Genotoxicity studies on gynecological illnesses among occupational beedi rollers of Northern Telangana

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Abstract– A cross sectional study was made on 126 occupational female beedi rollers from Nizamabad and Adilabad districts of Northern Telangana and many health problems have been identified by interviewing them using questionnaire. The study revealed that 17.4% suffered from respiratory problems, 24.6% from Head ache, 49% reported back pain, 38.8% from shoulder pain, 44.4% suffered from joint pain, 82.5% reported neck pain, 26.1% suffered from eye problems. 7.1% reported numbness of the fingers, 69% were anemic, few of them reported that they had liver problems (2%), 35.7% reported kidney problems, 5.5% reported symptoms of cervical cancer and 22.2% of the female beedi rollers have reported that they have undergone hysterectomy, which was alarming and further study was carried out on these female beedi rollers to find genotoxicity. DNA damage was assessed by comet assay method. Single cell gel electrophoresis resulted 5.8% limited damage of DNA and 2.2% extensive damaged DNA in female beedi rollers who have under gone hysterectomy.

Key words - Comet assay, DNA damage, Genotoxicity, Health hazards, Occupational beedi rollers,

1 INTRODUCTION

Beedi industry is a significant unorganized small-scale industry in most of the rural areas and under developed arid areas of urban localities. Beedi industry provides employment especially to women and girls, which is expected to be more than million people only from Nizamabad and Adilabad districts (1). Female beedi rollers collect raw materials like tendu leaves (*Diospyrox melanoxylon*), tobacco flakes, colour thread etc., from either kharkhana or contractors and handover the finished products i.e., prepared beedi bundles to the contractor or in kharkhana within stipulated time (2). Workers roll 500 to 1000 beedis daily. In this process of rolling beedis the beedi rollers are exposed to the tobacco dust for prolonged hours through cutaneous and nasopharyngeal routes (3). Due to continuous exposure to low ventilated and tobacco dust filled atmosphere in their work areas let it be houses or kharkhanas, they face diversified health problems like asthama (4), back pain, neck pain due to continuous sitting posture (5), head ache, eye problems, anemia, liver problems, generalized problems (6) and gynecologic problems

(Hysterectomy, cervical cancer etc.,) due to unhygienic practices(7). 42% of women were suffering from various gynecological problems There is strong association between increasing age, increasing experience in beedi rolling and gynecological morbidity(8). Most of them have undergone hysterectomy for menorrhagia and other symptoms. Even though lot of work is done on various aspects like socio-economic status of beedi-rollers, their literacy rates, working conditions and hematological studies, but little research work is observed in case of genotoxicity among occupational beedi rollers. So, the research study was taken up to find the genotoxicity studies on gynecological illnesses among these occupational beedi rollers in northern Telangana.

2. **Materials and methods:** 6 villages namely Bejjora, Kasbagalli, Bheemgal. Siripuram, Mudhirajgally and Pochammagally from Nizamabad and 5 villages from Adilabad districts Vatoli, Gundampally, Kadthal, Venkatapur, Temborni were selected for the present research study.

The female beedi rollers those who had a minimum work experience of 15 years with an average exposure of 8 hrs per day as a roller were selected for the study. Beedi rollers who had gynecological problems and undergone hysterectomy were specifically selected as an experimental group for the research study. Women who carried household activities without any exposure to tobacco were taken as control group for the study.

The research activity and comet assay tests were carried out in the laboratory of Genetics Research centre, Institute of Genetics and Hospital for Genetic diseases, Osmania University, Hyderabad from January, 2022 to June,2022. June, 2022. 5 ml of the blood sample from the selected study samples were collected early in the morning and dispensed into EDTA tubes for analysis of DNA damage by comet assay.

The Comet assay was performed according to the protocol outlined by Singh *et al*., (1988).

1. Clear plain slides dipped in 1% NMPA, wiped and dried at 37℃ over night were taken.
2. Pre coated slides were layered with a mixture of 110 µl of 0.5% or 0.75% or 1% LMPA and 20 µl of whole blood. The cover slip was placed and gel was allowed to set for 4 to 10 minutes. The cover slip was slide off.
3. The slide was layered with 110µl of LMPA for the third agarose layer. The cover slip was placed on the gel and allowed to set for 4 –10 minutes. The cover slip was slide off.
4. The slides were placed in cold lysing solution at 4^0c for 1-24hrs
5. The slides from lysing solution were taken and placed them side by side with gel touching with each other in horizontal electrophoresis box. The electrophoretic chamber was filled with buffer until the buffer level completely covered the slides. Bubble formation was avoided. The slides were kept in alkaline buffer for 40 min to allow the DNA for unwinding.

