



Influence of Dietary Ascorbic Acid Intake in Women Bidi Rollers by Comet Assay

P. Minny Jael

Human Genetics and Molecular Biology Lab, Department of Zoology, Osmania University, Hyderabad -500007

K. Rudrama Devi

Human Genetics and Molecular Biology Lab, Department of Zoology, Osmania University, Hyderabad -500007

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. A population monitoring study was conducted in human lymphocytes by analysis of comet tail length to investigate whether occupational exposure to tobacco dust is genotoxic in women bidi rollers exposed to tobacco work environment. There was significant increase in the frequency of chromosomal aberrations in human lymphocytes of women bidi rollers. In addition, a study on the antimutagenic effect of Vitamin C administered orally to women bidi rollers exposed to tobacco dust was investigated by measuring the frequency of comet tail length had a three month daily intake of Vitamin C. The results clearly showed a significant reduction on comets frequency evaluated after vitamin-C treatment. The results of the present study indicate the 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. AA supplementation plays an important role in inhibiting the DNA damage in women bidi rollers exposed to tobacco dust useful findings to public health as lakhs of women are engaged in bidi rolling

KEYWORDS : Tobacco dust, Occupational exposure, comet tail length, genotoxic AA supplementation

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 (Tice et al, 1995). 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 biomonitoring (meller., 2000). Ascorbic acid (vitamin C) is an essential nutrient in feeds, and is an indispensable nutrient required to maintain the physiological processes of different animals (Tolbert, 1979). Small amount of this vitamin is sufficient to prevent and cure scurvy; however, larger amount may be essential to maintain good health during environmental adversities, situation of physiological stress and conditions of infectious and parasitic diseases (Mc Dowell, 1989, Lim, 1996). Ascorbic acid (vitamin C) is essential for producing collagen and bone minerals, assists in metabolizing iron and helps in activation of vitamin D. It also assists in reducing the harmful effects of hormones produced by the adrenal gland during prolonged periods of stress (Navarre and Halver, 1989). Also, it has an important role in a great number of biochemical processes such as synthesis of collagen which is an intercellular protein and princi-

pal constituent of skin, scales, mucosa, cartilaginous tissues, bones and conjunctive tissue formation, which involves all the organs of the body (Mc Dowell, 1989). Hence in the present studies the influence of dietary ascorbic acid intake has been investigated in women beedi rollers using comet assay.

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 clastogenic 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 (Singh et al, 1988).

Estimation of ascorbic acid

Plasma ascorbic acid levels were measured by an ion pairing HPLC method in control and as well as in workers occupationally exposed to tobacco dust.

Single Cell Gel Electrophoresis (SCGE)

The comet assay was conducted under alkali conditions accord-

ing 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).

RESULTS

Table 1: Characteristic profile of control and women bidi rollers.

Groups	Sample	AA	No.of examines	Age in Years Mean±SD	Employment (Years)
Control	Feb 2014	-	50	32.80±0.80	22.95±1.90
	April 2014	+	42	34.70±1.20	22.10±0.80
Exposed	Feb -2014	-	84	36.80±1.20	29.80±1.20
	April - 2014	+	74	38.20±0.06	22.10±0.80

P>0.05

Table 2. Levels of Ascorbic Acid in women bidi rollers

Groups	No. of samples	Levels of Ascorbic Acid mg/dl
Control	50	0.46±0.07
Women Bidi workers	84	0.36±0.06*
Group A	46	0.58±0.06**
Group B	38	1.10±0.04**

a) * P<0.05 Significant with control group

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 17: Effect of ascorbic acid for 12 weeks on the frequency of comet tail length in women bidi rollers

Group	Sample timing	Ascorbic acid	No.of-examinees	Frequency of comet tail length
Control	Feb -2014	-	42	0.86±0.52
	April- 2014	+	40	0.92±0.86
Exposed group A	Feb -2014	Pre	36	5.82±0.96a
	April - 2014	Post	34	4.12±1.12b

Exposed group B	Feb- 2014	Pre	38	5.60±1.10a
	April - 2014	Post	32	2.46±1.20b

The frequencies of comet tail length prior to AA supplementation was 0.86±0.52 and increased to 0.92±0.86 after 12 weeks of AA supplementation. However the differences between the percentages of micronuclei between two groups were found to be insignificant (P>0.05).

The percentage of comet tail length is significantly increased from 0.86% to 5.82% and 5.60 % in tobacco dust exposed workers (Group A & B). However when subjects were given vitamin c there is a decrease in the percentage of comet tail length to 4.12 % in group A 2.46 % in group B respectively. The differences in the percentages of comet tail length between tobacco dust and controls were found to be significant where as the frequency of comet tail length between tobacco dust (prior to AA administration) and AA treated group where showed statistically significant (p<0.05). Hence the above results clearly indicate the protective role of Vitamin - C in tobacco dust exposed women. Use of Vit-c 1000mg is safer to women particularly for occupationally exposed population.

