

Genotoxic Effects of Tobacco Chewing

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ABSTRACT

Tobacco chewing is a widespread habit which leads to DNA damage. We are reporting a case of a tobacco chewer in which chromosomal aberrations, DNA breakage, buccal micronuclei and urinary thioether excretion level were studied. The study was carried out on a 28 year old male subject who is polio affected since his childhood. He has been chewing tobacco since the last 17 yrs @ 4 g, 08 times per day. The medical report of the subject indicates no abnormalities except post-polio paralysis in both lower limbs. He has no family history of any genetic disorder. He is not occupationally exposed to tobacco. The findings of the present investigation indicate increased incidence of chromosomal aberration % and micronuclei in buccal epithelial cells than the control values obtained from a subject of similar age and socioeconomic condition but not addicted to tobacco chewing. However, the urinary thioether values of the subject were lower than control values indicating a depression of the detoxification pathway.

Key words: Chromosomal aberrations, comet assay, micronucleus, tobacco chewers, urinary thioether

Tobacco chewing seems to be genotoxic as per the parameters studied in this case. The net harmful effects depend on the efficiency of DNA repair mechanism. The continued habit may lead to probable malignancy later in the life.

INTRODUCTION

Tobacco (Gutka chewing) is a very wide spread habit in Jabalpur, M.P. and its adjoining areas. It is one of the established risk factors for oral cancer. The carcinogenic potential of tobacco is well known^[1,2] However, not all tobacco consumers suffer ultimate consequences. This might be due to the genetic polymorphism or different

DNA repair capability of individuals towards the causative factor.^[3] A case study of a 28 year old healthy (but Polio affected) male who has been chewing tobacco since the last 17 years is presented. His blood and urine samples were taken with his consent. It shows an elevated occurrence of chromosome aberrations, micronuclei in buccal epithelial cells, higher percentage of cells showing DNA damage (studied by comet assay) as compared to a normal individual of the same age and socio economic conditions. His urinary thioether excretion was also examined. Cases of genotoxicity due to tobacco chewing have been reported taking buccal micronuclei or chromosome aberrations into consideration^[1,2,14] but the reports of such cases including the composite effects of the chewing habit on buccal micronuclei, chromosome aberrations, DNA damage by comet assay and urinary thioether excretion levels have not been come across.

CASE REPORT

The investigators were primarily working on occupational genotoxicity in bidi rollers for which purpose a control blood sample of a normal male was brought to the lab.

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When his chromosome slides (after peripheral blood culture and air drying/hypotonic/Giemsa technique) were examined, high incidence of aneuploidy was found apart from out of phase divisions and some evidence of

fragmentation and stickiness of chromosomes in prophase nuclei [Figures 1-6]. Upon enquiry, he turned out to be a tobacco chewer. The study was carried out on this 28 year old male subject who is polio affected since his childhood.

