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A COMPARATIVE STUDY OF DIFFERENT RISK FACTORS WITH ORAL CARCINOMA-IN SPECIAL REFERENCE WITH ARSENIC TOXICITY

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ABSTRACT

Introduction: Oral cancer ranks sixth among all the cancers worldwide. West Bengal being the most arsenic affected state in India, arsenic toxicity is also important in this carcinogenesis, which can be detected through micronuclei (MN) assay.

Objective: The objective of this study is to find out a possible correlation between arsenic toxicity and the development of this disease, assessing it through MN assay.

Methods: In this study, 104 oral malignant, 103 premalignant, 101 post operative cases along with 100 controls were included. The collected hair samples were processed for arsenic estimation by flow injection hydride generation atomic absorption spectrometry and the buccal smear samples were Giemsa stained and observed under microscope for calculating MN frequency.

Results: 67% of malignant, 54% of premalignant, 66% of post operative cases while only 4% of controls showed their arsenic count above the safe limit. The MN frequency was highest in malignant cases, but it was negligible in healthy controls.

Conclusion: A considerable percentage of cases showing higher arsenic count than controls may indicate a possible impact of arsenic toxicity on oral cancer, also detected through the significant difference of MN frequencies among the groups.

Keywords: Arsenic, Oral cancer, West Bengal, Micronuclei

INTRODUCTION

Oral squamous cell carcinoma (OSCC) is the 6th most common cancers worldwide (Kadashetti *et al.*, 2015; Coelho K.R., 2012) and 3rd most common cancers in developing world (Fazeli *et al*., 2011). India has world's highest number (nearly 20%) of oral cancers with an estimated 1% of the population having oral premalignant lesions (Chaturvedi P., 2012; Singh *et al.*, 2016), contributing to one-third of the world oral cancer burden (Gupta *et al.*, 2014). In West Bengal, this disease ranks one among men and fifth among women, where it is reported that 2 in every 5 men have been diagnosed with oral cancer (Sinha, 2016). OSCC accounts for the second most common cancer in adult males and third most common cancer in adult females worldwide. In West Bengal, it has been reported that there has been a substantial difference between the oral cancer detection rate among females and males (Sinha, 2016). Around 75% of oral cancers are linked to the exposure to various behavioral habits like smoking/smokeless tobacco use, excessive alcohol consumption, betel quid chewing (El-Zaemey, Schüz & Leon, 2015) etc. But, in recent years, many research studies suggest that there have been many cases developing this cancer with or without any history of habits related to these risk factors, which brings out the rise of another potent factor, namely metal toxicity (Su *et al.*, 2010). These metals include lead, nickel, arsenic etc. whose presence in soils may exert their effects on human health through the food grown on them, which may put people under a higher risk of cancer development, if the metal is proved to be a carcinogen (Navarro Silvera & Rohan, 2007). Out of these, we have chosen arsenic in our study since West Bengal is the only state of India which is on the alarming knock of arsenic toxicity. Several works have been done to claim the groundwater in different districts of West Bengal contain arsenic above the permissible limit, recommended by WHO (Chatterjee & Sarkar, 2011; Mahanta, 2005; Chakraborti *et al*., 2009). Arsenic exerts its toxicity mainly by inhibiting the enzymes involved in cellular detoxification and signaling pathways, DNA replication

and repair (Ratnaike, 2003). Its carcinogenicity in skin, ling and bladder cancer has been established, but its impact on oral carcinoma is not yet well established. Arsenic toxicity can be detected through different cytogenetic damage assays like micronuclei (MN) assay, assays for detecting sister chromatid exchanges, chromosome nondysjunction or presence of any chromosomal abnormality. So, in this study, we have carried out MN assay to find out any correlation between the metal toxicity and cytogenetic damage.

