

Antioxidant Status in Serum of Long-term Areca Nut chewers and Oral Squamous Cell Carcinomas Patients

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ABSTRACT

Aim: This study was carried out to investigate implications of serum antioxidant status in people with long-term areca nut chewers and oral squamous cell carcinoma (OSCC) patients. **Materials and Methods:** Serum samples were collected from areca nut chewers, OSCC patients, and control of both genders between the ages of 15 and 60 years. Superoxide dismutase (SOD), total antioxidant capacity (TAC), uric Acid, and protein were estimated using ELISA reader, semi-automatic biochemical analyzer, and spectrophotometer. **Results:** SOD level in OSCC patients was decrease when compared to control and areca nut chewers indicate a low antioxidant profile. Yet, a decrease in SOD level in areca nut chewers when compared to control suggests that areca nut chewers are also prone to oxidative stress. TAC a known cancer biomarkers are known to increase in tobacco and areca quid's consumers. Increase serum TAC level in OSCC and areca nut chewers when compared to healthy control could further justify that areca nut chewers population are prone to OSCC. Our studies also showed that decrease uric acid level in areca nut chewers corresponds to decrease in free radical scavenging activity which relates to our SOD level in our earlier finding. Increase protein level was also observed in OSCC patients when compared to areca nut chewers and controls. Although, there was no significant change between areca nut chewers and controls, increase protein level in areca nut chewers indicates violation to the integrity of capillary bed or mucosal lining as occurs in oral neoplasia which can cause increase protein synthesis. **Conclusion:** Areca nut chewers can alter serum antioxidant enzymes and increase OSCC cancer biomarker. Hence, maintaining the antioxidants status can help limit the progression of precancerous condition toward malignancy.

Keywords: Antioxidant, Areca nut, Lipid peroxidation, Oral squamous cell carcinoma, Reactive oxygen species

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INTRODUCTION

Around 95% of cancer of the head-and-neck are squamous cell carcinomas in the oral cavity.^(1,2) A major regional inducing factor is the chewing of areca nut and "Paan" in India. The North-east region especially Assam and Meghalaya is turning to be the stock house of oral cancer, one of the most important risks can be their tradition of chewing raw areca nut. The most of the people in this region consume raw areca nut with the betel leaf and slaked lime, which are dangerous and contribute to causing the highest number of oral cancers in the region. The lime contains a strong chemical compound that often burns the soft tissues or mucosa in the oral cavity that forms a scars or ulcers. Frequent and regular scratches of areca nut and areca leaf form ulcers in the oral cavity are the contributing factors for developing oral cancer.⁽³⁾

Oral squamous cell carcinoma (OSCC) is the 6th most common human cancer, with an increasing incidence in younger people, a high morbidity rate, and a 5-year mortality rate of about 50%. Free radicals, such as reactive oxygen and nitrogen species (ROS and RNS), that induce oxidative and nitrostatic stress contribute to the development of oral carcinogenesis from leukoplakia through DNA damage. The purpose of the present study was to evaluate various oxidative stress-related parameters and the antioxidant profile of the saliva in OSCC patients. This analysis may well of great importance to further understand the relationship between serum and free radicals in the genesis of oral cancer pathogenesis. Squamous cell carcinomas are important from a clinical point of view and have relevance to a wide variety of fields, including medicine, pathology, and surgery, nuclear medicine, and radiation therapy. The study has been conducted to have a good insight in establishing a

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relationship between serum antioxidant status and the early detection of the malignancy. This can help reduce the incidence and prevalence of different squamous cell carcinoma after the certain intervention of course.^[4-6]

MATERIALS AND METHODS

Sample Collection

The study was performed in accordance with proper approval by the Institutional Human Ethics Committee (Ethical memo no: ADTU/Ethics/Stdnt-Lett/2017/063). The sample collection criteria were the individual who is control (Group 1), habitual areca nut chewers (Group 2), and oral cancer patients (Group 3). Out of the 120 samples, 40 patients sample selected for Group 3 were diagnosed with OSCC and were collected from North East Cancer Hospital, Assam in India. Eighty patient's samples selected for

control and areca nut was obtained from Garo and Mayang village, Guwahati, Assam. Group 1 and Group 2 were divided accordingly bases on the exclusion and inclusion criteria. Each collected sample inclusion criteria were age group, history of areca nut abuse for at least 2 years, history of present lesion (OSSC) to be <3 months, and agreeing blood examination. The exclusion criteria were patients not willing to participate in the study and the patients suffering from systemic diseases or having clinical manifestations of areca nut abuse in the form of potentially malignant diseases.

