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Assessment of Oral Cellular proliferative activity among Tamol chewers population in Assam, India

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ABSTRACT

In North-East India, variety of Areca nut, locally called as "Tamol" in Assam, "Kwai" and "Kuba" in Meghalaya and Mizoram, is raw, unprocessed betel nut, lime and betel leaf without tobacco, which are more effective as compared to the dried. Average age of onset of chewing among tribes is 15-20 years and people swallow the whole quid instead of splitting it out which could be an important factor for OSCC. In this study 62 apparently healthy individual were selected, who Tamol chewers without tobacco. Oral smear are obtain from the mucosa and the smear is histologically stained and cytomophologically assessed for the any morphological variation in the oral epithelial cell. Morphological variations in the oral epithelial cell are observed in 93.5% of the cases, which are significantly associated with duration of Tamol chewing in a year. There variables were positively correlated with grading of epithelial dysplasia. Grade of epithelial dysplasia were found to have significant positive correlation with duration of Tamol consumption in a year (r=0.267, p=0.036) Tamol chewing is a major risk for the occurrence of cellular proliferative activity which may progress to cancerous lesions. Cytomophologic analysis of oral cellular proliferative cells may serve as a useful tool for the early diagnosis of oral squamous cell carcinomas.

It is important to aware North-East community on the risk factor such as betel nut and its impact on the incidence of cancer in this region. Therefore the aim of this study was to measure the cellular proliferative activity which is possible induce by the habit of "Tamol" chewing to enable early prediction of carcinogenesis process using exfoliative cytology as an easy innovative diagnostic and screening procedure.

ABBREVIATION

OSCC, Oral squamous cell carcinomas; **OPMD**, Oral potentially malignant disorder; **DPX,** Distrene Polystyrene Xylene

KEY WORDS: Tamol, exfoliative cytology, oral squamous cell carcinoma, betel nut.

INTRODUCTION

Currently oral cancer is the Sixth most common malignancy in the world (Parkin et al, 2000). In worldwide oral cancer incidence is around 500,000 new cases every year, accounting approximately 3% of all malignancy and creating a world health problem significantly. In India it is most common malignancy among men and one of the five most common malignancy among women (Boyle et al, 1986). Etiology of oral squamous cell carcinomas is multifactorial, several risk factor for the development of OSCC are tobacco product, alcohol, infection, dietary factors, chemical irritants etc. A lesser known risk factor of oral cancer in India is the uncontrolled use of areca nut chewing (Winstock AP et al 2000). In north east India, variety of Areca nut, locally called as "Tamol" in Assam, "Kwai" in Meghalaya and "Kuba" in Mizoram, is raw with higher contents of all the chemicals like alkaloids, polyphenol and tannins as compared to the dried one (Phukan RK, 2001). The betel- quid used in north east India especially in Assam contains raw betel nut, lime paste and piece of betel leaf without tobacco and other ingredient commonly called as Tamol pan. The average age of beginning of chewing among tribes is very less i.e. 15-20 years and an important factor for OSCC could also be the reason that people swallow the whole quid after chewing without splitting.(AK Rai, 2012). Aqueous extract of betel nut had taken to study the genotoxic potency in relation with the endogenous glutathione level in the mouse bone marrow and human peripheral blood lymphocytes and reported that longer the exposure of aqueous extract of raw betel nut showed higher level of p53 expression on mouse bone marrow cells. The level of endogenous glutathione level and the level of p53 protein could act as effective biomarkers for OSCC in relation with raw betel nut chewers.(Kumpawat and Chatterjee et al, 2003). The North East region especially Assam and Meghalaya is the major region. In local language a fresh betel nut is known as Tamol in combination with betel leaf and slaked. Frequent and regular scratches of betel nut and betel leaf forms ulcers in the oral cavity. The slaked lime contains strong chemical compound which further form scars or ulcers by burns the soft tissue in the oral cavity. Which can further be a main contributing factor for developing oral cancer.(Chatterjee A et al, 2003)

Therefore the aim of this study was to assess the cellular proliferative action which is potential induced by the habit of Tamol pan chewing to enable early prophecy of carcinogenesis progression using exfoliative cytology as an easy novel diagnostic and screening procedure.