METHODOLOGY

Study Population

This is a prospective study, where a stratified sampling method was used to select 408 participants (104 oral malignant (OM), 103 oral premalignant (OPM), 101 post operative cases and 100 control) who were asked about their daily lifestyle habits and epidemiological data, after taking their proper consent. A total of 3451 patients were screened in the Out Patient Departments of ENT-Head & Neck Surgery, Oral Maxillo Facial Surgery of Vivekananda Institute of Medical Sciences, Ramakrishna Mission Seva Pratishthan, Kolkata. Among these, 104 patients with histopathologically confirmed cases of oral carcinoma, 103 with premalignant oral lesions and conditions and 101 post operative cases were recruited for this study. Adults with non-treated frank and premalignant oral and oropharyngeal squamous carcinoma as well as control individuals, exposed/not exposed to arsenic toxicity were included in the study. Whereas, we did not include pediatric patients, advanced oral malignant cases, cases with other oral lesions and post treatment cases. All cases were newly diagnosed. All were residents of different districts of West Bengal. About100 controls (cancer free) were recruited simultaneously from the relative of the patients residing in similar geographic area. Controls were selected among the relatives of the cases who accompanied them and staying in the same localities. Age distribution for the controls was comparable to that of the cases. Cases and controls were matched primarily by frequency of geographic and social origin and secondly by age distribution. They mostly belong to medium to low economic classes having similar lifestyle and level of education.

Ethics

The study was ethically cleared by the respective institutional committee.

Questionnaire

The demographic data of the concerned subjects were collected (age, gender and residential history). Data of the extent of arsenic contamination in different blocks/wards of different districts of this state were obtained from literature (Chakrabarti *et al.*, 2009).

Sample collection and analysis

Buccal smear and hair samples were collected after obtaining informed consent from all the subjects. The collected hair samples were treated by the method of flow injection-hydride generation-atomic absorption spectrometry for proper arsenic count. The buccal smears were taken on slides, air dried, fixed with 80% methanol and stained with Giemsa stain, which were then examined under microscope for detecting the presence of micronuclei.

Statistics

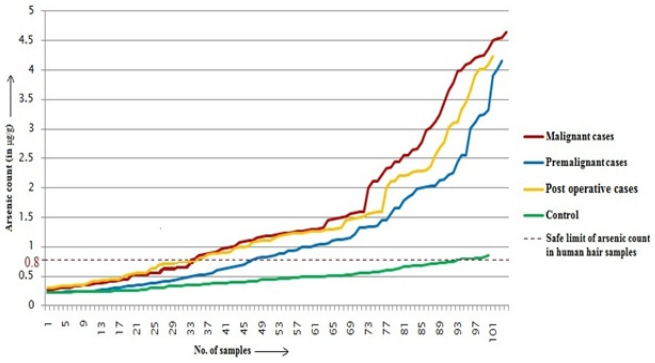
Statistical analysis was performed comparing the cases with malignant and premalignant oral lesions to the control on the basis of demographic factors, arsenic level in hair samples and the presence of micronuclei and apoptosis in buccal smear. A Student's *t*-test was used to compare the case and control groups for arsenic level in hair. The Arsenic (As) level mean, standard deviation (SD), quartiles and medians for all these groups were calculated (Table 1). Statistical analysis was done using Graph-pad prism software. Every test was 2-sided with a level of p value < 0.001, which prove out to be significant.

RESULTS

The distribution of As level in hair samples of malignant & premalignant cases and control is shown in Table 1, which includes the mean, SD, medians, range and the quartiles. 67% of malignant cases, 54% of premalignant cases & 66% of post operative cases showed their arsenic count above the safe limit (0.8 µg/g; recommended by WHO), whereas, 96% of the controls' arsenic count were within the safe limit. This is shown in Figure 1.

Table 1: Statistical representation of arsenic count among malignant, premalignant and control groups

	MALIGNANT	PREMALIGNANT	CONTROL
No. of values	104	103	100
Minimum	0.65	0.30	0.32
25% percentile	0.91	0.76	0.44
Median	1.55	1.17	0.52
75% percentile	2.54	2.05	0.65
Maximum	4.78	4.23	0.78
Mean	1.91	1.47	0.54
Standard deviation	1.14	0.97	0.11
The difference of mean values between control group and case (premalignant & malignant) group w.r.t. arsenic count is highly significant [*p* value < 0.001]			



The overall association of various risk factors with the cases and controls is illustrated in Figure 2.