Biochemical Estimation

Estimation of protein by Eze and Dumbroff,^[7] biochemical determination of enzymatic antioxidants such as superoxide dismutase (SOD), was estimated according Marklund *et al.*^[8] For uric acid, uric acid colorimetric assay kits were used according to provided protocol (my BioSource.com) and for total antioxidant capacity. Antioxidant assay hydrogen peroxide kits were used according to provided protocol (Cayman chemical company). Analyzes were performed using semi-automatic biochemical analyzer and ELISA reader.

RESULT AND DISCUSSION

Oral cancer is one of the most common cancers in the world, with approximately 274,300 new cases and 127,500 deaths occurring each year.^[9] Over 90% of oral cancers are squamous cell carcinomas.^[10,11] Identification of a biomarker with high sensitivity and specificity for detecting oral cancer from serum could dramatically improve the early detection of OSSC. The most common antioxidant SODs are class of enzymes that catalyze the breakdown of the superoxide anion into oxygen and hydrogen peroxide, thereby preventing free radicals mediated oxidative damage. Results obtained in Figure 1 showed SOD level in OSSC patients when compared to areca nut chewers and controls. Although there was no significant change between areca nut chewers and controls, a decrease in SOD activity observed in areca nut chewers and cancer patients indicates a low antioxidant profile. The decrease serum antioxidants levels showed that the deficient in natural antioxidant defense can cause high oxidative stress/lipid peroxidation and leading to the genesis of carcinogenesis. Serum total antioxidant capacity (TAC) is also known as one of the several cancer biomarkers are being evaluated in different neoplastic conditions. Furthermore,^[12] have reported reduce serum TAC level in tobacco and areca nut consumers. In our finding, increased TAC level in OSSC and patients areca nut chewers

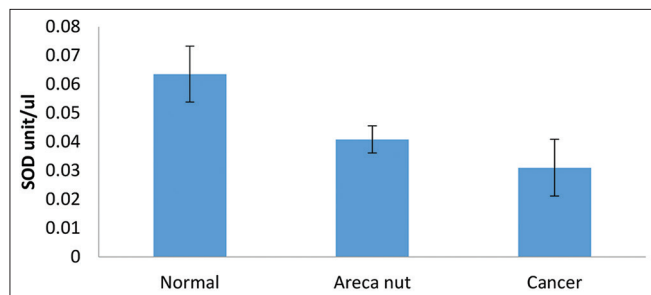


Figure 1: Superoxide dismutase activity in serum of normal individuals, areca nut chewers, and oral squamous cell carcinoma. All quantitative data were expressed as mean±SD. Tabulation and graphical presentation of the result were carried out for oral cancer and control groups

when compared to controls as depicted in Figure 2 further confirms that areca nut chewers population are prone to OSSC. The studies have shown that uric acid can eliminate free radicals as an essential antioxidant in the human plasma.^[13-15] Manifar *et al.*^[16] reported that uric acid has shown to increase inflammation and oxidative stress, which can in turn increase tumor cell proliferation and angiogenesis and support invasion and metastasis. Our studies in Figure 3 showed that decrease uric acid level in areca nut chewers corresponds to decrease in free radical scavenging activity which relates to SOD level in our earlier finding. Antioxidants can interfere with activation of pro-carcinogens, prevent binding of carcinogens to DNA, inhibit chromosome aberrations, restrain replication of the transformer cells, and suppress action of pre-cancerous lesions. Identification of these oxidative stress biomarkers such as SOD and antioxidants status in clinical samples of cancer patients and defining their roles in cancer initiation and progression holds great promise in promoting the development of targeted therapeutic approaches and diagnostic strategies evaluating disease status. Several studies determined proteins concentration in the supernatant of saliva in patients with breast cancer, ovarian cancer, oral cancer, and leukoplakia.^[17-20]

