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Changes in buccal micronucleus cytome parameters associated with smokeless tobacco and pesticide exposure among female tea garden workers of Assam, India

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ABSTRACT

Assam is the highest tea producing state in India. A large number of workers are engaged in various units of tea industry. There are few reports on the health status of the tea garden workers. The present cytogenetic biomonitoring study was undertaken to investigate the genotoxic effect associated with workers in tea industries in southern Assam. Smokeless tobacco chewing along with betel nut is very common practice among the workers. Workers also get exposed periodically to mixture of pesticides. Employing buccal micronucleus cytome assay, exfoliated buccal cells were analyzed in 90 female tea garden and compared to 90 age and sex matched non-chewer control as well as 70 chewers who are not tea garden workers. Statistically significant ($p < 0.001$) increase in genotoxic and cell death parameters was observed in tea garden workers compared to both the control groups. The frequency of cell proliferation biomarkers was highest in the chewer controls whereas genotoxic and cell death parameters were highest in tea garden workers. Linear correlation analysis revealed strong positive correlation between the duration of occupation and the frequency of micronucleus ($r = 0.597$; $p < 0.001$) as well as cell death parameters ($r = 0.588$; $p < 0.001$). Amount of chewing also had significant positive correlation with micronucleus frequency ($r = 0.243$ or 5.9%; $p < 0.05$) and cell death parameters ($r = 0.217$; $p < 0.05$). A statistically significant decrease in total RBC count, haemoglobin content as well as acetylcholine esterase in the blood of exposed individuals was observed. The average BMI among the tea garden workers was relatively lower compared to the control group. Pesticide exposure and chewing areca nut along with smokeless tobacco use may be responsible for changes in cytome parameters in exfoliated buccal cells.

Introduction

Tea is an important agro-industry of Assam, which contributes immensely to the state's economy. Population biomonitoring is becoming an extremely powerful approach to determine the effect of environmental mutagens or occupation on human populations. Although tea garden is an important industry, there is no report on the effect of this occupation on cytogenetic biomarkers among the workers. The buccal micronucleus cytome assay is a non invasive method for studying DNA damage, chromosomal instability, cell death, and the regenerative potential of buccal mucosal tissue, and is widely used in biomonitoring studies (Bonassi et al., 2011; Celik et al., 2003; Thomas and Fenech, 2011). The use of exfoliated cells for micronucleus assays has become well established in epidemiological studies aimed at defining genotoxic effects on target tissue following chronic exposure to genotoxic and cytotoxic agents (Chakraborty et al., 2006; Smith et al., 1993). A major goal of our study is to acquire information on the effect of this occupation on the exfoliated buccal cells so that a proper health risk assessment can be done that will be helpful for developing effective health care strategies. Agriculture workers, either in open fields or greenhouses, get exposed to pesticides or are in constant risk of exposure by accident. Besides, the general population in agricultural areas as well as in urban and suburban sites are exposed through trophic chains by eating food contaminated with pesticides (Bolognesi et al., 1993; Falck et al., 1999). Southern Assam is hot and extremely humid region which may cause pesticides to remain in air for longer duration. Furthermore, in many cases norms of pesticide handling, use of protective equipments and hygiene regarding the practice of washing hands before eating and after handling the pesticides is completely ignored (Falck et al., 1999). Although there are several reports on cytogenetic biomonitoring studies from different parts of the world, each one is unique because of differences in the levels of exposure, type of pesticides mixtures, geographic and meteorological characteristics of the agricultural areas etc. (Bolognesi et al., 1993; Pastor et al., 2003). Besides the lifestyle factors needs consideration during such study.

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In southern Assam, all the tea garden workers consume areca nut with "*sadagura*" (a roasted formulation of tobacco prepared at home with flavouring agents) (Kausar et al., 2009). Sometimes other smokeless tobacco chewing habit is also added to this habit. We investigated the percentage of micronuclei and other nuclear parameters in exfoliated buccal epithelial cells among female tealeaf collectors. Micronuclei are chromosomal fragments or the whole chromosomes that are not included into the daughter nuclei during cell division and are incorporated as a much smaller nucleus. The formation of micronucleus is therefore induced by substances that cause breakage of chromosomes (clastogens) as well as by agents which affect the spindle apparatus (aneugens) (Ghosh et al., 2008). The micronucleus analysis in epithelial cells is relevant because about 92% of cancers are of epithelial origin (Rosin and Gilbert, 1990) and is a reliable indicator for genomic instability. In addition to micronucleus, nuclear bud, was used as cytological biomarker for genotoxicity assessment.

Acetylcholine esterase (AChE) function in the central and peripheral neuron system to terminate nerve signal transduction at the neuromuscular junction by rapid hydrolysis of the acetylcholine (ACh) receptor released into the cholinergic synaptic cleft. AChE is also found in human red blood cells (RBCs) (Brauer and Root, 1945), and it is one of the typical extrinsic membrane bound enzymes (Heller and Hanahan, 1972).