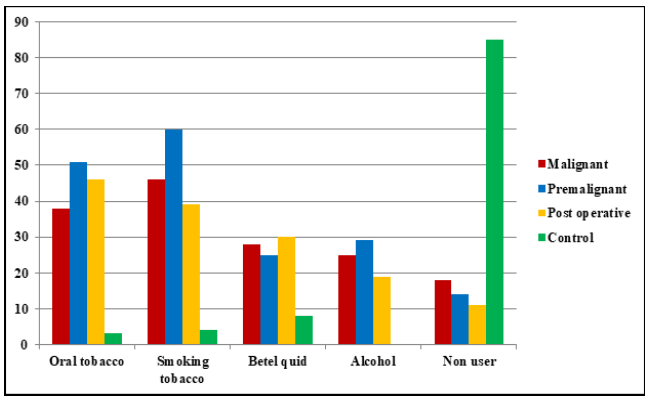


Figure 2: Histogram showing the association of different risk factors with the groups

There is a significant difference (*p* value < 0.05) of the micronuclei frequency between the cases and controls (Table 2). In our study, we have found no significant correlation between the As content in hair and micronuclei frequency in the malignant as well as premalignant patients, Spearman's 'r' value is found to be 0.116 and 0.19 respectively. In both the malignant and premalignant cases, distribution was found to be non-parametric by Kolmogorov-Smirnov test. One case and all the controls showed the presence of apoptosis. The micronuclei can be clearly seen through Figure 3.

Table 2: Tabular representation of range of MN frequency of the groups, indicating the p value

Cases / Data	Premalignant (Range)	Malignant (Range)	Post operative (Range)	Control (Range)
Micronuclei frequency (%)	0.2 - 0.6	0.4 - 1.5	0.1 - 0.3	0 - 0.1
p value	< 0.05*			
* denotes that the difference of mean values of micronuclei frequency w.r.t. malignant & premalignant cases and post operative & control groups is significant				

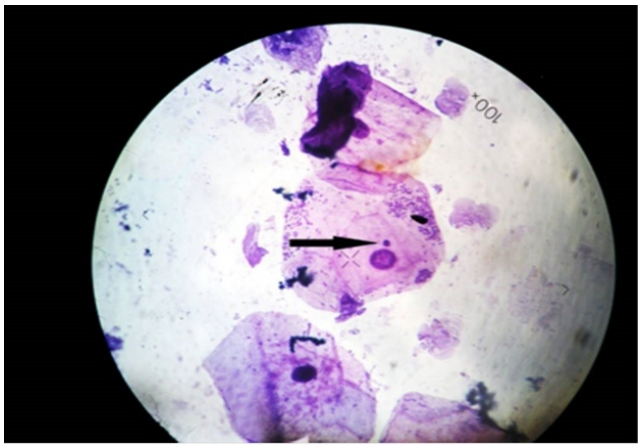


Figure 3: Micrograph showing human buccal cells stained by Giemsa and observed at 100X magnification. The solid black arrow indicates micronuclei in the cell

DISCUSSION

Arsenic contamination in drinking water has become a major concern worldwide, being considered as a potent risk factor in various countries like Bangladesh, Taiwan, India, Mexico, China, Chile, Argentina and