DISCUSSION

Bidi rollers, mostly women, are exposed to tobacco constituents via the cutaneous route or through inhalation of tobacco dust (Bhisey and Govekar 1991). 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. 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. Many naturally occurring substances in plants and other sources have protective effects on environmental mutagens / carcinogens and also on endogenous mutagens (Ferguson, 1994). It has been reported that the common use of antimutagens and anticarcinogens in everyday's life will be the most effective against the genetic and other related diseases (Vitamin supplements have a marked potentiality against toxic effects of diversified environmental chemicals. Antioxidant supplements decrease oxidative DNA damage in humans (Duthie et al., 1996), as do antioxidant-rich foods (Pool-Zobel et al., 1997; Mitchell and Collins, 1999; Collins et al., 2001). Earlier we have been published on the incidence of chromosomal aberrations and sister chromatid exchanges in women bidi rollers exposed to tobacco dust (Rudrama devi et al., 2011, 2012).induction of increased comet tail lengths were noted in women bidi rollers. (rudrama devi and minny, 2015)

The present investigated results are in agreement with that of Sram et al (1983a) reported a high frequency of chromosomal aberrations in peripheral lymphocytes of coal-tar workers, occupationally exposed to polycyclic aromatic hydro carbons and benzene was reduced by ascorbic acid prophylaxis at a daily dose of 1 gr. Further, Sram et al (1983b), who reported a significant decrease in the frequency of chromosomal aberrations in peripheral blood lymphocytes of workers occupationally exposed to halogenated ethers. Furthermore, the present results are in augmentation with Pohl and Reidy (1989), where the intake of vitamin-C decreases the chromosomal damage in bleomycin drug induced in cancer patients. The inhibitory effects of ascorbic acid in various mammalian test systems were reported with insecticide Ragor (Hoda et al 1993), Endosulphan, Phosphanidon and Mancozeb (Khan et al 1993) Cisplatin (Giri, 1998) and Cyclophosphamide and Bleomycin (Vijaya Laxmi et al 1992). Further Cohen et al, (1993) reported that the chromosomal damage and DNA strand breaks were reduced in mammalian cells in the presence of an antioxidant ascorbic acid.

A second relevant aspect of our results is the clear inhibitory effects of the genotoxicity of lead exposure by a continuous treatment with a complex polyvitamin mixture. These results agree with recent studies in Chernobyl clean-up workers (Oganesian et al., 1997, Emerit et al., 1997 Lazutka 1996), where the use of multivitamins as dietary sup-

plement significantly decreases the frequency of chromosome aberrations, specially chromatid breaks; and confirming previous results where a decrease in occupational induced chromosome damage in lymphocytes after prophylaxis with vitamin C (Sram et al., 1983), vitamins A and E, (Mierauskiene et al., 1993) or polyvitamin complexes (Vaglenov et al., 1997) has been detected. Earlier we reported the protective effects of ascorbic acid in drugs and heavy metals induced cytotoxicity in *in vivo* and *in vitro* mammalian test systems (Reddy et al., 2002, Rudrama devi et al., 2003, a.b.c., Madhavi and Rudrama Devi 2003, Shoba Rani and Rudrama Devi 2006, Shoba Rani et al., 2009,

Vitamin C is a known free-radical scavenger and has been shown to inhibit lipid peroxidation in liver and brain tissue of lead-exposed animals. In lead-exposed rats, a minimal 500 mg/L concentration in drinking water was able to reduce ROS levels by 40 percent (Hsu PC et al 1998). In other animal studies, the toxic effects of lead on heme production were reversed by a vitamin C dose of 100 mg/kg (Vij et al 1998). Other studies indicate vitamin C might have significant chelation capacity for lead. One rat pharmacokinetic study found intravenously administered vitamin C lowered lead tissue levels in rats that were continuously administered lead (Dalley et al 1990). A human study, evaluating blood lead levels in pregnant women, found that 1,000 mg vitamin C per day, in addition to a prenatal multivitamin supplement, significantly lowered blood lead levels from a mean of 5.1 to 1.1 µg/dL during the course of pregnancy (West et al, 1994). In a study of silver refining (involving lead smelting), workers with mean blood lead levels of 32.84 µg/dL and symptoms of lead toxicity (anemia, muscle wasting, abdominal colic) were given thiamine (vitamin B1) or vitamin C to evaluate the ability of these supplements to affect lead exposure. With continuous lead exposure and either 75 mg thiamine once daily or 250 mg vitamin C twice daily for 30 days, both vitamins significantly lowered blood lead levels (Tandon et al, 2001).

In a study assessing the mechanism of vitamin C's lead-lowering capacity, 75 male smokers with no known occupational or residential lead exposure were given 1,000 mg vitamin C daily for 30 days. Blood lead levels were effectively lowered from a mean of 1.8 µg/dL to 0.4

µg/dL within one week and remained at that level for the remainder of the study (Dawson et al, 1999). Vitamin C was effective at inhibiting lead uptake and reducing lead cytotoxicity (Fischer et al, 1998). Vitamin C, in combination with silymarin, has also been shown to effectively reduce the hepatotoxic effect of acute lead poisoning (Shalan et al, 2005). The hypothesis that dietary antioxidant vitamins, minerals and trace elements play a significant role in reducing the incidence of human cancer, has received special interest during the last decades, but, although the overall results are promising, data seems insufficient to extract conclusive results (van't Veer et al., 1996, van Poppel et al., 1997).

However there are many results showing the antioxidant vitamin supplementation exhibits an overall protective effect against DNA damage induced in human cells by X-ray or H2O2 treatments, as demonstrated by using the comet test (Sweetman et al., 1997) or the micronucleus assay (Umegaki et al., 1994). These results indicate that the effects of oxidative stress have the potential to be modified by the presence of antioxidants, the level of protection appear to depend on the nature and intensity of the oxidative stress. Supplementation of the chemo-preventative compounds has been known to be a strategy for protection against oxidative damage caused by environmental agents. The research development in this field has been established for the detection of anti-risk factors in human beings.

CONCLUSIONS

This work shows a clear genotoxic effect associated to the occupational exposure to high tobacco levels that can be significantly reduced by 3 month Ascorbic acid supplementation hence to the industry management it is advised to use the vitamin supplementation to the workers as the occupational exposure is genotoxic and the workers are continuously inhaled the fumes of tobacco dust has been proven as carcinogenic. The safety measures such as wearing gloves and masks and maintenance of the work place is very important for the health of the individuals. The results of our studies are useful for public health and medical community.

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