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Evaluation of genetic damage in tobacco and arsenic exposed population of Southern Assam, India using buccal cytome assay and comet assay

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ABSTRACT

Ground water is the principal source of drinking water in Assam. Ground water contamination of arsenic in drinking water is a great concern for human health and considered as a human carcinogen. The present cytogenetic biomonitoring study was undertaken to investigate the genotoxic effects associated with people of southern Assam consuming arsenic contaminated water and chewing tobacco. Employing the buccal cytome assay, exfoliated cells were analyzed in 138 individuals of age range 22–42 years and divided into four groups. Group I ($n=54$) are participants residing in localities where ground water contains arsenic concentration below the permissible limit (< 10 μg/l) and without any tobacco chewing history. Group II ($n=32$) participants from the same area but they are tobacco chewers. Group III ($n=24$) participants from localities where significantly high arsenic contamination in ground water were observed. Whereas the Group IV ($n=28$) consists of participants from the arsenic contaminated area and also tobacco chewers. Body mass index (BMI) in all the groups are found to be nearly same and in normal range. Statistically significant ($P<0.001$) increase in genotoxic, cell death parameters and cell proliferation biomarkers were observed in the Group IV compared to other groups. In the comet assay, percent of tail DNA gradually increases among the groups and has statistical significance. Spearman correlation revealed strong positive correlation between the arsenic exposed peoples and the binucleated cells ($r=0.4763$; $P<0.001$). Amount of chewing tobacco had significant positive correlation with micronucleus frequency ($r=0.268$; $P<0.05$) and karyolitic cells ($r=0.217$; $P<0.05$) and also in the percentage of tail DNA ($r=0.5532$, $P<0.001$). A statistically significant increase in glucose content and decrease in hemoglobin content as well as acetylcholine esterase in the blood of exposed individuals was observed. Our preliminary study indicate that population exposed to arsenic through drinking water may become more susceptible towards chewing tobacco induced nuclear damage as evaluated by buccal cytome assay and comet assay.

1. Introduction

Arsenic is a natural constituent of the earth's crust. Arsenic is a chemical that is widely distributed in nature and principally occurs in the form of inorganic or organic compounds. Arsenic is released in the environment through natural processes such as weathering and volcanic eruptions and may be transported over long distances through water and air. The source of arsenic contamination is geological. Incidents of arsenic contamination in the ground water have been reported from widespread areas throughout the world such as Taiwan, Mexico, Chile, Argentina, Thailand, Bangladesh, USA, Hungary, Japan and also India.

Naturally occurring arsenic that contaminates drinking water is the major source of this ongoing global public health problem. Ground water plays a key role in meeting the water needs of the Southern Assam, India. Arsenic in ground water is becoming an arising issue in the water supply and health sectors of various parts of India. In rural areas of Assam high percentage of people depend on pumped tube wells for drinking purpose. Humans can be exposed to arsenic through the intake of air, food and water (Tchounwou et al., 1999). Inorganic arsenic is classified as Group 1 human carcinogen by the International Agency for Research on Cancer (IARC, 1987, 2004, 2012). This classification is based on several epidemiological studies which show an association of exposure to arsenic and the development of cancer.

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Many human epidemiological studies on arsenic poisoning are available. To our knowledge, there was no genetic monitoring study in the arsenic affected areas of three districts of Southern Assam, we first reported an elevated frequency of micronuclei and other nuclear anomalies in oral mucosa cells. Previous work reports that in West Bengal, India where a large population is drinking arsenic contaminated water and the symptomatic individuals are associated with number of toxicity with ingestion of arsenic-contaminated drinking water (Basu et al., 2002). Chronic exposure to arsenic has been associated to cancer in skin, lung, bladder and liver (Abernathy et al., 1999). The greater number of populations who had a history of relatively long periods of arsenic exposure confirmed higher incidences of chromosomal aberrations (Ostrosky-Wegman et al., 1991), sister chromatid exchanges (Hsu et al., 1997) and micronucleus formation (Dulout et al., 1996). Arsenic can induce DNA damage in multiple test systems (Schaumlooffel and Gebel, 1998). There are reports regarding increase in liver cancer mortality in children who had exposure to high concentrations of arsenic in drinking water starting soon after birth (Liaw et al., 2008). Reductions in birth weight have been found in a low-arsenic-exposure study in Chile < 50 μg/L (Hopenhayn et al., 2003). Chronic arsenic exposure increased the risk of infant mortality were reported by Hopenhayn-Rich et al., 2000 Ehrenstein et al., 2006; Rahman et al., 2007. Arsenic concentration in the range of 35–40 μg/L in drinking water in Nevada may influence the incidence of childhood cancer (Moore et al., 2002).

