



Is There any Effect of Addictions in Arsenic Induced Oral Carcinoma?

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Abstract

Background- The association of various addictions with oral squamous cell carcinoma (OSCC) has been well established. But it's any possible link with metal toxicity has not been studied in the context of this carcinoma.

Aim- To correlate the variation in the effect of various addictions with arsenic toxicity in the development of OSCC in the study population.

Settings and design- It is a case-control study. Ethical clearance was obtained from the institutional committee. A total of 104 oral malignant, 103 premalignant and 200 age and sex matched healthy individuals were selected for the study. The history of addictions of case and controls was collected from questionnaire.

Methods and material- On proper consent of cases & controls, the hair samples were collected for arsenic estimation by flow injection hydride generation atomic absorption spectrometry and the peripheral venous blood samples were processed for human leukocyte culture followed by Giemsa staining for the detection of chromosomal abnormality.

Statistical analysis used- Student t test, logistic regression by R software and correlation analysis by Graph Pad software.

Results- Significant correlation was observed between arsenic toxicity & OSCC (p value = 2.18×10^{-6} ***). Smoking tobacco (r value 0.5365) was more significant over chewing tobacco (r value 0.4804). 3% of malignant cases showed chromosomal break.

Conclusion- Statistically significant correlation among arsenic, addictions & OSCC in this study may indicate an associative role between the two factors. However, the study could not define any correlation with cytogenetic damage.

Keywords: oral carcinoma, arsenic toxicity, addictions, chromosomal abnormality.

Introduction

The 6th most common cancer worldwide ([Kadashetti et al., 2015](#), [Coelho, 2012](#)) and 3rd most common cancer in developing world ([Fazeli et al., 2011](#)) stands in oral squamous cell carcinoma (OSCC). Being the most common cancer in South East Asia (India, Bangladesh, Pakistan and Sri Lanka), it accounts for one-third of various types of body carcinomas ([Atkinson et al., 1964](#)). Previous studies have already well established the effect of different addictions on the development of oral malignancy worldwide. The main risk factors include the intake of oral and smoking tobacco, betel quid, alcohol, snuff, ganja etc. The most used addictive product being tobacco, its use in developed countries mainly lies with its smoking counterpart, while in developing countries like India, its chewing practices are mainly in use in the form of paan masala, guthka, zarda, khaini, snuff etc. ([Rodriguez et al., 2004](#), [Juntanong et al., 2016](#)), along with the use of smoking tobacco like bidi, cigarette to some extent. In India, the mass ignorance and poverty coupled with long standing bad oral habits ([Bouda et al., 2000](#)) and poor oral hygiene mainly accounts to the increased incidence of OSCC cases in this country. Apart from the main addictions, source of drinking water should also be considered as an important factor in such malignancies since it is connected with the involvement of oral cavity, ultimately affecting the whole human body system. Moreover, it is reported that over 200 million people in the whole world are currently at a high risk of arsenic contamination, out of which more than half resides in Bengal Delta Plain including West Bengal and Bangladesh ([National Research Council, 1999](#)). These two areas are the worst affected areas in the world ([Ratnaik, 2003](#)). It has also been reported that arsenic toxicity may lead to the occurrence of oral carcinoma in various places ([Su et al., 2010](#), [Arain et al., 2015](#), [Pal et al., 2017, 2018](#)) apart from skin, lung and bladder carcinoma. So, our aim of this study is to find out whether the effect of addictions or the metal toxicity through drinking water intake is acting as the most potent factor in the development of oral

carcinoma and correlate these three; selecting the population of West Bengal, India.