6. Supply was turned on to 25 volts and current was adjusted to 300 mA by raising or lowering the buffer level. Electrophoresis was carried out for 30 min. Power supply was turned off and the slides were lifted gently and placed on a staining tray
7. The slides were flooded with 0.4 M Tris neutralizing buffer for 10 min and the buffer was drained off (This step was repeated twice)
8. The slides were placed in fixing solution for 10 minutes and washed with distilled water for every five minutes. The slides were air dried at 37^0 C for one hour to overnight.
9. PBS – 5 ml, Giemsa -5 ml, Distilled water - 40 ml (9)these components were mixed thoroughly and added to the coupling jar containing slides. Kept for 5 to 10 minutes. After that the slides were removed from coupling jar and carefully the slides were washed with distilled water to remove excess stain.
10. The slides were dipped in a jar containing stopping solution for 10 minutes until yellowish brown colour was developed. The slides were washed with distilled water and dried in inclined position at room temperature. The slides were stored in dust free environment for a long period.
11. The slides were observed under microscope.

Note: Step 2 & 4 were done under dim light.

3. Results:

In the present study, percentage of limited damaged cells is high (5.8%) in case of beedi rollers compared to control group (2.2%). High percentage of extensively damaged cells (2.2%) in beedi rollers was noticed, whereas in control group it was 0% Predominantly total damaged cells in beedi rollers were significantly increased and difference is considered to be statistically significant. The findings of the present study showed that beedi rollers had an increased level of DNA damage as compared to control group of people. The results of the study provided confirmation that beedi rollers seem to be facing the occupational hazard of genotoxicity due to inhalation and handling of tobacco dust.

CometAssay

Figure 1 shows the variation in cells of control and beedi rollers after comet assay. Tables 1 and 2 show the results of comet assay of control and beedi rollers respectively. The percentage of no migration (undamaged), limited migrated (limited damaged) and extensively migrated (extensively damaged) cells in control group is 97.8, 2.2 and 0 respectively. The percentage of undamaged, limited damaged and extensively damaged cells in beedi rollers group is 92, 5.8 and 2.2 respectively. The percentage of total damaged cells is high (5.8%) in case of beedi rollers compared to control group.

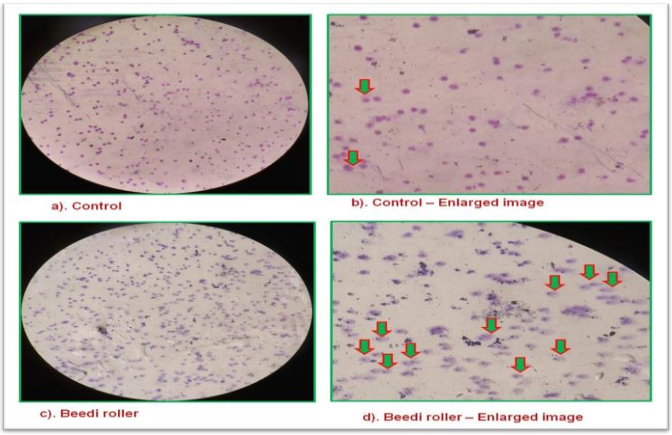

Figure 1: Comparison of microscopic images of Comet assay in control (a, b) and beedi roller (c, d) under 400X magnification - Comets are clearly visible in b and d (marked with arrows). Huge variation in number of comets [Beedi rollers have higher number (d) compared to control (b)] are noticed.

Table 1: Results of Comet assay for Control group

S.No.	Age (Yrs)	Years of experi-ence in beedi rollers	Grade of damage of DNA per 100 cells		
			No migration	Limited migration	Extensive migration
1	46	0	99	1	0
2	49	0	97	3	0
3	32	0	98	2	0
4	36	0	97	3	0
5	47	0	98	2	0
Mean	**42.00**	**0.00**	**97.80**	**2.20**	**0**
SD	**7.52**	**0.00**	**0.84**	**0.84**	**0**
SE	**3.36**	**0.00**	**0.37**	**0.37**	**0**

Table 2: Results of Comet assay for Experimental group (Women Beedi rollers)

