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EFFECT OF ASCORBIC ACID PROPHYLAXIS ON THE FREQUENCY OF CHROMOSOMAL ABERRATIONS IN PERIPHERAL LYMPHOCYTES OF WOMEN BIDI ROLLERS EXPOSED TO TOBACCO DUST

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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 84 women bidi workers and 50 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 chromosomal aberrations.* A population monitoring study was conducted in human lymphocytes by analysis of chromosomal aberrations 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 chromosomal aberrations had a three month daily intake of Vitamin C. The results clearly showed a significant reduction on chromosomal aberrations frequency evaluated after vitamin-C treatment. The results of the present study indicate the protective nature of AA supplementation in Women bidi rollers exposed to tobacco dust.

KEYWORDS: Chromosomal aberrations, ascorbic acid, women bidi rollers.

1. 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.

2. MATERIALS AND METHODS

2.1 Selection of population

The antimutagenic nature of prophylactically administered ascorbic acid (A.A) preparations was studied in a group of 134 women bidi rollers, exposed to tobacco dust. A daily dose of 1g for 5 consecutive days in a week was given systematically to the women bidi rollers in tablet form with brand name –Galen Pharmaceuticals pvt. Ltd. The effectiveness of ascorbic acid prophylaxis was evaluated in human peripheral blood lymphocytes obtained from the women bidi rollers, examined before and after 3 months treatment with ascorbic acid. Group of 50 unexposed people belongs to the same age group and socio economic status was collected as controls for the purpose of comparison. Out of 46 subjects, only 38 individuals were responded to take these preparations and continued for 3 months (Group A). Out of 38 subjects, only 36 individuals were responded to take these preparations and continued for 3 months (Group B). The blood samples were collected under medical supervision the clinical data was collected using standard questionnaire. The workers exposed to Tobacco dust are divided into 2 groups. Group A – administered with 500mg group A,AA daily for 3 months. Group B- administered with 1000mg, AA daily for 3 months.

2.2 Estimation of ascorbic acid

Plasma ascorbic acid levels were measured by an ion pairing HPLC method.[1] in control and as well as in workers occupationally exposed to tobacco dust.

2.3 Human lymphocyte culture

Human lymphocyte culture method is a sensitive tool in evaluating the adverse effects of chemicals on the genetic component of man. Human peripheral blood contains a large number of B and T- lymphocytes, which can be stimulated to proliferate in *in vitro* conditions with the suitable mitogen. PHA (Phyto haemoagglutinin) is the most commonly used mitogen, which is having a marked capacity to inhibit T-lymphocytes. Generally the lymphocytes are always in Go-stage of cell cycle i.e., in the form of unreplicated chromosomes. In culture systems, the cells reenter into the cell cycle, under the influence of mitogen. The mitogens are generally inducing aberrations in G1, and S-phase of cell cycle. Lymphocyte cultures were prepared and harvested by adopting the standard method of Moorhead et al,[2] with slight modification.

2.4 Preparation of culture medium

The RPMI-1640 culture media was prepared to culture the peripheral human lymphocytes, which contains the following chemicals. (i) RPM-(10X) 10ml, (ii) Phytohaemoagglutin P-form 0.5ml, (iii) foetal Bovine Serum 5 ml, (iv) Streptomycin 0.25ml, (v) Gentamycin 0.25 ml, (vi) Sterile double distilled water 90 ml. The pH was adjusted to 7.2 using 10% sodium bicarbonate solution 6 to 8ml of medium was distributed in each 30 ml vial.

2.5 Collection of blood samples and initiation of cultures

Intravenous blood from the Bidi workers were collected in 10 ml heparinized (100 units/ml) sterile syringe. After the blood drawn, initiate the cultures within 24 hrs. 0.5ml blood of each individual was added in to the culture vials, containing 8 ml of RPMI-1640 culture media and duplicated cultures were maintained for each sample. Control batch of cultures wee setup simultaneously for each batch of experiment and all the cultures were incubated at 37^{o}C for 72 hrs.

2.6 Harvesting of the cultures

0.5 ml (0.6µg/ml) of colchicines was added to all the cultures, before 2 hrs of culture termination to inhibit the spindle formation. At the end of 72 hrs, each culture was transferred into 15 ml centrifuge tube and centrifuged at 1000 r.p.m. for 10 minutes. The supernatant was

discarded and 5 ml of pre-warmed hypotonic solution (0.75M Kcl) was added to the pellet and the cells were incubated at 370C for 25 minutes. After the hypotonic treatment the cell suspension was recentrifuged again at 1000 r.p.m. for 10 minutes. Supernatant was removed and the pellet was suspended to 5 ml of pre-chilled fixative (3:1 methanol: acetic acid). The fixative was added carefully from the walls, in order to avoid clumping of the cells. The cells were allowed to settle for 10 minutes. After 10 minutes, the cell suspension was centrifuged at 1000 r.p.m. for 10 minutes. The supernatant was removed and fresh fixative was added to the pellet. This process was repeated for 3 to 4 times and the final cell suspension as made up to 1 ml of fresh fixative.