MATERIAL AND METHODS

In this study, oral scraps were obtain from Garo Gaon, Guwahati, Assam through the Health camp organised by Downtown Hospital and conducted in central facility laboratory Assam down town University, Guwahati, Assam as per the ethical guidelines. Total of 62 samples have been collected. The study group include healthy female volunteers female aged above 18 years. The sample collection criteria was the individual who are non-smoker, non-tobacco chewer and habitual tamol chewers. The subject was instructed to rinse the oral cavity with water and the smear was taken from the buccal mucosa by using wooden spatula. The material collected were smeared on two slides and immediately fixed on 95% ethyl alcohol for 15 minutes. One slide was stained according to the Haematoxylin and Eosin staining method and PAP staining method. The smear were the observed under 40X and 100X magnification The cytological features of the smear has been observed and graded according to the morphological variation give by Speight et al (Speight et al, 2007). The staining procedure has been described below.

Haematoxylin and Eosin stain

Ethyl alcohol fixed smear were hydrate in descending concentration of 95% alcohol through 70% alcohol to distilled water, for two minutes in each stage. The smear were then treated with Harris Alum Haematoxylin for 10 minutes to stain the nuclei, rinsed in distilled water and differentiate in 0.5% aqueous hydrochloric acid for a few seconds to remove the excess stain. this was immediately followed

by rinsing in distilled water to stop the action of discoloration. Then the smear were blued in alkaline water for seven minutes and The smear were next treated with Eosin for four minutes. Dehydrate in ascending alcoholic concentration from 70% to 95% with two minutes each. The smear is cleared in Xylene and mount in DPX

Papanicolaou staining method

Ethyl alcohol fixed smear were hydrate in descending concentration of 95% alcohol through 70% alcohol to distilled water, for two minutes in each stage. The smear were then treated with Harris Alum Haematoxylin for 10 minutes to stain the nuclei, rinsed in distilled water and differentiate in 0.5% aqueous hydrochloric acid for a few seconds to remove the excess stain. this was immediately followed by rinsing in distilled water to stop the action of discoloration. Then the smear were blued in alkaline water for seven minutes. The smear was treated with Papanicolaou Orange G6 for two minutes and dehydrate in ascending alcoholic concentration from 70% to 95% with two minutes each. The smear is cleared in Xylene and mount in DPX

RESULT

In this study, 62 tamol chewers both male and female were included. Oral epithelial dysplasia or OMPD was observed in habitual tamol chewers. The subjects were compared with the period of Tamol chewing in a year with a grade of epithelial dysplasia. The correlation was described between the severity (grade) or oral mucosal dysplasia or OMPD and Tamol consumption in year using r (rho-0) as null hypothesis. OPMD were found to have significant positive correlation with duration of Tamol consumption in a year (r=0.267, p=0.036) (Table 1). Cytological features of epithelial dysplasia or OMPD along with the grades were observed which were then relate with parameters with tamol consumption like total duration of tamol pan chewing to determine the dose response relationship between tamol chewing and oral epithelial dysplasia and we observed that the longer the duration of tamol pan chewing, greater the likelihood of epithelial dysplasia or OMPD. (Table 2)

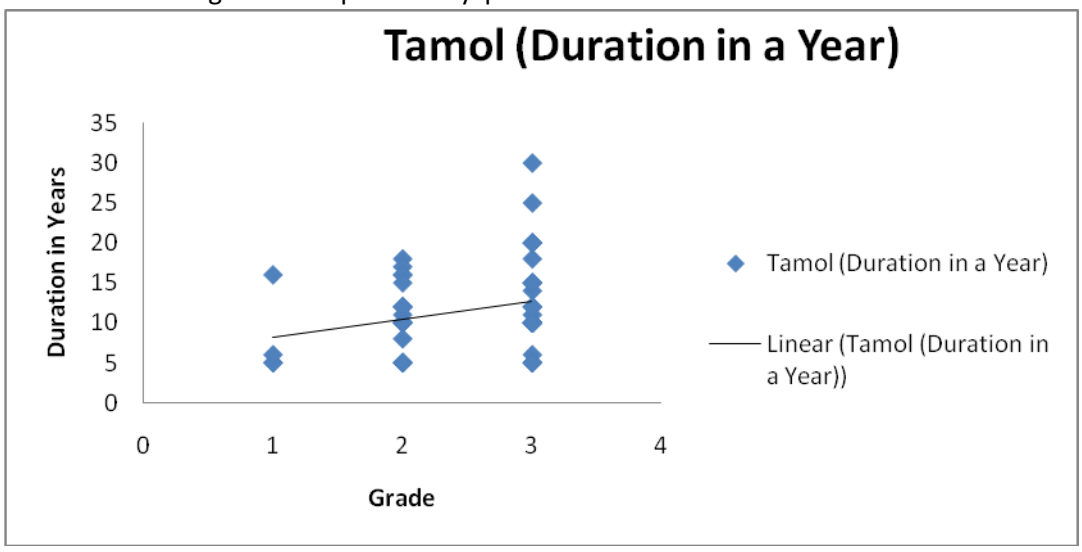
Frequency associated between duration of Tamol chewing in a year with the development of OPMD or oral epithelial dysplasia. In cross tabulation which indicates the longer the duration of Tamol exposure greater the chance of developing OMPD or oral epithelial dysplasia. In the group of individual chewing Tamol for more than 26 years have 100% development of OPMD or oral epithelial dysplasia as evaluate with the group of individual who chew tamol between 1-5 years develop OMDP or oral epithelial dysplasia as 81.8%. (Table no 3)

