International Journal of Research in Health and Allied Sciences

Journal home page: www.ijrhas.com

Official Publication of "*Society for Scientific Research and Studies*" [Regd.]

ISSN 2455-7803 Index Copernicus value 2016 = 68.10

ORIGINAL Article

Estimation of Serum and Salivary Albumin and Uric Acid Levels in Oral Submucous Fibrosis and Oral Squamous Cell Carcinoma: A Biochemical Study

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***ASBTRACT*:**
Introduction: - Life style and diet play an important role in the pathogenesis of oral cancer. Unfortunately, under the present day life style conditions, many people run an abnormally high level of oxidative stress that could increase their probability of early incidence of decline in optimum body functions and lead to a number of pathologies. Hence this study aimed to estimate serum and salivary albumin and uric acid levels in healthy individuals, oral sub mucous fibrosis and oral squamous cell carcinoma patients.
Methodology: - Study comprised of three groups as follows: **Group I:** Comprised of 10 healthy individuals. **Group II:** 20 otherwise healthy and consenting patients with Oral Submucous Fibrosis, **Group III**: 20 otherwise healthy and consenting Oral Squamous Cell Carcinoma patients. Venous blood was drawn and Unstimulated whole saliva was collected after the clinical diagnosis followed by biochemical estimation of serum and salivary albumin and uric acid with the help of Semiautomatic Analyzer. The results were analyzed using students -t test and were averaged as mean ± standard deviation with p- value < 0.05 considered to be statistically significant. The normality of data was checked before the statistical analysis was performed. **Results -** Inter comparison of serum albumin and uric acid levels between all the three groups revealed that the difference was statistically significant, indicating that serum albumin and uric acid levels decreased in Oral Submucous Fibrosis and Oral Squamous Cell Carcinoma compared to healthy individuals. Salivary albumin levels significantly increased in Oral Submucous Fibrosis and Oral Squamous Cell Carcinoma cases compared to healthy individuals. Whereas salivary uric acid levels significantly decreased in Oral Submucous Fibrosis and Oral Squamous Cell Carcinoma cases compared to healthy individuals. **Conclusion -** The results obtained emphasize the need for more studies with larger sample size to be conducted before a conclusive role could be drawn in favor of serum and salivary albumin and uric acid as diagnostic markers of significance in oral sub mucous fibrosis & Oral Squamous Cell Carcinoma.
Key words: Saliva, Albumin, Uric Acid, free radicals, Oral Sub Mucous Fibrosis ,Oral Squamous Cell Carcinoma.

Received: 12 November 2018 Revised: 8 December 2018 Accepted: 10 December 2018

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This article may be cited as: Tiwari P, Khajuria N, Metgud R. Estimation of Serum and Salivary Albumin and Uric Acid Levels in Oral Submucous Fibrosis and Oral Squamous Cell Carcinoma: A Biochemical Study. Int J Res Health Allied Sci 2019; 5(1):5-9.

INTRODUCTION

Oxidative stress is defined as "an imbalance between pro-oxidants and antioxidants resulting in an overall increase in cellular levels of ROS such as O_2^-, H_2O_2 and OH^-". ROS can cause DNA base alterations, strand breaks, damage to tumor suppressor genes. The primary targets of peroxidation by ROS are the membrane lipids, yielding in end products such as lipid hydroperoxides and malondialdehyde. To combat such damage, the body possesses several antioxidant systems in body fluids such as saliva and serum that are important in the prevention of oxidative stress.[1]

Oral squamous cell carcinoma (OSCC) is a multistage process, from normal to dysplastic cells (precancerous lesions) and ultimately to squamous cell carcinoma.[1] Despite the recent treatment advances, oral cancer is reported as having one of the highest mortality ratios amongst other malignancies and this can much be attributed to the late diagnosis of the disease.[2]