USA (Singh *et al.*, 2011). The WHO guidelines declared safety limit of arsenic concentration in drinking water being 10µg/l and a maximum permissible limit of arsenic in drinking water is 50µg/l (Steinmaus *et al.*, 2005). It is reported that a number over 200 million people in the whole world are at a risk of getting arsenic contamination, out of which more than half resides in Bengal Delta Plain including West Bengal and Bangladesh (Washington, 2001). These two areas are the worst affected areas in the world (Ratnaike, 2003). One plausible mechanism of arsenic accumulation in the Bengal delta Plain can be the deposition of arsenic containing alluvial sediments by rivers like Ganga, Brahmaputra, Meghna and other small rivers flowing across this plain into the Bay of Bengal during the late Quaternary age or Holocene age (Mukherjee & Bhattacharya *et al.*, 2001). After intake of arsenic through drinking water or food grown on such soils, it gets absorbed in the internal organs like kidney, liver, lung and others, leading to various disorders and even cancerous diseases like bladder, lung cancer. Its effect is acute within 2-3 days of exposure, readily excreted through urine or resident in blood. However, its impact is chronic in skin or keratin rich tissues like nails or hairs for a long term exposure. The outbreak of chronic exposure starts from a period of 4-6 months, which can be said as a silent killer and easily not detectable in the attacking span. This can even lead to carcinogenesis, clearly evident in case of established skin cancer. However, its impact on oral carcinoma is an emerging fact which requires more studies from various parts of this world and proper establishment. While this effect is established in Taiwan (Su *et al.*, 2010) and Pakistan (Arain *et al.*, 2015), prominent consequences in the form of cytogenetic damage have been reported from acute and chronic arsenic toxicity in various countries like Mexico, Finland, Argentina etc. (Ghosh *et al.*, 2006). Arsenic involvement in the mutation of glutathione- S- transferases hampering the cellular detoxification can be detected through assays of chromosomal abnormalities and micronuclei (Ghosh *et al.*, 2006). Various cytogenetic tests including the micronucleus test in addition to tests of various degenerative alterations indicative of apoptosis (karyorrhexis, pyknosis, and condensed chromatin), give a promising method to detect the occurrences of cytogenetic alterations in the oral epithelium due to this carcinoma (Kingsley *et al.*, 2008).The authors observed MN frequencies in exfoliated buccal cells in normal mucosa, precancerous lesions and squamous cell carcinoma of Indian subjects and concluded that the MN frequency may be a marker of epithelial carcinogenic progression (Stich, Curtis & Parida, 1982). However, no correlational studies between the cytogenetic damage imposed by oral carcinoma and the arsenic toxicity have yet been performed, making this study more novel in its own stand. But, the use of soil As concentration data relating to each patient does not actually indicate the true environmental exposure, which acts as a limitation of this study.

CONCLUSION

In our study, we have shown a possible correlation between As toxicity in the occurrence of premalignant and malignant oral lesions, on examination of this metal's level in hair samples of the concerned cases. However, a high count has also been found out in post operative cases due to their exposure just before the operation, or as an effect of arsenic containing medicines taken for treatment on medication. We have collected the hair samples from the post operative cases just after a period of a few days of operation, which accounts to such exposure being remained even at the absence of malignant condition after the operation. This was totally reversed in case of the control individuals, where a very negligible number of them showed a high arsenic count, even on staying in same level of arsenic exposed/non-exposed areas of this state like those of the cases. This may incur on the capability of different individuals in retaining the inorganic arsenic in their hair samples or any concerned organs, which actually depends on individual to individual. Moreover, due to the possible faulty result in cases of the post operative individuals, whether the arsenic count may relate to the residential arsenic in the hair samples on the course of carcinogenesis or the impact of medicines, we have opted out them from the statistical analysis for coming into a proper correlation even statistically. However, no statistically significant correlation has been found out between the arsenic toxicity and the occurrence of MN frequency among the cases and the controls, observed in this study may nullify the fact of estimating the extent of this metal toxicity in terms of MN assay, in case of oral squamous cell carcinoma. Although, there remains a possible link between the occurrence of MN and the arsenic impact in cases and control, since the highest range of MN frequency has been observed in malignant cases, followed by a moderate range in premalignant cases and a low range in post operative cases and a negligible

range in controls. However, a higher sample with an extensive statistical analysis is definitely required to exempt all the limitations of this study and come to a proper conclusion.

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