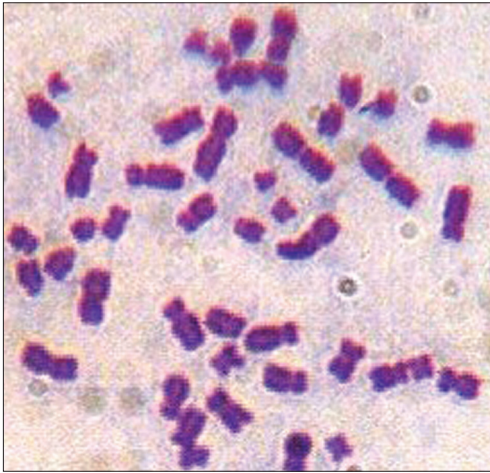


Figure 1: Normal metaphase plate

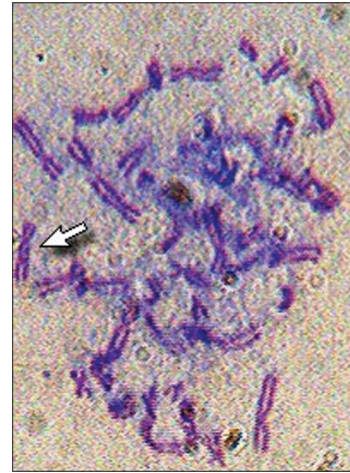


Figure 2: End to end association in tobacco chewer

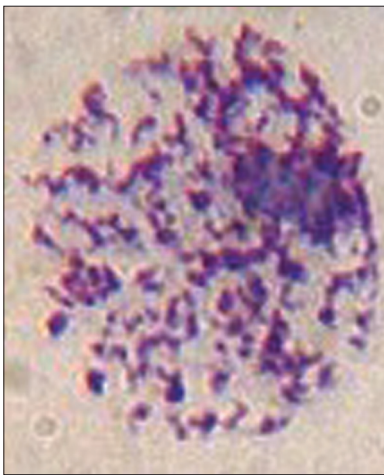


Figure 3: Metaphase plate showing sticky and pulverized chromosomes in tobacco chewer



Figure 4: Early metaphase chromosomes showing loop formation in tobacco chewer

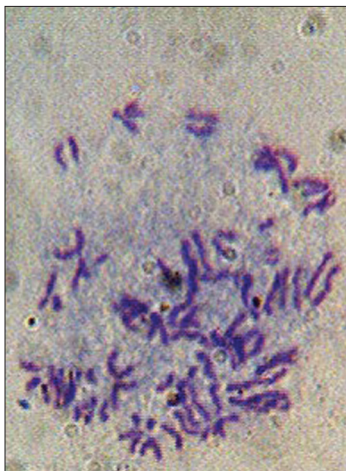


Figure 5: Centromeric separation and fragmentation in tobacco chewer

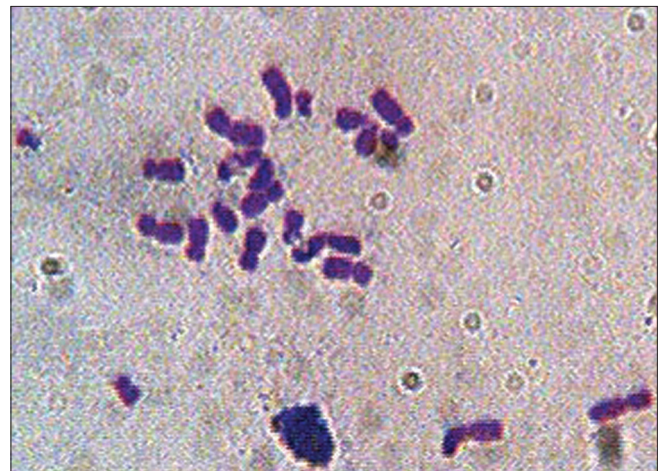


Figure 6: Haploid metaphase chromosomes of the tobacco chewer

On medical examination by a registered medical practitioner (MD Medicine), no abnormalities except post-polio paralysis in both lower limbs were found. His temperature was normal, pulse rate was 72/min, BP was 120/80. No pallor, cynosis, clubbing, icterus and oedema was found. No abnormalities were found in per abdomen examination, both lungs were clear and both heart sounds were normal. His blood profile shows Hb 12.2 g%, Serum creatinine 1.2 mg/dl, SGOT was 22.7 IU/L and SGPT was 31.0 IU/L. All these values are in the normal range. His urine creatinine clearance test is also normal (95 ml/min) He has no family history of any genetic disorder.

MATERIALS AND METHODS

Since the subject had been chewing tobacco since the last 17 yrs @ 4 g, 08 times per day, his LTE (life time exposure=frequency per day × no. of years) calculated by the method of Patel *et al.*, (2010)^[4] comes out to be 136. The chromosome aberrations were studied from preparations of chromosomes from lymphocyte culture (Moorhead *et al.*, 1960).^[5] Venous blood of the subject was cultured in TC-199 Medium supplemented with foetal bovine serum. Cells were harvested after 72 hrs but one hour before harvesting, colchicine was added @ 10 µg/ml. Chromosome spreads were made by the usual air drying, hypotonic Geimsa technique. The stained slides were scored for chromosomal aberrations by scoring 100 metaphase plates each for the subject and the control. Urinary thioether excretion levels were determined after alkaline hydrolysis by Ellman's spectrophotometric method as described by Vainio *et al.* (1978).^[6] The urinary thioether levels were expressed as µmole/mmol of creatinine. The urinary creatinine levels were determined by alkaline picrate method using commercial (Cogent) kit. Comet assay for the assessment of DNA damage was done according to the alkaline single-cell gel electrophoresis method (Singh *et al.*, 1988, Dhawan *et al.*, 2002)^[7,8] Comets were visualized by silver staining method (Silvina, 2001).^[9] DNA damage was quantified by visual classification of cells into categories of comets corresponding to the DNA damage (Nandhakumar *et al.*, 2011 and Khanna *et al.*, 2011).^[10,11] The buccal micronuclei

slides were prepared (Surrals *et al.*, 1997)^[12] and buccal epithelial cells (2000 cells each) were scored for the chewer and the control.