Materials & Methodology

This is a case-control prospective study, where a stratified sampling method was used to select a total of 407 individuals (104 oral malignant (OM), 103 oral premalignant (OPM), and 100 controls for OM (CM) and 100 controls for OPM (CPM)). The study was ethically cleared by the Ethics committee of Vivekananda Institute of Medical Sciences, Ramakrishna Mission Seva Pratishthan, Kolkata. Newly diagnosed and previously untreated 1070 out of 36785 patients attending the Out Patient Departments of ENT- Head & Neck Surgery & Oral & Maxillo Facial Surgery of our hospital were screened for the presence of oral malignant and premalignant lesions. The rest of the patients had reported with other oral disorders. A total of 104 patients with histopathologically confirmed cases of oral squamous cell carcinoma and 103 patients with premalignant oral lesions and conditions were selected for this study between 2013 and 2017. All the clinical characteristics of the individuals including their basic medical data were obtained from their corresponding medical records. All the selected individuals were residents of different districts of West Bengal, India. 200 control individuals (without any oral lesions or other oral disorders) were recruited simultaneously from the relatives and accompanying persons of the patients residing in similar districts. Cases and controls were all matched primarily by frequency of geographic and social origin and secondly by age-sex distribution. They mostly belong to the medium to low economic classes having similar lifestyle and level of education. The participants for this study were interviewed with a proper detailed questionnaire and on their informed consent, their addictive habits were collected. After obtaining proper consent from the corresponding participants, their hair and peripheral

venous blood samples were collected. All the case and control hair samples were analyzed by the method of flow injection-hydride generation-atomic absorption spectrometry for arsenic count. The corresponding peripheral venous blood samples of the cases and controls were processed for human leukocyte culture followed by Giemsa staining for 10mins for the detection of the presence of chromosomal abnormalities.

All the case and control group observations were analyzed on using descriptive analysis in terms of demographic factors, arsenic level in hair samples, and history of different addictions and presence of cytogenetic damage in the form of chromosomal abnormality. The arsenic count in hair samples were analyzed by Student's t test and all the case data were compared with those of controls'. Statistical analysis of arsenic level of case and control groups was done using Graph-pad prism software and the mean value, standard deviation (SD), quartiles and medians for all the groups were calculated. The analysis was two-sided with a highly

significant level of p value <0.001. The statistical analysis of correlation between arsenic toxicity and the occurrence of OSCC & correlation between addictions and arsenic toxicity w.r.t. the carcinoma is performed by logistic regression analysis done by using R software.

Results

A moderately high percentage of 78% of oral malignant and 62% of oral premalignant cases showed their arsenic count above the safe limit (range: 0.08-0.25 mg/kg) (Arnold et al., 1990), whereas, 96% of the controls' arsenic count were within the safe limit. It has been observed that the difference of mean values between the cases (OM & OPM) and the controls (CM & CPM) w.r.t. the arsenic count is statistically highly significant (p < 0.001). Moreover, it is also statistically observed that arsenic toxicity bears a highly correlation with the occurrence of oral carcinoma in this study population (p value = 2.18e-06 ***; Table 1).

Table 1. Statistical analysis of correlation of arsenic toxicity with the occurrence of OSCC in the study population

Parameters	Estimate	Std. Error	z value	P value
Arsenic toxicity	19.408	6.151	4.736	2.18e-06 ***
(*) denotes significant correlation between the said parameter and OSCC [*- significant; **- highly significant; ***- very highly significant]				

Another most interesting finding lays in the fact that out of 81 OM & 64 OPM cases with elevated arsenic count, 15 (18.51%) OM & 13

(20.3%) OPM cases were non users (not having the history of addictions).

Table 2. Statistical analysis of the data of different addictions of the individuals of oral malignant, oral premalignant and control groups.