Blood cholinesterases have been widely used for monitoring exposure to pesticides. There are strong association between exposures to pesticides and cholinesterase as it is significantly reduced in exposed populations (Ali et al., 2008; Mourad, 2005; Nigg and Knaak, 2000; Singh et al., 2007).

In the present cytogenetic biomonitoring study conducted among the female tea garden workers exposed to pesticides as well as indulge in smokeless tobacco use, the effects were evaluated using cytome assay and blood AChE. Besides, other haematological parameters were used to assess the health status of the population.

Materials and methods

Chemicals

Phosphate buffered saline (Ca^{2+}, Mg^{2+} free; PBS) (CAS No. TL 1029) and acetylthiocholine iodide (CAS No. RM 773) were obtained from Hi-Media Ltd., Mumbai, India. Dithiobisnitrobenzoic acid (DTNB) (CAS No. 044883) was procured from Sisco Research Laboratories Pvt. Ltd. (Mumbai, India). Sodium bicarbonate (CAS No. 144-55-8) was obtained from Sigma–Aldrich, India. Methanol and glacial acetic acid were obtained from Qualigens Pvt. Ltd., India and were of analytical grade. Schiff's reagent was purchased from S-d Fine Chemicals, Mumbai.

Study population

The study was approved by the Institutional Ethics Committee of Assam University, Silchar and carried out between December 2009 and December 2011. The study involved 250 participants divided into three groups, 90 females employed in tea industry involved in tealeaf collection, 90 age and sex matched control individuals without any chewing habits and 70 age and sex matched subjects with chewing habits who are not tea garden workers. Selection of non-chewer workers from tea garden was not possible as all of them consume tobacco. The exposed group consisted of tea garden workers employed in two tea plantation units covering the districts of Cachar and Hailakandi in the southern part of the state of Assam in India. All the groups in the study population belonged to similar socio-economic and educational backgrounds. Participants were asked a structured questionnaire to obtain information regarding lifestyle and demographic parameters. The workers worked during the day in open in two shifts that is morning (9 am to 12 noon) and afternoon (1–4 pm). All the workers stay within or nearby garden areas and collect the leaf by hand. Pesticides used are shown in Table 1. Generally pesticides are sprayed directly at an interval of 7–10 days depending upon the necessity and sprayed by hand by male workers all throughout the year. The female workers collect leaves after 5–6 days of pesticide spraying and are engaged in leaf collection in other sectors of the garden throughout the working week while spraying is going on in one sector. We have always tried to collect the samples when the workers return from the field at the end of the first shift of work following a pesticide application.

The control group was selected from the general population from same geographical area, mostly housewives, with or without betel quid/tobacco chewing habit without having known exposure to pesticides or any other environmental genotoxic agents. Individuals suffering from mouth ulcers and oral lesions or those under any medication or X-ray exposure during past two months were not included in the study.

Sampling procedure

Cell sampling and staining was done following the method of Thomas and coworkers (Thomas et al., 2007) with minor modifications. Briefly, pre-moistened cotton swab (Johnson & Johnson, India) were rotated in a circular motion against the cheek following prior rinsing of the buccal cavity. The swabs were dipped into microcentrifuge tubes containing 2 ml normal saline solution (0.9% NaCl, Qualigens Pvt. Ltd., India). The cells were centrifuged at 1000 rpm for 10 min in a microcentrifuge machine (Spinwin, Tarsons). Supernatant was discarded and centrifuged again after addition of fresh buffer solution. The process is repeated thrice until a clear pellet is obtained. The pellet is then agitated to dislodge the cells by drawing in and out for six times with a syringe. Smears were prepared in pre-cleaned slides, air-dried for 10 min and fixed in 3:1 Methanol: Glacial acetic acid mixture for 10 min prior to staining.

Staining and scoring

Fixed slides were treated for 1 min each in 50% and 20% ethanol and washed using deionized water for 2 min prior to staining. These were then treated with 5 M hydrochloric acid for 30 min followed by washing for 3 min in running tap water. Moist slides were treated with Schiff's reagent at room temperature in dark for 60 min, washed in running tap water for 5 min and rinsed for 1 min in deionized water. Slides were stained in 10% Fuelgens stain for 10 min and air dried. Then slides were stained in Fast green for 30 s. Slides were allowed to air dry at room temperature and mounted in DPX. Coded slides were scored by a single scorer to eliminate inter-observer variations using a light-microscope (Leica DMLS) at 1000× magnification. The observer is an expert having several years of experience in such analyses. However, we have also performed a follow up analysis to look for possible inter-observer variations. For this, three observers simultaneously assessed various parameters in real time (one using the microscope and two others on displayed images on separate monitor screens) and we did not find any significant variations in scoring pattern among the observers. The details of the analysis and findings are summarized in the form of a table (Table S1) and included as supplementary material. Two thousand mucosal cells were counted per individual to determine the micronucleus, nuclear bud, basal cells, binucleated cells, karyolytic cells, condensed chromatin, pyknotic cells and karyorrhectic cells.

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ijheh.2013.04.007.