S. No.	Age (Yrs)	Years of experience in beedi rollers	Grade of damage of DNA per 100 cells		
			No migration	Limited migration	Extensive migration
1	46	18	88	8	4
2	49	21	90	8	2
3	39	16	93	6	1
4	36	13	97	2	1
5	49	25	92	6	2
6	43	22	94	5	1
7	48	24	92	6	2
8	37	17	91	6	3
9	42	19	93	4	3
10	45	20	90	7	3
Mean	**43.40**	**19.50**	**92.00**	**5.80**	**2.20**
SD	**4.84**	**3.69**	**2.49**	**1.81**	**1.03**
SE	**1.53**	**1.17**	**0.79**	**0.57**	**0.33**

Statistical analysis of Comet assay results

Table 3 shows the results of Kolmogorov-Smirnov test for comet assay. *The* Kolmogorov-Smirnov test shows there is difference among control and beedi rollers in the aspect of undamaged cells ($D = 0.743$, $p < 0.05$), limited damaged ($D = 0.787$, $p < 0.05$) and extensive damaged cells ($D = 0.859$, $p < 0.05$). The values indicate the null hypothesis is rejected and suggesting that the two populations are from different distributions.

Table 3: Kolmogorov–Smirnov (K-S) normality test for Control *Vs* Beedi roller of comet assay results

Variable	Kolmogorov- Smirnov[a]	
	Statistic (D)	Sig. (p)
Undamaged cells	0.743	0.009**
Limited damaged cells	0.787	0.009**
Extensive damaged cells	0.859	0.008**

- Represents the lower limit of the true significance
- a. With the correction of Lilliefors

****Significant at p<.05**

Table 4 shows the statistical analysis of Student's t-test for the comet results in control and beedi rollers indicates a significant difference in number of damaged cells. The difference among the undamaged cells (no migration) of control and beedi rollers is significant (t = 4.97, p = 0.0002). The difference between limited migrated cells of control and beedi rollers is significant (t = 4.163, p = 0.0011). Similarly, the difference among the extensive migrated cells of control and beedi rollers is significant (t = 4.67, p = 0.0004. Limited migrated and extensive migrated cells in women engaged in beedi rolling were higher than those of control population and is significant (t = 3.633, p<.0011). Over all total damaged cells in beedi rollers were significantly increased and difference is considered to be very statistically significant.

Table 4: Student's *t*-test for data of comet assay (control *Vs* Beedi roller)

Statistics	No migration		Limited migration		Extensive migration	
	Control	*Beedi rollers*	*Control*	*Beedi rollers*	*Control*	*Beedi rollers*
Mean	97.80	92.00	2.20	5.80	0.00	2.20
Variance	0.70	6.22	0.70	3.29	0.00	1.07
Popula-tions (n)	5.00	10.00	5.00	10.00	5.00	10.00
Pooled Variance	4.52		2.49		0.73	
Hypothe-sized Mean Difference	0		0		0	
Df	13		13		13	
t Statistics	4.979		4.163		4.674	
t Critical one-tail	1.770933		1.770933		1.770933	
P(T<=t) one-tail	0.000126**		0.000556**		0.000218**	
t Critical two-tail	2.160368		2.160368		2.160368	
P(T<=t) two-tail	0.000252**		0.001113**		0.000435**	

****Significant at p<.05**

Discussion

Due to illiteracy, poor socio-economic status, unawareness on risk factors during handling of tobacco, inadequate ventilation, improper lighting and unhygienic practices followed by the rollers have led to the gynecological problems which are identified as infections in uterus, symptoms of cervical cancer and led to hysterectomy.

Several studies made earlier supported the results of present research study. Beedi rollers showed increased chromosome aberration and lengthy comet tail compared to control group(10). a significant increase of DNA damage was observed in beedi rollers when compared to their relevant controls (11). Increased mean comet length and tail length with exposure of time during beedi rolling was observed. beedi rollers are exposed to xenobiotics during their occupation and have increased level of DNA damage as contrast to control (12). A significant increase in deletion fragments and chromatid gaps in the beedi rollers indicating that occupational exposure to tobacco imposes substantial genotoxicity(13).

Conclusion:

Occupational exposure for prolonged period of 8- 10 hours to tobacco dust was found to be associated with the risk of developing many gynecological problems.

This was due to unhygienic practices followed by female beedi rollers which have led to development of many gynecological infections, symptoms of cervical cancer and ultimately resulted in removal of uterus (hysterectomy). There is urgent rush on creating awareness on simple hygienic practices to be followed by occupational beedi rollers.

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