Addictions	r value	95% CI	P value	Significant	R ²
Oral tobacco	0.4804	0.4019 to 0.5518	<0.0001	significant	0.2307
Smoking tobacco	0.5365	0.4635 to 0.6023	<0.0001	significant	0.2878
Betel quid	0.2426	0.1489 to 0.3320	<0.0001	significant	0.05886
Betel leaf	0.2043	0.1093 to 0.2957	<0.0001	significant	0.04175
Alcohol	0.3885	0.3028 to 0.4681	<0.0001	significant	0.1510
Snuff	0.02039	0.07699 to 0.1174	0.6817	non- significant	0.0004157
Ganja	0.1392	0.04253 to 0.2333	0.0049	significant	0.01937
Non user	-0.6952	-0.7423 to -0.6414	<0.0001	significant	0.4834

The statistical analysis states that the involvement of various addictions with the causation of oral / oropharyngeal squamous cell carcinoma in the population of West Bengal is highly significant, the highest being found in case of tobacco (smoking over chewing). Except snuff, all other addictions play a positive role in this malignancy. However, since the percentage of non-users is

small enough, it bears a non-significant relation with this carcinoma. This is depicted in Table 2.

Out of 104 OM cases, only three (~3%) showed the presence of chromosomal abnormality (break). Two metaphase plates one showing normal set of chromosomes and another showing chromosomal break are depicted in Figure 1.

Figure 1. Micrographs show metaphase plates; one showing normal set of human chromosomes and the other showing the presence of chromosomal break



Discussion

The high incidence (54.48%) of oral and oropharyngeal carcinoma in North-Eastern India has been reported by several studies (Sharma *et al.*, 2016). This accounts to the dietary habits and high tobacco use by the concerned population. On account of this high incidence, various cancer summits have been arranged with an effort to increase the awareness among the population, the latest being carried out in Agartala, Tripura, India. Moreover, West Bengal in India is the most arsenic effected state. The state has different levels of this metal contamination in its different districts, which divides it into three zones of arsenic affected areas: highly affected (Nadia, North 24 Parganas, Murshidabad, South 24 Parganas, Hooghly, Maldah, Howrah, Kolkata and Burdwan), mildly affected (North Dinajpur, South Dinajpur,

Cooch Behar, Darjeeling and Jalpaiuri) and unaffected (Purulia, Birbhum, East Midnapur, West Midnapur and Bankura) (Chakraborti *et al.*, 2009).

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, Qat chewing (El-Zaemey *et al.*, 2015) etc. In developing countries like India, where chewing practices are more common, oral cancer represents up to 40% of all cancer compared to just 4% in United Kingdom. A study from South India stated that 74.8% of all the OSCC cases had at least one predisposing habit, mainly chewing areca nut (Ranganathan *et al.*, 2015). Many studies point out the combination of more than one habits as the

cause of oral malignancy, the fact being explained as the presence of one risk factor (smoking) enhancing the effects of the second risk factor (chewing) and showing synergism in development of oral cancer or potentially malignant oral disorders (Kadashetti et al., 2015, Ho et al., 2007). In a North Indian study, a strong correlation has been found out between the presence of chewing habit and the occurrence of both oral premalignant and malignant lesions (Gupta et al., 2014). In western countries, the use of smoking tobacco is more prevalent than in India and Indian subcontinent countries, where smokeless tobacco are more consumed. This is also consistent with another study in Kerala, where the use of smokeless tobacco has been found out potent in creating oral mucosal lesions (Aslesh et al., 2015). Even the occurrence of oral premalignant conditions like OSF, leukoplakia, lichen planus gets enhanced by the use of the predisposing habits (Pratik et al., 2015). Other factors include poor oral hygiene, irritation caused by ill-fitting dentures and rough surfaces of teeth, poor nutrition, dietary habits like low consumption of fruits and vegetables (Epstein et al., 2002), life style habits (Fazeli et al., 2011), wood dust exposures, consumption of certain slated fish and others (NCI Factsheet 2013), some, chronic infections by fungi, bacteria viruses etc. The use of tobacco is mostly prevalent in West Bengal, which is evident from the fact that 40% men from different districts like Hooghly, Nadia, Howrah, South 24 Parganas, North 24 Parganas and Burdwan have suffered from oral cancer due to excessive intake of chewing tobacco. Out of these, 60% have grown buccal mucosa carcinoma as a result of continuous chewing of tobacco. In the present study, it has been observed that the main addiction of the individuals of this population is intake of tobacco (smoking and smokeless), followed by intake of betel quid and alcohol, leading to the occurrence of oral/oropharyngeal malignancy. However, a small percentage of non-users also fall in these two categories, which indicates the involvement of causative factors of this malignancy other than these addictive habits. The observations totally contradicted with the control group. The study indicates the greatest

influence of smoking tobacco over chewing tobacco in the occurrence of this malignancy in this population, which contradicts with most of the studies carried out in developing countries like India, where chewing practices are common. The data also depicts even the effect of only betel leaf on this malignancy.