DISCUSSION

In the present study, the mean chromosomal aberrations (mean CA%) in tobacco chewer was found to be 2.4±0.69 whereas, in the controls this value was 1.2±0.41. This showed a significant increase ($P<0.01$) in the chewer as compared to the control. The spontaneous chromosomal aberrations (SCA) per cell was found to be 0.27 in the chewer as compared to 0.12 in the control. The results of chromosomal aberrations %, SCA per cell are summarized in Tables 1 and 2.

The measurement of urinary thioether excretion provides an index of the extent of exposure to mutagens. Exposure of man to electrophilic compounds may ultimately lead to the excretion of thioethers by mediation through glutathione. The urinary thioether (expressed as µmole/mmol of creatinine) was found to be 9.0 in the chewer as compared to 14.6 in the control which indicates a depression of the detoxification pathway in the subject [Table 3].

The global thioether levels in lamination workers exposed to styrene was found to be 8.4-7.5 µmole/mmol of creatinine by some workers.^[13] They found the global thioether values ranging from 1.2 to 14 µmole/mmol of creatinine in such workers. This agrees with our findings.

The buccal micronucleus is a small, round or oval cytoplasmic chromatin mass next to the nucleus. The micronuclei originate from aberrant mitosis and consist of chromosome fragments/acentric fragments that have failed to incorporate into daughter nuclei. It is a commonly evaluated index of genotoxicity.^[14] The mean buccal epithelial micronucleus value was found to be 1.5±0.5% in the tobacco chewer as compared to the normal healthy control value of 0.05% which shows a significant increase [Figure 7]. A significant increase in micronucleus % (MN %) in chewers (1.90±1.03) as compared to controls (0.81±0.66) has been reported by some workers.^[14] Similar observations were made by other workers in tobacco processing.^[1,15]

Comet assay (done by the process of alkaline single-cell gel electrophoresis) is a well-known technique for the assessment of double stranded DNA breaks. In the present study after assessment by comet assay [Figure 8a-e] the % of damaged

Table 1: Chromosomal aberrations %

Sample	CA % Mean±SD	Remarks
Control	1.2±0.41	-
Tobacco chewer	2.4±0.69	Significant increase as compared to control $P<0.01$

Table 2: Spontaneous chromosomal aberrations per cell

Sample	No. of metaphase plates counted	Aneuploidy	Dicentric	Satellite associations	Ring chromosomes	Chromatid break	CA per cell
Control	100	11	-	01	-	-	0.12
Tobacco chewer	100	21	02	02	01	01	0.27

Table 3: Urinary thioether levels

Sample	Urinary thioether expressed as $\mu\text{mole}/\text{mmole}$ of creatinine
Control	14.6
Tobacco chewer	9.0

Table 4: Results of comet assay: Percentage of cells in different categories of DNA damage (A-E showing progressively greater degree of DNA damage)

Sample	Cell % in different categories				
	A	B	C	D	E
Control	44 \pm 3.9	38.4 \pm 3.24	14 \pm 1.87	2 \pm 0.70	1.2 \pm 0.83
Tobacco chewer	14.2 \pm 3.3	30.4 \pm 1.97	59.16 \pm 2.84	15 \pm 2.38	5.8 \pm 1.93

