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Original Research Article

Clinical Significance of Antioxidant Levels in Saliva of Raw Betel Nut Chewer's: an Experience from Assam

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ABSTRACT

Background: Saliva as a diagnostic component is emerging as powerful diagnostic tool. Saliva is delivered from various salivary glands. It is always considered to be the first line of defense against oxidative stress and often provide the perfect medium to explore for health and disease surveillance. Various sources of oral free radicals and reactive oxygen species (ROS) are oral disease or/and tobacco consumption.

Materials and methods: we have determined the salivary antioxidant specially targeting uric acid and total antioxidant capacity in three groups i.e. Control population (No exposure to betel nut and healthy), raw betel nut chewers and oral cancer patients**.**

Result and **Conclusion:** Significant variation is seen in all the three categories. Mean value uric acid and Total Antioxidant in cancer patients and Tamol chewers are less as compare to control with the mean value of 2.7330, 3.6185 and 6.8545 for uric acid and .98635, 51500 and 1.19500 for total antioxidant capacity respectively which state an oxidative state in the oral cavity of the respective categories. Uric acid and total antioxidant capacity can be the important and promising salivary biomarker for raw betel nut associated oral cancer.

Key words: Saliva, Betel Nut, Biomarker, Oral cancer, North East

INTRODUCTION

In past few decades saliva has been used as a new diagnostic tool. The saliva fluid contains water, normal protein, lysozyme, peroxidase, immunoglobulin and other small molecules. The use of saliva as an alternative tool for monitoring the oral diseases and as a diagnostic tool represents various advantage as it is easily assessable and non-invasive method. [1]

Oral stress indicates the imbalance between the production of highly reactive molecular species (ROS), reactive nitrogen species (RNS) and antioxidant defense system. Where the formation of ROS or RNS increase and/or decrease antioxidant. Oxidative stress is now implicated in the pathology of several oral diseases such as oral cancer. Antioxidant resent in the saliva represents the first line of defense mechanism against Oxidative Stress. Saliva is rich in antioxidant such as Uric acid, albumin, ascorbic acid, glutathione and antioxidant enzyme. [2]

Various sources of oral free radicals and reactive oxygen species (ROS) are tobacco chewing and smoking that further can lead to oral cancer. Reactive oxygen species (ROS) are generating in significant amounts in oral cavity during chewing of betel quid/ betel nut. ROS can be determine in the oral mucosa and are reported to be

directly involved in promoting carcinogenesis, either by inducing mutations or by making the oral mucosa vulnerable to environmental toxicants. International Association for Research on Cancer (IARC) has classified areca nut and betel quid without tobacco as Group 1 carcinogens to humans and a cause of oral cancer. [2, 3]

Currently 10% of the world population or approximately 700 million people might be consuming Betel Nut in different forms across the globe. Epidemiological studies indicate that in the past 2 to 3 decades, 20-40% of the population in India, Nepal and Pakistan have used BQ. The main constituents of Betel nut are crude fiber, carbohydrates, fats, polyphenols, alkaloids, tannins, proteins and water. The amounts of these constituents are varies in produce of different area as well as in the dry or raw/wet variety of Betel Nut. The raw and wet variety of Betel nut is relatively rich in all constituents as compared to the dry variety. The active components of both forms of BN, which produce various effects, are primarily the alkaloids, polyphenols, and tannins (Figure 3). Figure 3 also highlights the outlines of the main events triggered in a living cell upon exposure to BN and/or its components that eventually lead carcinogenic transformation of the cell. [3] The North East region especially Assam and Meghalaya is tuning to be the stock house of Oral cancer due to many reasons. The tradition of chewing Betel Nut in North Eastern states of Assam and Meghalaya is very old, which are locally called as "Tamol" and "Kwai" in Assam and Meghalaya. Most of the people in this region like to have betel nut with the betel leaf. But many of them like the combination of Slaked lime and raw tobacco along with the betel nut and leaf which is very dangerous and contributes causing highest number of oral cancers in the region. The average age of onset of chewing among numerous tribes of north east India varies from 15-20 years. Again, ingestion of the quid after chewing instead of splitting it out which could be an important factor for progression to oral cancer. [3, 4] According to a British Journal 358 male and 144 females are found to be suffering from oral cancer, just due to the habit of chewing betel nut. Even the International Agency for Research on Cancer or IARC has declared the betel nut as one of the major Carcinogen, that provide aid to cancer and it has reached to the conclusion that there are sufficient evidences that chewing of betel nut leads to cancer. [3,4,5]

