BIDI ROLLERS USING COMET ASSAY

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ABSTRACT

A study was conducted on a group of employees from small scale bidi rolling industries in three different districts of telangana state were tested for comet tail lengths that are well established as indicators of early biological effects. To investigate whether occupational exposure to tobacco dust is genotoxic, a total of 182 women bidi workers and 182 control groups of individuals in the age group of 16 to 65 years and 6-30 yrs of tobacco dust exposure were recruited; a questionnaire based survey was conducted. In the present study, the assessment of Primary DNA damage hosted by peripheral blood leukocytes of workers employed in tobacco based bidi rolling industry was performed using the alkaline comet assay, the tail length and long-tailed nuclei thereby being the primary outcome of the measure. A significant increase in the incidence of DNA damage was observed in the experimental subjects when compared to their respective controls. The processing of tobacco leaves generates a lot of dust and facilitates the release of numerous tobacco components in to ambient air. The results obtained in this investigation indicate that bidi rollers seem to be facing the occupational hazard of genotoxicity due to inhalation and handling of bidi tobacco dust.

INTRODUCTION

In India more than five million individuals are engaged in the bidi rolling. A beedi is a thin South Asian cigarette made of 0.2-0.3 g of tobacco flake wrapped in a Diospyrox melanoxylon leaf and secured with colored thread at both ends. As it is a cheap form of tobacco consumption, it is extremely popular among the non-affluent but it carries greater health risks as it delivers more nicotine, carbon monoxide and tar than conventional cigarettes.These individuals' work in small factories or at house-hold based enterprises in an environment filled with tobacco dust. The processing of tobacco leaves generates a lot of dust and facilitates the release of numerous tobacco components in to ambient air. In the present study, the assessment of Primary DNA damage hosted by peripheral blood leukocytes of workers employed in tobacco based bidi rolling industry was performed using the alkaline comet assay, the tail length and long-tailed nuclei thereby being the primary outcome of the measure.

Comet assay can sensitively detect DNA single strand break and alkali-labile site [1]. It was used in this study to examine lymphocyte DNA damage of CT users. This technique has suggested a positive role of the comet assay in the human monitoring of DNA damage from environmental and /or occupational exposure to carcinogenic and mutagenic agents, and has been shown to be a very sensitive method to detect genetic damage at the individual cell level and in human biomointoring [2].

MATERIALS AND METHODS

Chemicals

Agarose-normal melting, agarose-low melting, sodium chloride, potassium chloride, disodium hydrogen phosphate, potassium dihydrogen phosphate, disodium ethylenediaminetetraacetic acid (disodium EDTA), tris, sodium hydroxide , sodium dodecyl sulphate / sodium lauryl sarcosinate, tritron X 100, trichloro acetic acid, zinc sulphate, glycerol, sodium carbonate, silver nitrate, ammonium nitrate, silicotungstic acid, formaldehyde.

Subject Recruitment and Sample Collection:

The study was conducted on 182 females aged 16-66 years from Wrangal, Nizamabad & Adilabad districts of Telangana. The control groups consisted of 182 healthy females aged 16-66 with no history of exposure to clastogenci and/or aneugenic agents and socio-economic level also similar to that of experimental subjects. At the time of sample collection (3ml/individual) all the bidi rollers signed a term of informed consent and replied to Questionnaires elaborated to determine the profile and habits of study population. The protocol has been approved by local ethical committee. The exposed women to tobacco dust, the duration of service was taken more than five years. Peripheral blood samples (V = 5 ml) were collected under sterile conditions by venipuncture into heparinized tubes for comet assay [3].

Single Cell Gel Electrophoresis (SCGE)