Saliva has been found to contain constituents that reflect the diseased or physiological state of the human body, and hence could be utilized for diagnostic purposes. The search for reliable salivary biomarkers for early detection of OSCC has developed rapidly, spurred on by the fact that collecting saliva is relatively easy and non-invasive, compared to the drawing of blood.[4,5]

Saliva is the first line of defense against oxidative stress and contains various antioxidants including albumin (Johnson2001). The roles of albumin in the body are understood well enough to allow it to be used as a reliable marker of oxidative stress; it could be useful for early diagnosis of various oral precancerous lesions and for determination of the tendency toward transformation into frank oral malignancy (Lannitti 2012).[6]

Uric acid the next demonstrated important antioxidant and a free radical scavenger in humans is one of the major radical trapping antioxidants in plasma and is reported to protect the erythrocyte membrane against lipid peroxidation.[3]

AIMS AND OBJECTIVES-

1. To estimate serum & salivary albumin and uric acid levels in healthy individuals, oral submucous fibrosis and oral squamous cell carcinoma.
2. To correlate serum and salivary albumin, uric acid levels in healthy individuals, oral submucous fibrosis and oral squamous cell carcinoma.

MATERIAL AND METHODS

The study was designed to estimate the serum and salivary albumin and uric acid levels in healthy individuals, Oral Submucous Fibrosis (OSMF) & Oral Squamous Cell Carcinoma (OSCC) patients by using assay kits and Semiautomatic Analyzer.

Inclusion criteria

- 20 patients with clinical manifestations of OSMF.
- 20 patients with clinical manifestations of untreated OSCC

Exclusion criteria

- Patients undergoing cancer therapy.
- Oral and systemic conditions known to alter serum and salivary albumin, uric acid levels.
- **For serum albumin and uric acid -**5ml of venous blood sample was collected from individuals of all the groups under aseptic precautions. Clotted blood was centrifuged. The clear serum was separated and stored at -20^{0}C until analyzed.
- **For salivary albumin and uric acid**- 3ml of unstimulated whole saliva was collected from individuals of all the groups. Prior to saliva collection oral cavity was rinsed out with distilled water and unstimulated whole saliva was collected with the subject leaning forward and spitting saliva into a sterile container. Immediately after collection, saliva sample was spun (3000 rpm, for 10 min at 4^{0}C), the upper parts were drawn and stored in small laliquots at -20^{0}C and protected from light until analyzed. The Assay Buffers were warmed to room temperature before use.

Thus obtained samples were thawed, and the antioxidant activity of both serum and saliva was analyzed by biochemically estimating albumin and uric acid levels using the kits BCG Albumin Reagent CE IVD and Uric Acid Ver 2 Cobas Integra and semiautomatic analyzer. Two sample student t-tests with unequal variance was applied for testing the significance of difference between pair wise combinations of three groups (healthy individuals, OSMF and OSSC patients).

RESULTS

A wide variation in age was noted amongst the study groups, ranging from 22-60 years with a mean age of 23.8 years in group I, 33.1 years in Group II and 49 years in Group III.

Group I comprised of 5(25%) male and 5(25%) female subjects, Group II comprised of 18 (90%) male and 02 (10%) female subjects and group III comprised of 15 (75%) male and 05 (25%) female subjects.

In Group II 13 (65%) cases of oral submucous fibrosis were in S1 group (buccal mucosa) and 7 cases (35%) in buccal mucosa with other regions retromolar, gingival, hard palate, commissural region.(Table 7a) In group III, most common sites were alveolus 07 (35%) and buccal mucosa 06(30%) followed by tongue 2(10%), buccal vestibule 1(5%), labial vestibule 1(5%), floor of mouth 01(5%), angle of the mouth 01 (5%) & 1(5%) retromolar region.

Among the 20 OSMF patients 1(5 %) was with clinical stage I, 14(70%) were with clinical stage II and 5(25%) were with stage III.