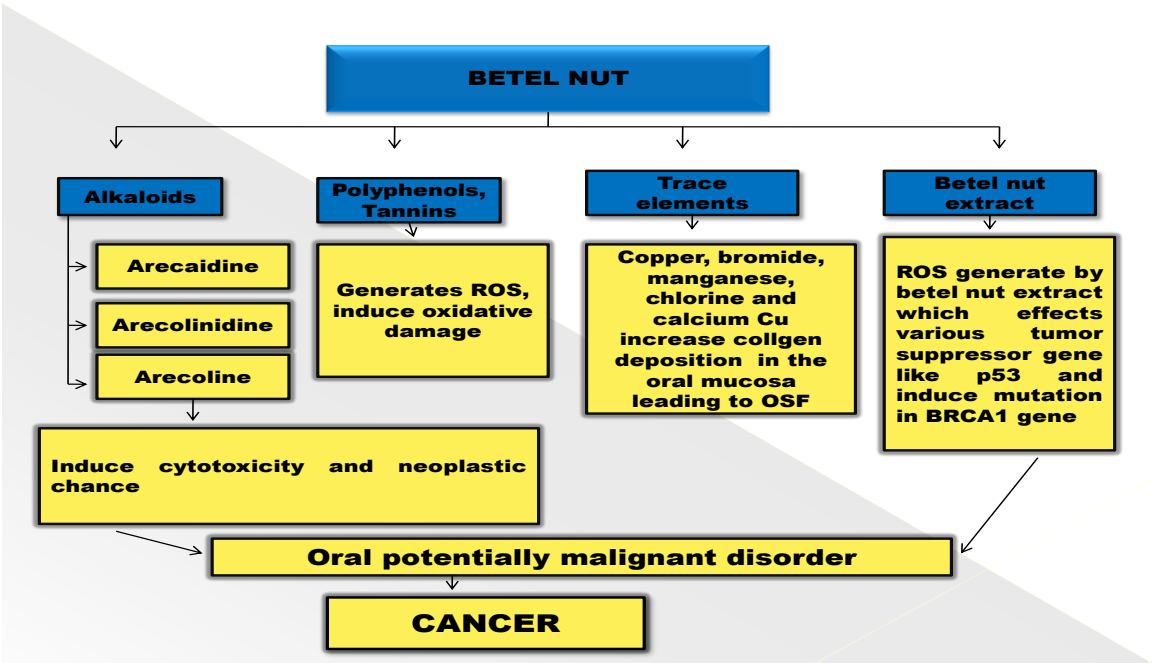

Figure 1: Flow Chart on Betel nut constituent and its effects [3]

Assessment of antioxidant profile using saliva sample can provide great information especially in tamol chewers to enable the prophecy of oxidative stress which may lead to oral cancer. With this background knowledge the current study was designed to assess the antioxidant profile by using saliva sample, specially targeting uric acid and Total antioxidant capacity.

METHODOLOGY

The sample collection criteria were the individual who are non-smoker, non-tobacco chewers (group 1), habitual tamol chewers (group 2) and oral cancer patients (group 3).

Unstimulated whole saliva from each subject was obtained from Garo Village and Mayang Village, Guwahati, Assam. A total of 120 samples were collected (40 each from three groups). Collected sample were immediately placed in ice and then centrifuged at 3,000 rpm and supernatant were used for analysis. For uric acid, uric acid colorimetric assay kits were used according to provided protocol (my BioSourse.com) and for total antioxidant capacity, Antioxidant assay hydrogen peroxide kits were used according to provided protocol (Cayman chemical company). Analyses were performed using semi- automatic biochemical analyzer and ELISA reader.

RESULT

The mean value of the three categories are different and represent the variation in all categories, Uric acid and TAC in cancer patients and Tamol chewers are less as compare to control, which state an oxidative state in the oral cavity of the respective categories (table no 1, figure 2 and 3 shows).

Table No. 1: Mean value of all the three groups

Group	TAC(µM/L)	Uric Acid (mg/dl)
Oral cancer Patient	.98635	2.7330
Tamol Chewers	.51500	3.6185
Control	1.19500	6.8545

Salivary uric acid and Salivary Antioxidant where shown statistics significance in between mention Group. The statistics value is (P<.002) in uric acid and (P<.001). In Antioxidant capacity that indicate there is the significance between three group in both uric acid and total antioxidant capacity (Table no 2)

Table no 2: Uric acid and TAC level with significance value P<.002 and P<.001.