In our study, we have also focused on estimating the serum protein to correlate with OSSC. Result depicted in Figure 4 reported increase protein level in OSSC patients when compared to areca nut chewers and controls. Although there was no significant change between areca nut chewers and controls, increase protein level in areca nut chewers when compared to control was observed. Salivary proteins may be originated as an ultra-filtrate

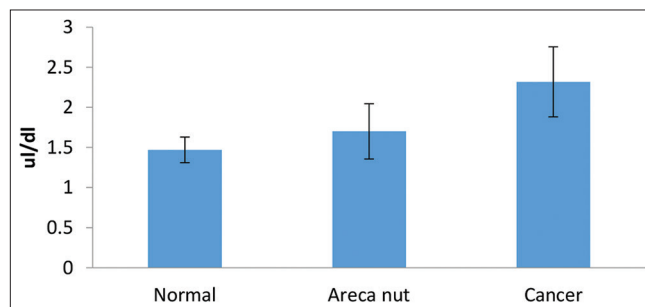


Figure 2: Total antioxidant capacity in serum of normal individuals, areca nut chewers, and oral squamous cell carcinoma. All quantitative data were expressed as mean±SD. Tabulation and graphical presentation of the result were carried out for oral cancer and control groups

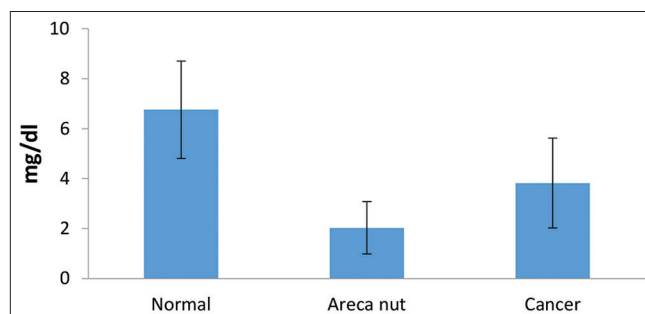


Figure 3: Uric Acid in serum of normal individuals, areca nut chewers, and oral squamous cell carcinoma. All quantitative data were expressed as mean±SD. Tabulation and graphical presentation of the result were carried out for oral cancer and control groups

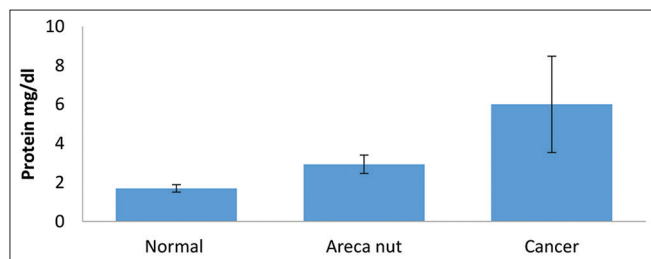


Figure 4: Protein level in serum of normal individuals, areca nut chewers, and oral squamous cell carcinoma. All quantitative data were expressed as mean \pm SD. Tabulation and graphical presentation of the result were carried out for oral cancer and control groups

of plasma proteins or through salivary and mucosal secretions, so any violation to the integrity of capillary bed or mucosal lining as occurs in oral neoplasia may be associated with an increase in salivary proteins especially albumin.

CONCLUSION

Cancer is the product of interaction of genetic factors and environment exposures. Areca nut chewers can violation to the integrity of capillary bed, mucosal lining, or cellular/biochemical hemostasis. As a result, assessment of these markers in areca nut chewers would help determine the tendency of the individual to develop OSSC and also limit the progression of precancerous condition toward malignancy by maintaining the antioxidants status.

