Salivary Albumin as a Biomarker for Oral Squamous Cell Carcinoma and Chronic Periodontitis

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Abstract

Background: Detection of cancer at the early stage is of great importance to decrease the morbidity and mortality of the disease. And as it is known that in neoplastic conditions, many biochemical substances deviate from their normal values; these may include proteins of body fluid. Analysis of salivary markers may provide an economical and noninvasive approach for screening large population. Periodontal infection has been implicated as risk factor for systemic diseases such as coronary heart diseases and diabetes. Aim: The purpose of this preliminary study is to investigate whether salivary albumin levels can be used as an indicator for early detection of oral squamous cell carcinoma and in early detection of chronic periodontitis. Methods: In this cross-sectional study a total of 90 participants were recruited from Dept. of Periodontics, A. B. Shetty Memorial Institute of Dental Sciences and Dept of Oncology, K. S. Hegde Medical Academy. The participants were divided into three groups of 30 each. Group A -healthy individuals, Group B -patients with chronic generalized periodontitis & Group C -patients with oral squamous cell carcinoma. Salivary Albumin levels were measured using commercially available kits. The difference in the albumin levels between the three groups were compared using Kruskall Wallis test followed by Mann- Whitney U test. P value of less than 0.05 was considered statistically significant. Results: The mean value of Albumin in group A (healthy) is 0.28(SD is 0.19), in group B (chronic gen periodontitis) is 0.31(SD is 0.19), and in group C (OSCC) is 0.82.(SD is 0.41). On comparing the three mean values it is seen that there is a significant rise in salivary levels of Albumin in OSCC patients, but there was no significant rise in salivary albumin levels in chronic periodontitis cases when compared with the healthy group. Conclusion: This study shows that Salivary Albumin levels could be used as a biomarker in Oral squamous cell carcinoma cases but may not be useful as a biomarker for early detection of chronic generalized periodontitis. Keywords: Oral squamous cell carcinoma; Albumin; Chronic periodontitis

Introduction

Oral squamous cell carcinoma (OSCC) is common and one of the most dreaded types of cancer with high rates of mortality and morbidity all over the world [1]. The increasing incidence of OSCC among women and young and middle-aged males is particularly challenging [2-4]. In India, the incidence rate is high due to consumption of tobacco in various forms, consumption of alcohol, cultural factors, and lack of awareness regarding oral health. Of all cancers in India, the incidence of OSCC is highest in males and third highest in females [3]. Despite advances in surgical and radiation treatments and chemotherapy, the average 5-year overall survival rate of advanced OSCC is not much higher than 50% [6]. Hence detection of OSCC in early stages is very important. Various cytokines, proteins’ and growth factors play a significant role in pathophysiologic regulation of tumor growth and progression.

Chronic periodontitis is considered a multifactorial disease. In the presence of systemic or environmental factors that may modify the host response to plaque accumulation, such as diabetes, smoking, or stress, disease progression may become more aggressive [7]. Chronic Periodontitis is characterized by tissue destruction, bone loss and periodontal attachment loss. Many bacterial components directly and indirectly cause tissue destruction. Along with them mediators such as proteases, cytokines and prostaglandins produced as a part of the host response also contribute to tissue destruction [8].

In the oral cavity albumin is regarded as a serum ultra-filtrate to the mouth, and it may also diffuse into the mucosal secretions. Salivary albumin has been shown to be increased in medically compromised patients whose general condition gets worse. Immunosuppression, radiotherapy, and diabetes are examples of states in which high concentrations of salivary albumin have been detected [9].

Therefore the purpose of this preliminary study is to investigate whether salivary albumin levels can be used as an indicator for early detection of oral squamous cell carcinoma and in early detection of chronic periodontitis.

Materials and Methods

The study protocol was reviewed and approved by the Ethical...
Immediately after the saliva sample was collected, it was centrifuged at 2,600X g for 20 minutes at 4°C. After centrifugation, the supernatant was separated from the pellet. Three proteinase inhibitors were added to each 1 ml of the supernatant: 1 ml aprotinin (10 mg/ml), 3 ml sodium ortho vanadate (400 mM), and 10ml phenyl methyl sulfonyl fluoride (10 mg/ml). The samples were then stored at -80°C until future analysis. All samples were analyzed within 6 months after the samples were collected and processed.

**Determination of salivary albumin levels**

After thawing, Albumin level in the samples was measured by Albumin Agappe kit done by bcg method, and the values were obtained.

**Statistical analysis**

The data was entered in Microsoft XL spread sheet and analysed using statistical package for social sciences, version 20 (SPSS 20). Descriptive statistics were presented in terms of mean, standard deviation, medians and quartiles. The difference in the albumin levels between the three groups were compared using Kruskall Wallis test followed by Mann-Whitney U test. P value of less than 0.05 was considered statistically significant.