2.7 Preparation of the slides

Air dried slides were prepared by dropping one or two drops of the final cell suspension on the grease free, pre-chilled slides with the Pasteur pipette. The slides were dried immediately by air-drying method, coded and stained in 2% Geimsa (2 ml of Geimsa + 2ml of sorenson's buffer+46 ml of distilled water) for 5 minutes.

3. RESULTS AND DISCUSSION

For each individual 100 well spread metaphases were scored for the presence of structural (gaps, breaks, fragments, exchanges dicentrics) and numerical aberrations (polyploids) and well spread metaphases were microphotographed. Structural aberrations such as (a) Gap, (b) Break, (c) Fragment, (d) Exchanges, (e) Dicentrics. Numerical aberrations such as Polyploids were observed in control and exposed group. The characteristics of the exposed groups are depicted in table 1. Further, the AA concentration (mg/dl) were measured in the control group and the values were 0.46 and reduced in bidi workers to 0.36 and when subjects were supplemented with vit-C the values showed higher than control data (Table 2). However, there is a significant increase in the frequency of Chromosomal aberrations AA supplementation: There was increase in the percentages of CA's in bidi rollers occupationally tobacco exposed group (from 0.65 in controls to 1.62) Table 3).Group A-the frequency of chromosomal aberrations increased to 1.72 in group B individuals 0.065% was observed in control population. However, when vitamin C is given for 3 months it significantly reduced the percentage of chromosomal aberrations to 1.20% in group A and it was found to be 0.78% in group B (Table 3).

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.

b) **P<0.01 Significant With tobacco exposed group (BW).

Table 3: Effect of ascorbic acid for 12 weeks on chromosomal aberrations in women bidi rollers exposed to tobacco dust (Adilabad district)

Group	Sample timing	Ascorbic acid	No. of examinees	No. of metaphases	No. of aberrant cells (%)
Control	Feb –2014	-	50	3850	0.65
	April- 2014	+	42	3920	0.72
Exposed group A	Feb –2014	Pre	46	4360	1.62*a
	April – 2014	Post	38	3700	1.20*b
Exposed group B	Feb- 2014	Pre	38	3720	1.72*a
	April - 2014	Post	36	3480	0.78*b

*P<0.05

a - denotes significance of data with control group.

b-denotes significance of data with exposed group.

Many naturally occurring substances in plants and other sources have protective effects on environmental mutagens / carcinogens and also on endogenous mutagens.[3] 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.[4] Vitamin supplements have a marked potentiality against toxic effects of diversified environmental chemicals. Antioxidant supplements decrease oxidative DNA damage in humans.[5] as do antioxidant-rich foods.[6-8]. Earlier we have been published on the incidence of chromosomal aberrations and sister chromatid exchanges in women bidi rollers exposed to toacco dust.[9,10]

The present investigated results are in agreement with that of Sram et al.[11] 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.[11] 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.[12] 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.[13] Endosulphan, Phosphanidon and Mancozeb.[14] Cisplatin.[15] and Cyclophasphamide and Bleomycin.[16] Further Cohen et al,[17] reported that the chromosomal damage and DNA stand breaks were reduced in mammalian cells in the presence of an antioxidant ascorbic acid. Antunes et al.[18] reported a significant reduction in chromosomal aberrations and number of abnormal metaphases induced by Doxorubicin in human peripheral lymphocytes cultures by Vitamin-C.

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.[19-21] where the use of multivitamins as dietary supplement significantly decreases the frequency of chromosome aberrations, specially chromatid breaks; and confirming previous results where a decreases in occupational induced chromosome damage in lymphocytes after prophylaxis with vitamin C.[11] vitamins A and E,[22] or polyvitamin complexes.[23] has been detected. Earlier we reported the protective effects of ascorbic acid in drugs and heavy metals induced cytotoxicity in *invivo* and *invitro* mammalian test systems.[24-31] 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.[32] In other animal studies, the toxic effects of lead on heme production were reversed by a vitamin C dose of 100 mg/kg.[33] 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.[34] 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.[35] n 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.[36]

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.[37] Vitamin C was effective at inhibiting lead uptake and reducing lead cytotoxicity.[38] Vitamin C, in combination with silymarin, has also been shown to effectively reduce the hepatotoxic effect of acute lead poisoning.[39] 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.[40,41]

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.[42] or the micronucleus assay.[43] 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.

4. 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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