Another factor, namely arsenic toxicity has also emerged now a days to be carcinogenic and has been well associated with various types of carcinoma. More than 26 million people in West Bengal are chronically affected by arsenic contamination in drinking water (Banerjee et al., 2014). The age range of cases under arsenic poisoning falls mainly among adults older than 19 years (Bronstein et al., 2011) and also in children younger than 6 years (NPDS 2007 data). The effect of this metal toxicity has already been well established in various carcinomas like skin, lung, bladder, liver, kidney worldwide. However, its effect in oral carcinoma is not well studied. A few studies in Taiwan, Pakistan and India have indicated its positive correlation with the occurrence of this carcinoma (Su et al., 2010, Arain et al., 2015, Pal et al., 2017, 2018). Since West Bengal is the most affected state in India, we have chosen this metal toxicity as a possible cofactor or an independent risk factor in the oral carcinoma. In our study, a high percentage (70%) of both oral malignant and premalignant cases bearing elevated arsenic count over controls may also focus on the arsenic toxicity effect in contributing to this malignancy. Moreover, its highly significant correlation coefficient observed with the occurrence of OSCC in this study population may also indicate its potent role in this malignancy.

Furthermore, a very small percentage of malignant cases showing the presence of chromosomal abnormality (chromosomal break) cannot exert the fact of this cytogenetic damage created as a result of the metal toxicity or addictions. This observation cannot imply a possible relation between these factors, rather just implementing the creation of DNA damage as a result of the interference of any other

risk factor in association with these two. A considerable number of malignant cases showing both the factors into play may indicate a possible impact of the metal toxicity on the DNA of the affected patients as an outcome of the presence of chromosomal break, or, may be the created environment of the arsenic factor playing a positive role in the interplay of different other risk factors like addictions finally leading to the DNA damage and malignancy. A very small percentage of malignant cases showing the presence of chromosomal abnormality in the form of chromosomal break, cannot confirm the co relational link between this metal toxicity or addictions individually and this cytogenetic damage, yet assessing the damage created by any other risk factor in association with these. To bring out such correlation, a much higher sample size with extensive statistical analysis is required.

Conclusion

It can be concluded that the present study bears the same trend with the literature implying the effect of different predisposing risk factors in the form of addictions in the oral malignancy, the most significant being the use of tobacco. From this study, it can also be stated that one can even not throw away the effect of only betel leaf playing a role in this malignancy. Furthermore, the effect of arsenic toxicity is also certain indicated from the highly significant correlation coefficient. However, in spite of the high correlation found between this metal toxicity and the development of OSCC in this study population, one cannot opt out the history of different addictions of the concerned

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cases, although a small percentage of nonusers (18.51% OM & 20.3% OPM cases) also fall in this category. So, this work also opens up into a new phase of research where further studies can be carried out to find out any possible correlation between these two factors (addictions and arsenic toxicity), may be suggesting their additive role or whether the effect of these addictions gets promoted in such in vivo environment of arsenic toxicity, contributing to the development of carcinoma on a larger scale. However, the presence of chromosomal break in only three OM cases (3%) may not indicate a proper correlation between the occurred cytogenetic damage and the effect of the metal toxicity or any addiction ultimately leading to this malignancy. This also focuses on the involvement or interference of other factors like viral infection or low immune response directly contributing or indirectly creating the environment suitable for such damage. For such conclusion, a higher sample size with extensive statistical analysis is definitely required.

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