**Results**

The mean value of Albumin in group A (healthy) is 0.28 (SD is 0.19), in group B (chronic gen periodontitis) is 0.31 (SD is 0.19), and in group C (OSCC) is 0.82 (SD is 0.41). On comparing the mean values of three groups it is seen that there is significant rise in salivary levels of albumin in OSCC patients, but there is no significant rise in salivary levels of albumin in chronic gen periodontitis patients when compared with group of healthy individuals [Table 1 and Figure 1].

**Discussion**

The present study is done to know whether levels of salivary Albumin can be used as potential biomarkers for early diagnosis of OSCC and CP detection. The results showed that Salivary Albumin levels could be used as a biomarker in oral squamous cell carcinoma cases but may not be used as a biomarker in chronic periodontitis patients.

Vast number of molecular markers has been correlated with OSCC outcome, illustrating the complex events leading to carcinogenesis and cancer progression. Some of the proposed markers are cytokines like inteleukin-6, interleukin-8, tumor necrosis factor etc.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Mean (SD)</th>
<th>Median (Q1-Q3)</th>
<th>Kruskall Wallis test</th>
<th>Group 1 vs. Group 2</th>
<th>Group 1 vs. Group 3</th>
<th>Group 2 vs. Group 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin (g/dl)</td>
<td>0.28 (0.19)</td>
<td>0.27 (0.15-0.33)</td>
<td>0.26 (0.19-0.38)</td>
<td>Group 3 vs. Group 1: &lt;0.001*</td>
<td>407.5</td>
<td>0.53 (NS)</td>
</tr>
</tbody>
</table>
|          | 0.31 (0.19) | 0.76 (0.48-1.24) | 32.84 | 125.0 | <0.001* | 338

Group 1 - Healthy; Group 2 - Chronic periodontitis; Group 3 - Squamous cell carcinoma with chronic periodontitis; *P<0.001 statistically significant; p>0.05 non-significant (NS)
Salivary levels of tumor necrosis factor-alpha indicate the presence of generalized chronic periodontitis and have also demonstrated significant changes after non-surgical periodontal therapy [18].

As the assessment of salivary levels of cytokines need ELISA kit which is quite expensive, not readily available (can get only on special order) and the kit should be opened only when all the sample size is collected, as the kit expires in a month once opened. Thus it cannot be used when immediate results are needed or when the sample size is small. Whereas salivary albumin levels can be assessed by Albumin Agappe kit this is not expensive and is easily available.

Among the various possible causes of cancer, ROS and reactive nitrogen species found in tobacco rank high as causes of the development of cancer. Plasma levels of antioxidants are reliable indicators of the antioxidant status, because they reflect both bio-availability and increased utilization of antioxidants to counter oxidative stress. Antioxidant levels also are influenced by tobacco and alcohol. Serum proteins have long been known to have antioxidant properties owing to their free thiol groups. Among them, albumin is the most potent and abundant extracellular antioxidant [19].

On the other hand, saliva has long been viewed as a unique, but complex, body fluid. Saliva is the first line of defense against oxidative stress and contains various antioxidants including albumin. The use of saliva as a diagnostic tool has many advantages: it is easy to collect using a noninvasive technique that can be performed at home, no special equipment is required for collection, and it has fewer complications than many alternatives.

Salivary biomarkers have many advantages over serum because they are inexpensive, non-invasive, cost effective and easily accessible media and play a vital role in diagnosis, prediction of prognosis and monitoring of patient’s health [20]. This advantage over other detection methods gives salivary biomarker screening the potential to identify patients with premalignant lesions. Collection is inexpensive & can be performed in any settings without the assistance of a health care practitioner. Identifying the proper salivary biomarker profile could serve as a remarkable addition to the current screen method of oral cancer. The ability to identify pre neoplastic states before reaching the stage of invasive disease could have incredible implications for the diagnosis and management of squamous cell carcinoma.

Similarly, salivary biomarker technology has potential to detect recurrence, a common cause of mortality in OSCC patients [21]. Monitoring a patient’s salivary levels post-treatment could be a successful tool for early identification of recurrence.

**Conclusion**

In this study the salivary albumin levels were raised to a significant level in OSCC subjects, but rise of salivary Albumin levels in chronic periodontitis subjects was not statistically significant.

Based on the results it can be concluded that albumin levels in saliva can be used as biomarker for OSCC but may not use for...
early detection of chronic periodontitis. More studies will be required to corroborate these findings and verify the possible association between Salivary Albumin and OSCC.

**Conflict of Interest**

All authors disclose that there was no conflict of interest.

**References**