		Sum of Squares	df	Mean Square	F	Sig.
uric acid (mg/dl)	Between Groups	188.284	2	94.142	6.823	**.002**
	Within Groups	786.419	57	13.797		
	Total	974.703	59			
TAC (µM/L)	Between Groups	4.854	2	2.427	11.469	**.001**
	Within Groups	12.062	57	.212		
	Total	16.916	59			

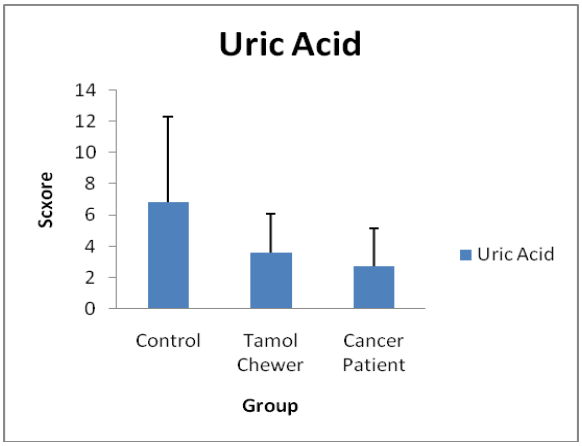

Figure 2: Concentration of uric acid in three groups

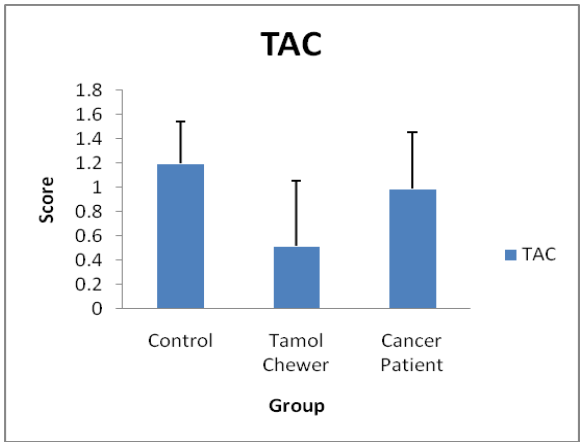

Figure 3: Concentration of total antioxidant capacity in three groups

DISCUSSION

In oral cancer, buccal mucosa carcinoma is the most malignant tumor in the south East Asia; At least 95% of cancer of the head and neck are squamous cell carcinoma rising most commonly in the oral cavity. A major predisposing factor is the chewing of betel quid and "*paan*" in India. Most oral cancers begin in the tongue or in the floor of the mouth and are squamous cell carcinomas. [6] Research has also generated sufficient evidence to implicate betel nut, with or without tobacco, as a suspected carcinogen to humans. Beside oral cancer, significant increase in the incidence of cancers of the esophagus, liver, pancreas, larynx and lung were seen among betel nut chewers. [9]

A study of esophageal squamous cell carcinoma (ESCC) in Taiwan revealed that subjects who chewed between 1 and 495 betel nut or more per year had 3.6-fold and 9.2-fold higher risk, respectively, of developing esophageal cancer compared to those who did not chew BN. [9]

A similar work was conducted by Jacob BJ et al, 2004 India, and it was reported that the chewing mixture of betel nut, alone or in combination with betel leaf and lime, can cause damage to the oral mucosa. Dose-response relationships were observed for both the frequency and duration of betel quid chewing without tobacco on the risk of oral precancer. [5] Unstable free radical species attack cellular components causing damage to lipids, proteins, and DNA which can initiate a chain of events resulting in the onset of a variety of disease. Living organisms have evolved a complex antioxidant system to curb ROS and to reduce their damage. Thus, all the antioxidant capacity may provide important biological information, as it considers the cumulative effect of all antioxidants present in plasma and body fluids. [3, 5, 7]

The study of antioxidants and its relation to tamol chewing suggest that antioxidant may play a major role in prevention of oral cancer and it utilization as diagnostic and prognostic marker. The result of this study suggests that patients of oral squamous cell of carcinoma and tamol chewers is associated with decreased level of uric acid and TAC as compared to control individuals and values between three groups were statistically significant.002 and.001. The mean value of the three categories are different and represent the variation in all categories, Uric acid in cancer patients are less as compare to tamol Chewers and control, which state an oxidative state in the oral cavity of the respective categories. However the total antioxidant capacity is less in tamol chewers as compare to cancer patients and control sample, it can be due to fact that during the process of tumor development, the level of oxidants and antioxidants changes depending on the stage of the cancer.

CONCLUSION

Our study supports the hypothesis that Tamol chewer elevates the risk of oral submucous and oral precancer lesions. The knowledge to help predict the probability of the individual to develop such harmful consequences in the future due to chewing habits can be used as an effective screening and education medium. The knowledge can be used to monitor and educate the individual in giving up the harmful habit of betel nut chewing. However, longitudinal studies need to be done to help in the quantification and support of the aforementioned contention. The study on antioxidants and its relation to tamol chewing suggest that antioxidant may play a major role in prevention of oral cancer and utilization as diagnostic and prognostic marker. The result of this study suggests that tamol chewing is associated with decreased level of uric acid and TAC. The increase in ROS and RNS may have been the event that lead led to the consumption and reduction of salivary antioxidant system, thus also explains the oxidative damage to the DNA and proteins, and possibly the promotion of Oral squamous cell carcinomas. This may be important for

better understanding the pathogenesis of the disease any may contribute to its prognostic, diagnosis and treatment. [7, 8]

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Conflict of Interest: No
Financial Disclosure: None

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