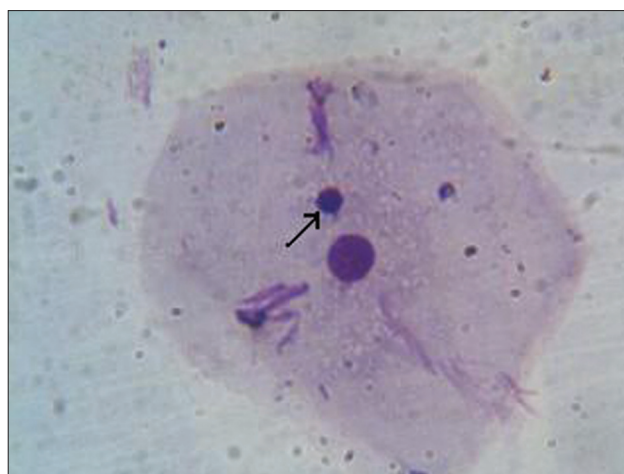


Figure 7: Buccal epithelial micronucleus of tobacco chewer

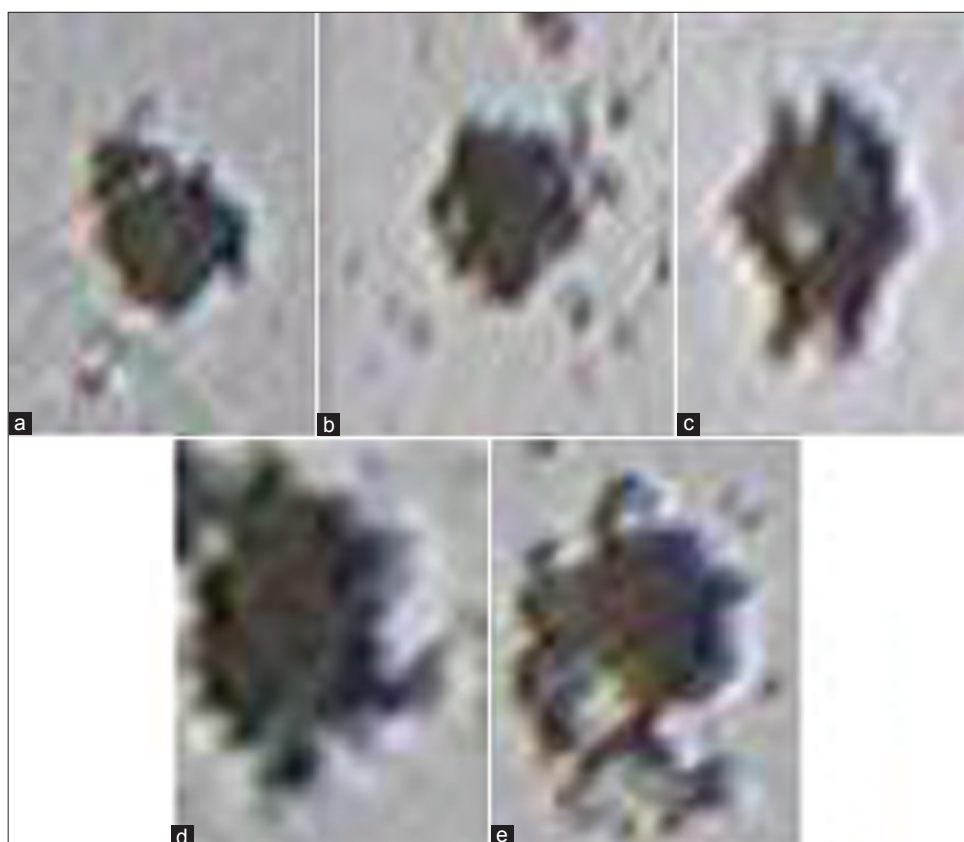


Figure 8a-e: Comet images from the tobacco chewer showing progressively greater DNA damage

cells (C-Category) is significantly higher (59.16 \pm 2.84) in the chewer than in the control (14 \pm 1.87). The chewer has only 14.2 \pm 3.3% cells in the non-damaged category as opposed to 44 \pm 3.9% cells in the control [Table 4].

A significant increase in the tail length of comets in bidi rollers exposed for more than 20 yrs (2.34 \pm 0.05 μ) as opposed to controls (1.68 \pm 0.01 μ) has been reported earlier by the first author.^[16,17]

Thus the chewer in the present case seems to have a higher

CA %, more damaged DNA as assessed by comet assay and higher percentage of micronuclei in buccal epithelial cells than the control. However his urinary thioether excretion seems to fall in the range of other environmentally affected persons. He has been advised to cut down his chewing habit which he is trying to do inspite of withdrawal symptoms.

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