The comet assay was conducted under alkali conditions according to Singh et al. (1988) [3]. All chemicals were obtained by Sigma. Two microlitre of whole blood were suspended in 0.5% low melting agarose and sandwiched between a layer, of 0.6% normal melting agarose and a top layer of 0.5% low melting agarose on fully frosted slides. The slides were kept on ice during the polymerization of each gel-layer. After the solidification of 0.6% agarose layer, the slides were immersed in lysis solution (1% sodium sarcosinate, 2.5 M NaCl, 100 mM Na2EDTA, 10 mM Tris–HCl, 1% Triton X-100 and DMSO 10%) at 4 °C. After 1hr, the slides were placed in the electrophoresis buffer (0.3 M NaOH, 1 mM Na2EDTA, pH 10) for 20 min at room temperature to allow for DNA to unwind. The buffers were then chilled and the electrophoresis was performed at 300 mA and 19V in a horizontal electrophoresis platform for 20 min. The slides were neutralized with Tris–HCl buffer (pH 7.5) and stained with 10% ethidium–bromide for 10 min. Each slide was analyzed by using Leitz Orthoplan epifluorescence microscope. For each subject 50 cells were analyzed by automatic digital analysis system Comet assay II (Perceptive Instruments Ltd., Suffolk, Halstead, UK), determining tail length and tail moment (tail length×tail % DNA/100). DNA damage was further quantified by visual classification of cells into categories of 'comets' corresponding to the amount of DNA in the tail according to Anderson et al. (1994) [4].

RESULTS

The frequency of comet tail length in women bidi rollers (site 1) is increased to 4.07 % from 0.90 % in control 1 whereas when the cells were screened from women residing in nizamabad (site 2) the percentage of comet tail length were 5.42% when compared with control value 0.98 %. The frequency of comet tail length in adilabad district (site 3) is 5.82% and 1.02% in controls who are not exposed to any pollutant and away from the tobacco dust region. The difference in the frequency of comet tail length between control and treated groups were statistically found to be significant ($P < 0.01$).

The effect of occupational exposure in bidi rollers on the level of DNA damage in lymphocytes of study group was assessed by the comet assay. The exposed women showed significantly higher levels of DNA damage than controls. The range of mean tail moment (MTM) was higher than controls respective. There was significant difference MTM ($P<0.01$) between experimental and controls. The slides were screened with help of fluorescent microscope available at Indian Institute of Chemical Biology, Tarkana, Hyderabad. ANOVA and simple linear regression analysis were performed to assess the association between endpoints and independent variables.

Table 1: Frequencies of comet tail lengths in women bidi rollers of Warangal district (Site 1)

Treatment	Controls	Exposed group				
		1-7yrs	8-14yrs	15-21yrs	22-28yrs	1-28yrs
No. of samples	61	20	19	12	10	61
Frequency of comet tail length	0.90±0.512	2.60±0.82	3.86±0.96	3.92±1.20	5.92±1.46	4.07±1.36

Table 2: Frequencies of comet tail lengths in women bidi rollers of Nizamabad district (Site 2)

Treatment	Controls	Exposed group				
		1-7yrs	8-14yrs	15-21yrs	22-28yrs	1-28yrs
No. of samples	58	13	18	15	12	58
Frequency of comet tail length	0.98±0.512	2.72±0.52	3.51±0.41	3.09±1.06	5.30±1.82	5.42±1. 84

Table 3: Frequencies of comet tail lengths in women bidi rollers of Adilabad district (Site 3)

Treatment	Controls	Exposed group				
		1-7yrs	8-14yrs	15-21yrs	22-28yrs	1-28yrs
No. of samples	63	18	20	16	9	63
Frequency of comet tail length	1.02±0.512	2.55±0.34	3.91±0.88	3.63±1.44	5.72±1.38	5.82±1.27

DISCUSSION

Bidi rollers, mostly women, are exposed to tobacco constituents via the cutaneous route or through inhalation of tobacco dust [5]. Female *bidi* rollers were monitored for possible genotoxic effects of prolonged exposure to *bidi* tobacco and to study the role of working conditions in occupational hazards. The comet assay can sensitively detect DNA single strand break and alkali-labile sites [1]. The results showed that occupational exposure to tobacco dust can significantly ($P<0.05$) increase DNA strand breakage. The findings of the present study show that *bidi* rollers had an increased level of DNA damage as compared to control population. Working condition is an important factor that contributes to the occupational hazard in the rollers, which is reflected in workers working in confined environmental conditions. Kopjar et al. [6] 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 [7] 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. Dayashankar Gautam and Asha Khanna [8] found 63.52 ± 1.27 µm comet length in non-tobacco exposed group. In the 51-65 year tobacco 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. [9] 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 the findings of Dayashankar Gautam and Asha Khanna [8] 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. Our findings suggest that occupational exposure to toxic components of tobacco dust causes genomic damage in somatic cells of bidi rollers with various etiological factors.

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