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REFERENCES

- Gupta PC, Ray CS. Smokeless tobacco and health in India and South Asia. *Respirology* 2003;8:419-31.
- Bhattacharjee A, Chakraborty A, Purkaystha P. Prevalence of head and neck cancers in the North East an institutional study. *Indian J Otolaryngol Head Neck Surg* 2006;58:15-9.
- Kumpawat K, Chatterjee A. The usefulness of cytogenetic parameters, level of p53 protein and endogenous glutathione as intermediate end-points in raw betel-nut genotoxicity. *Hum Exp Toxicol* 2003;22:363-71.
- Phukan RK, Ali MS, Chetia CK, Mahanta J. Betel nut and tobacco chewing; potential risk factors of cancer of oesophagus in Assam, India. *Br J Cancer* 2001;85:661-7.
- Sharan RN, Mehrotra R, Choudhury Y, Asotra K. Association of betel nut with carcinogenesis: Revisit with a clinical perspective. 2012;7:e42759.
- Bahar G, Feinmesser R, Shpitzer T, Popovtzer A, Nagler RM. Salivary analysis in oral cancer patients: DNA and protein oxidation, reactive nitrogen species, and antioxidant profile. *Cancer* 2007;109:54-9.
- Eze JM, Dumbroff EB. A comparison of the Bradford and Lowry methods for the analysis of protein in chlorophyllous tissue. *Can J Bot* 1982;60:1046-9.
- Marklund SL. Clinical aspects of superoxide dismutase. *Med Biol* 1984;62:130-4.
- Sankaranarayanan R, Ramadas K, Thomas G, Muwonge R, Thara S, Mathew B, *et al.* Effect of screening on oral cancer mortality in Kerala, India: A cluster-randomised controlled trial. *Lancet* 2005;365:1927-33.
- Brinkman BM, Wong DT. Disease mechanism and biomarkers of oral squamous cell carcinoma. *Curr Opin Oncol* 2006;18:228-33.
- Hasan HR, Abdelwahb NN. Evaluation of protein level's changes in saliva and sera of oral squamous cell carcinoma patients. *Baghdad Sci J* 2014;11:447-54.
- Bose KS, Vyas P, Singh M. Plasma non-enzymatic antioxidants- vitamin C, E, beta-carotenes, reduced glutathione levels and total antioxidant activity in oral sub mucous fibrosis. *Eur Rev Med Pharmacol Sci* 2012;16:530-2.
- Kuzkaya N, Weissmann N, Harrison DG, Dikalov S. Interactions of peroxynitrite with uric acid in the presence of ascorbate and thiols: Implications for uncoupling endothelial nitric oxide synthase. *Biochem Pharmacol* 2005;70:343-54.
- Wu X, Muzny DM, Lee CC, Caskey CT. Two independent mutational events in the loss of urate oxidase during hominoid evolution. *J Mol Evol* 1992;34:78-84.
- Kand'ár R, Žáková P, Mužáková V. Monitoring of antioxidant properties of uric acid in humans for a consideration measuring of levels of allantoin in plasma by liquid chromatography. *Clin Chim Acta* 2006;365:249-56.
- Manifar S, Rahimzamani A, Shirkhoda M, Ghamsari MN, Bakhshi M. Role of serum uric acid as a protective biomarker in patients with different histopathological grades of oral squamous cell carcinoma: A case-control study. *Biomed Res Int* 2020;2020:5185423.
- Streckfus C, Bigler L, Tucci M, Thigpen JT. A preliminary study of CA15-3, c-erbB-2, epidermal growth factor receptor, cathepsin-D, and p53 in saliva among women with breast carcinoma. *Cancer Investig* 2000;18:101-9.
- Gorelik E, Landsittel DP, Marrangoni AM, Modugno F, Velikokhatnaya L, Winans MT, *et al.* Multiplexed immunobead-based cytokine profiling for early detection of ovarian cancer. *Cancer Epidemiol Biomarkers Prev* 2005;14:981-7.
- Rhodus NL, Ho V, Miller CS, Myers S, Ondrey F. NF- κ B dependent cytokine levels in saliva of patients with oral preneoplastic lesions and oral squamous cell carcinoma. *Cancer Detect Prev* 2005;29:42-5.
- Brailo V, Vučićević-Boras V, Cekić-Arambašin A, Alajbeg IŽ, Milenović A, Lukač J. The significance of salivary interleukin 6 and tumor necrosis factor alpha in patients with oral leukoplakia. *Oral Oncol* 2006;42:370-3.