The mean and standard deviation values of serum and salivary albumin levels for Group I were 4.55 ± 0.86, 0.44 ± 0.35, Group II were 2.82 ± 0.49, 0.86 ± 0.12 and for group III were 2.56 ± 0.82, 2.20 ± 0.80. The mean and standard deviation values of serum and salivary uric acid levels for Group I were 5.88 ± 0.62, 4.66 ± 0.66, Group II were 2.67 ±1.10, 2.60 ± 0.81 and for Group III were 0.91 ± 0.12, 1.90 ± 0.80 respectively.

Inter group comparison for serum and salivary albumin levels between group I and II revealed statistically significant results with p values of 0.0021 and 0.001.On the other hand inter group comparison of serum and salivary uric acid levels between group I and II revealed statistically significant results with p values of 0.003 and 0.001.

Intergroup comparison for serum albumin levels between Group II & III revealed non significant results with p value of 0.081 whereas significant result was observed for salivary albumin levels with p value of 0.003.Also serum and salivary uric acid levels revealed statistically significant results with p values of 0.005and 0.013.

On inter group comparison for serum and salivary albumin levels between group I & III significant results were obtained with p values of 0.0064 and 0.022. Whereas comparison of serum and salivary uric acid levels revealed highly significant results with p values of 0.0001 and 0.0004. (Table 1)

Table 1- Mean and Standard Deviation values of Serum and Salivary Albumin and Uric Acid levels in the study groups

Groups	Mean ±SD Serum albumin	Mean ±SD Salivary albumin	Mean ±SD Serum UA	Mean ±SD Salivary UA
Group-I	4.55±0.86	0.44±0.35	5.88±0.623	4.66±0.66
Group-II	2.82±0.49	0.86±0.122	2.67±1.10	2.60±0.81
Group-III	2.56±0.82	2.20±0.80	0.913±0.125	1.90±0.80

DISCUSSION

Exceptional advances in biomedical sciences since the past century gives opportunities to understand the molecular basis of disease that could result in new strategies for treatment and prevention of diseases.

Nowadays, antioxidants are getting more and more attention as potential preventers of cell damage by free radicals. In fact, it is known that antioxidants may counteract the effects of free radicals in every part of the body. Epidemiological studies suggest that the intakes of antioxidants are inversely related to the cancer risk. While the cell culture studies confirm the effect of antioxidants against cancer, the clinical trials remain inconclusive.[7]

Delayed detection is likely to be a primary reason for the high morbidity rate of oral cancer patients, and this support the imperative need for sensitive biomarker to improve early detection of oral cancer.[8]

Non-invasive methods like analysis of saliva may provide a cost-effective approach for screening large population. Human saliva proteins can be informative for disease detection and surveillance of oral health.[7]

In the present study, of the 10 healthy individuals in Group I mean age was 23.8 years, 25% were males and 25% were females. Of the 20 patients in Group II, mean age was 33.1 years, 90% were males,10% were females and 70% patients presented with clinical stage II with buccal mucosa being the common site in 65% patients. These findings were consistent with reports of studies by Chaturvedi et al.(1991), Kiran Kumar(2007), vaneja reddy et al.(2011),fareedi mukram et al.(2013) who observed male predominance with mean age of 39.1 years. On the other hand rangnathan et al. (2001) observed female predominance in stage II with the mean age of 38.8 years.

With respect to Group III mean age of patients was 49 years, 75% were males and alveolus was the most common site with 35%. Our observations were consistent with reports of study by Salman Shafique et al.(2010) who reported predominance of males with the mean age of 48.3 years. However A.O. lawal et al.(2012)[16] and Prashanthi Chippagiri et al.(2014) have reported predominance of males with the mean age of 53.7 years and 53.4 years respectively.

In the present study we observed that serum albumin levels decreased significantly in both oral submucous fibrosis patients and oral squamous cell carcinoma patients compared to healthy controls. Our observations are consistent with the reports of following studies. Yasunori Iwao et al. (2006) reported decreased levels of albumin in different histological grades of oral cancer patients.