Table 1: Correlation between Tamol consumption and grade of epithelial dysplasia or OMPD

Variables		OPMD	Duration in Years
Grades of OPMD		1	.267*
	Sig. (2-tailed)		.036
	N	62	62
Duration in Years		.267*	1
	Sig. (2-tailed)	.036	
	N	62	62

* Correlation is significant at the 0.05 level (2-tailed).

Table 2: Scatter chart representing the correlations between the duration of Tamol pan chewing and its relation with the grades of epithelial dysplasia or OPMD.



Grade 1= Mild
Grade 2= Moderate
Grade 3= Severe

Table 3: Duration in Years * Presence and absence of OPMD Cross tabulation

Parameters	OMPD	
	Absent	Present
Duration in year		
1-5(n=11)	18.2%	81.8%
6-10(n=25)	4.0%	96.0%
11-15(n=14)	0.0%	100.0%
16-20(n=10)	10.0%	90.0%
20-25(n=1)	0.0%	100.0%
26-30(n=1)	0.0%	100.0%

DISCUSSION

In Oral cancer, buccal mucosa carcinoma is the most malignant tumor in the South East Asia (Gupta et al, 2003). At least 95% of cancer of the head and neck are squamous cell carcinomas rising most commonly in the oral cavity. (Bhattacharjee A et al, 2007). A major regional predisposing influence is the chewing of betel quid and "paan" in India. World Health Organization and International Agency for research on cancer classified areca nut as a Group1 human carcinogen with enough verification of increased risk of precancerous oral lesion and cancer of the oral cavity(Jacob BJ, 2004). The presence epithelial dysplasia in 57.7% of the habitual pan chewers and it has a positive correlation with pan

consumption per day and in a year. These variables were positively related with grading of epithelial dysplasia and study conclude that epithelial dysplasia was common among chronic pan chewers (Waris S and Nagi AH et al, 2014) has reported Considerable association was found between epithelial dysplasia and number of pans consumed per day as well as duration of pan consumption. White patches (leukoplakia) on the oral mucosa is reported to be a most characteristic sign of oral potentially malignant lesions. The malignant progression in these lesions overall is only of the order of 5% and there are no currently accepted markers to distinguish those that may progress or not(Speight et al. 2003). Role of quid in promoting proliferative activity in a normal buccal mucosal cells has been studied in which cytological evaluation has been done in the combination of AgNOR counts and PAP, which can predict the early quid-associated cellular changes before the expression of clinical premalignant and malignant lesions(Mohan BC et al, 2013). The betel quid used in north east India specially in Assam contains raw betel nut, lime paste and small part of betel leaf without tobacco and other constituents commonly called as tamol pan.

Aqueous extract of betel nut had taken to study the genotoxic potency in relation with the endogenous glutathione level in the mouse bone marrow and human peripheral blood lymphocytes and reported that longer the exposure of aqueous extract of raw betel nut showed higher level of p53 expression on mouse bone marrow cells. The level of endogenous glutathione level and the level of p53 protein could act as effective biomarkers for OSCC in relation with raw betel nut chewers.(Kumpawat and Chatterjee et al, 2003). About 1300 head & neck cancers are reported every year to Dr. B. Borooah Cancer Institute, Guwahati, Assam (Clinical Society of BBCI, Assam, Press release, 2/12/13). The basic knowledge and awareness is very necessary. Each and every individual should have a primary knowledge in Oral Cancer. Any abnormality in the oral cavity should be immediately consulted with ENT specialist and experienced physician. The warning signs and symptoms of oral cancer are very important.

CONCLUSION

Tamol chewing represents great burden and enormous health problem in the North East India especially Assam. Early stage of cancer cells can be revealed by oral exfoliative cytology. In addition PAP and H&E smear could be sensitive and useful tool for early identification of any morphological variation of oral epithelial cells by making oral smear and can be implemented in a routine investigation in dental clinic.

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