Tobacco use in various forms always have been a major component of life style factors. Smokeless tobacco users have increased risk of ischaemic heart disease (Bolinder et al., 1994). Experimental evidence also suggests that chewing tobacco may be carcinogenic (Going et al., 1980). Numerous studies have shown a strong association between smokeless tobacco and adverse pregnancy outcomes, particularly low birth weight (Krishnamurthy and Joshi, 1993). Cardiovascular disease and asthma demonstrate a strong etiologic association with smoking (Benovitz, 1991). Considering the widespread reports of arsenic and tobacco induced carcinogenicity in human beings, we recognize the need to biomonitor the genotoxic effects of arsenic and tobacco in human beings exposed to arsenic and tobacco through drinking water and chewing habits. The Buccal cytome (Byct) 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 (Celik et al., 2003; Thomas and Fenech, 2011; Bonassi et al., 2011).

MN assay in epithelial cells have shown to be a sensitive method for monitoring genetic damage in human populations (Sarto et al., 1990; Karahalil et al., 1999; Majer et al., 2001). It is the only biomarker that allows the simultaneous evaluation of both clastogenic and aneugenic effects in a wide range of cells. Exfoliated epithelial cells have traditionally been used for cancer screening and biomonitoring of genotoxic effects in humans (Guzman et al., 2003). The frequencies of micronuclei observed in the exfoliated cells of oral mucosa serve as an appropriate index to monitor the genotoxicity induced by arsenic and tobacco because these cells are in direct contact with the carcinogen.To bring homogeneity and to establish the clastogenic effects of arsenic exposure and tobacco chewers, we broadened the study using samples from individuals inhabiting the same area and still using the arsenic contaminated water. Mucosal cells are not only in the direct route of exposure to ingested pollutants, but also capable of metabolizing chemical agents to reactive species (Zhang and Mock, 1989; Zhang, 1994). Arsenic-induced genotoxic effects are implicated in carcinogenic outcomes (NRC, 1999). The exact mechanism of arsenic-induced carcinogenicity still remains elusive; however, it acts as a clastogen, inducing the formation of chromosomal aberrations and micronuclei in animal and human systems (Basu et al., 2001).

The comet assay is a delicate and rapid technique for quantifying and analyzing DNA damage in individual cells. It is also known as Single Cell Gel Electrophoresis (SCGE). This assay can sensitively detect DNA single strand break (Hecht et al., 1978; Oesch et al., 1994). It is widely used as a standard technique for evaluation of DNA damage, biomonitoring, molecular epidemiology and genotoxicity testing. In the present study, comet assay determines the percent tail DNA to measure DNA damage causing by chewing tobacco and arsenic exposure. Blood acetylcholine esterase (AchE) have been widely used for monitoring exposure to different chemicals and tobacco (Giri et al., 2014).

In the present study, we have used the buccal cytome assay in exfoliated buccal cells, the comet assay in peripheral leukocytes, AchE in blood and other hematological tests to assess whether prolonged exposure to arsenic and chewing tobacco could lead to an increase in cytogenetic damage in peoples of Southern Assam, India.

2. Materials and methods

2.1. Chemicals

Sodium chloride (NaCl), Histopaque and Sodium bicarbonate (CAS no.144-55-8) were purchased from Sigma-Aldrich, India. Ethanol was obtained from Ajax Finechem, India. Hydrochloric acid (HCl), Light Green solution, DPX mounting medium were obtained from Merck, India. Sodium hydroxide, sodium chloride, acetic acid, Dithiobisnitrobenzoic acid (DTNB) (CAS no. 044,883), Normal melting point (NMP) agarose, low melting point (LMP) agarose, di-sodium salt of ethylene diamine tetra acetic acid (EDTA), Tris buffer, ethidium bromide (EtBr), thiobarbituric acid (TBA), trichloroacetic acid (TCA), Triton X-100 and DMSO were procured from Sisco Research Laboratories Pvt. Ltd. (Mumbai, India). Feulgen stain, Phosphate-buffered saline (Ca^{2+}, Mg^{2+} free; PBS) (CAS no. TL1029), acetylthiocholine iodide (CAS no. RM 773) and trizma base were obtained from Hi-Media Ltd., Mumbai, India. Methanol and glacial acetic acid were obtained from Qualigens Pvt. Ltd., India and were of analytical grade. The chemical solutions were freshly prepared in distilled water prior to experimentation.

2.2. Estimation of arsenic in Water by atomic absorption spectroscopy

All water samples are collected from underground sources and not from surface or rainwater. The ground water (100 ml) samples collected in acid-washed (nitric acid-water, 1:1) plastic bottles and in to which nitric acid (1.0 ml/L) was added later as preservative (Chatterjee et al., 1995). The arsenic content in water was analyzed in the laboratory of Sophisticated Analytical Instrument Facility (SAIF), North Eastern Hill University, Shillong. A Perkin Elmer 3110 Spectrometer at SAIF, Shillong were utilized for the estimation of arsenic in the collected samples. At regular intervals instrument was calibrated especially for the estimation of arsenic. For each sample, reading was taken in duplicate for the accuracy.

2.3. Study population

The study was approved by the Institutional Ethics Committee of Assam University, Silchar and carried out between June 2010 and June 2012. The study involved 138 participants divided into four groups, 54 control individuals without any chewing habits and ground water arsenic concentration below (WHO, 1996) permissible limit (Group I), 32 subjects with chewing habit (Group II),