On the other hand studies by Lawal et al. (2010) reported that risk of oral cancer was 32 times more in patients with low serum albumin levels compared to oral cancer with alcohol & tobacco consumers. Nayyar et al. (2011)[27] reported significant decreased level of albumin in histologically proven cases of poorly differentiated oral squamous cell carcinoma. V Dharkey et al.(2012) [22] reported the decreased sera albumin level associated with increased risk of oral cancer. Also Rukzan et al.(2013)[9] reported significantly decreased levels of sera albumin in oral tumours (benign and malignant).and R.Metgud et al.(2014)[8] reported that the albumin level decreases in oral leukoplakia and OSCC cases compared to healthy individuals and albumin may play a role in early diagnosis of oral premalignant and malignant lesions.[7,9]

Rajendran R.et al. (1990)[13] reported lower concentration of serum albumin in oral submucous fibrosis patients as compared to healthy individuals. Whereas V.dharkey et al. (2012) observed decreased levels of serum albumin and increased levels of total iron capacity in oral submucous fibrosis patients.

The reduction in serum albumin concentration that was observed in the current study may be explained as follows: - Liver is the site for the synthesis of most proteins including both negative APR (pre albumin and albumin) and positive APR. It is well known that the concentration of positive APR in serum increases in malignancy, which means that liver will be busy with synthesis of this type of APR leaving behind the synthesis of other proteins like albumin, so the synthesis of inflammatory cytokines which is increased in malignancy, such as tumor necrosis factor-α (TNF-α) and interlukines and C-reactive protein seems to cause a reduction in albumin concentration. Moreover, nutritional deficiency and weight loss in association with psychological distress and lower quality of life may cause reduction in sera albumin concentration. [10,22]

The other cause of the observed reduced albumin level in sera of patient group may be due to the role of albumin as one of the extracellular antioxidants, where albumin constitutes up to 49% of total plasma antioxidant status.[9]

Albumin acts as sacrificial antioxidant by inhibiting the generation of free radicals through an immediate attack of albumin molecule itself, so the radical reaction continue on albumin surface and cause damage to albumin molecule, such damage is probably biologically insignificant, due to which the albumin is present in plasma in high concentration.[9]

It has been shown that diet and nutrition are etiological factors for oral cancer. Winn (1995) suggested that inadequate nutrition enhances cancer risk.[35] These findings are consistent with a study in India in which

subjects with oral cancer were less likely to consume nutritious food compared to a control group (Lawal 2010). Because serum albumin may reflect nutritional status and protein intake (Knekt 2000), it is possible that low serum albumin levels are associated with increased risk of oral cancer (Ko 1986). [21]Therefore, serum albumin is not only a window into the patient's nutritional status, but also a useful factor for predicting patient prognosis.[7] The potential advantages of serum albumin as pre-treatment prognostic factor in cancer patients are that it is inexpensive, reproducible and powerful (Gupta and Christopher 2010).

In contrast to serum albumin, throughout the present study, salivary albumin concentration revealed a significant increase in oral submucous fibrosis and oral squamous cell carcinoma patients in comparison to that of the healthy individuals.This findings was consistent with the reports of following studies. Meurman JH et al (2002)[25] reported significantly higher salivary albumin concentrations in the oral diseased patients. Miricescu et al. (2011)[12] reported increased levels of albumin in pre-cancer and oral cancer. Also Rukzan et al.(2013) [9] reported increased concentration of salivary albumin in oral tumours (benign and malignant) and R. Metgud et al.(2014)[8] reported significantly increased salivary albumin concentration in patients diagnosed with leukoplakia and OSCC. [22,8]

Roche 2008, Miricescu 2011 [19] suggested that albumin and uric acid appear to contribute to most of the antioxidant protection in whole saliva with uric acid being principle antioxidant and in cases of oral precancer and oral cancer the levels of salivary uric acid decreases and the levels of salivary albumin increases as a compensatory antioxidant defence system to counteract oxidative stress.[19]

In the oral cavity, albumin in saliva reported to be blood derived protein [9] may diffuse into the mucosal secretions [8], so the marked increase level of albumin may be related to the increase of tissue damage and loss of epithelial barrier function and increased vascular permeability leading to leakage or escape of many plasma proteins including albumin to extracellular spaces (interstitial) and through crevicular fluid to saliva causing increase in salivary albumin. [9]

In the present study we observed significantly decreased serum levels of uric acid in patients with oral submucous fibrosis and oral squamous cell carcinoma as compared to healthy individuals, which was consistent with the results by Nagini et al. (1997)[16] who reported decreased serum uric acid levels in oral cancer patients, A.O. Lawal et al.(2012)[16] reported lower level of serum uric acid concentration in oral cancer patients as compared to healthy individuals and suggested that low serum uric acid in oral cancer patients may be due to nutritional compromise of the patients due to Tumour necrosis Factor (TNF) and Interleukin 6 (IL-6) produced in cancer patients, which cause loss of appetite.

In the present study we observed that the salivary uric acid decreased significantly in both OSMF and OSCC patients, which is consistent with the results of following studies. Tsuchiya et al.(2002), presented that uric acid concentration drastically goes down even with the single consumption of cigarette. Maria Greabu et al.(2011)[15], observed that exposure to the gas phase of cigarette smoke (CS) caused a statistically significant fall down in salivary uric acid.

Gideon Bahar et al. (2006)[15] reported decreased concentration of salivary uric acid levels in premalignancy and OSCC. Joanna et al (2011) [18] reported that the concentration of salivary uric acid significantly decreases in oral squamous cell carcinoma. Also Hanspal Singh et al.(2014)[10] observed statistically significant changes in the salivary uric acid levels of clinical staging and histological grading between well to moderate and moderate to poorly differentiated OSCC patients.

The concentrations of albumin and uric acid are lower in saliva than in serum. This may indicate an active secretion system for salivary antioxidants rather than passive diffuse from the circulation. Stimulated saliva contains a lower concentration of antioxidants, but when flow rates are taken into account, the antioxidant capacity is greater than in unstimulated saliva (Moore 1994).[24,27]

Changes in the salivary antioxidant enzymes suggest that saliva may be appropriate marker for monitoring of oral diseases compared to our conventional invasive serum antioxidant enzyme. [29,32]

The advantages of salivary monitoring include ease of collection, elimination of fear of needles, inexpensive testing and reduced risk of disease transmission between healthy and diseased patients. In addition salivary diagnostic is fast, highly sensitive and specific portable, users friendly and can screen for multiple agents.[25,30,34]

It is difficult to measure oxidative stress using histochemical, spectrophotometric or proteomic and genomic assays, and uncertainty about its etiological link with pathological conditions have slowed research and development in this field. Recently, new practical and reliable instruments have become available that require only a drop of blood or a sample of urine or saliva to assess a patient's oxidative stress by quantifying free radicals.[7,31,32]

Oral cancer develops from precancerous conditions and lesions. If treatment acted at the beginning of disease then it can result in a high cure rate. Antioxidants have possibility to avert, inhibit and inverse some of multiple steps involved in oral carcinogenesis.[27,28]

CONCLUSION

The search for reliable salivary biomarkers for early detection of OSCC has developed rapidly, spurred on by the fact that collecting saliva is relatively easy and non-invasive, compared to the drawing of blood. The result obtained emphasize the need for more studies to be conducted in this regard for the assessment of sera and salivary albumin and uric acid to accept their utility and to assess their role in pathogenesis and their impact on the diagnosis of oral cancers providing a scientific ground for justifying the use of diverse chemo preventive strategies in controlling damage at genetic and molecular levels to

prevent the ongoing transition of various oral premalignant conditions into malignant degenerations.

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