Evaluation of salivary total proteins, albumin, globulin and A/G ratio among healthy individuals and patients with oral cancer: A pilot study

M. Pooja Umaiyal¹, R. Gayathri²*, V. Vishnu Priya²

INTRODUCTION

A large number of medical and dental practitioners are finding that saliva is a non-invasive diagnostic medium and easily available medium for a rapidly widening range of diseases and clinical situations. The principal defensive factor in the mouth is saliva, and the orodental health is influenced by its rate of flow, that is, a reduction in its flow rate affects orodental health. A variety of symptoms are produced by the reduction in the salivary flow and so the establishment of patients’ saliva flow is of primary importance in oral medicine and dentistry.

Cancer is by no means a modern disease. It was known to the ancient Egyptians as early as 1500 B.C. 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. Over 90% of oral cancers are squamous cell carcinomas.

Systemic diseases such as cancer, cardiovascular, metabolic and neurological diseases may be diagnosed

ABSTRACT

Background: Oral cancer is a type of head-and-neck cancer. It may originate in any of the tissues of the mouth and may be of varied histology types: Teratoma, adenocarcinoma derived from a major or minor salivary gland, lymphoma from tonsillar or other lymphoid tissue, or melanoma from the pigment-producing cells of the oral mucosa. Hence, estimation of the salivary proteins can help in indication of health status of the patient. Objective: The objective of this study was to estimate the level of salivary total proteins (TPs), albumin (alb), globulin, and A/G ratio among healthy individuals and patients with oral cancer. Materials and Methods: This study had been carried out on patients attending the Hospital of Saveetha Dental College in Chennai city, Chennai. Patients were evaluated by full medical history to exclude any existing of systemic disease or taking any drug that may affect the parameters to be examined. Three individuals were included in the present study; they were clinically and histologically diagnosed as patients with oral epithelial tumors (benign and malignant). This was conducted as a pilot study to determine the sample size and feasibility of the research. Samples of saliva were collected from two groups of subjects as follows: Group A condition of healthy people with no smoking and alcohol habits and people who have no pre-cancerous lesions and no history of systemic diseases. Group B consists of subjects who have been diagnosed for carcinoma of buccal mucosa and currently undergoing treatment. All patients were verbally explained the nature of the study and an informed written consent was obtained before the study. The study protocol was approved by the Ethical Committee of the Institution. Result: Analyses of the acquired detail were done and the mean values of the three samples from both control group [Table 1] and oral cancer patient were obtained. The mean value of albumin in oral cancer patient was found higher when compared to the concentration of albumin of the control group. The mean value of total protein in oral cancer patient was higher than the mean value of total protein among the control group. A/G ratio’s mean value was higher among oral cancer patient. The difference among the globulin levels in oral cancer patient and control group was not clearly distinguishing. Conclusion: The TP concentration and A/G ratio were found to be higher in cancer patients compared to the normal individuals.

KEY WORDS: A/G ratio, Albumin, Globulin, Oral cancer, Salivary total protein

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using other biological fluids than blood.\[9\] The most commonly used fluid for the analyses of the cellular and chemical constituents in a laboratory is blood. For the past few years, saliva has been used as a new diagnostic fluid. Saliva is a complex fluid produced by a number of specialized glands and secreted into the oral cavity of mammalian vertebrates. A small contribution of saliva production is made by the numerous small labial, buccal, and palatal glands which line the mouth, but majority of its production is produced by the major salivary glands (parotid, submandibular, and sublingual). The contents of salivary fluid contain water, normal proteins, post-translationally modified proteins (glycoproteins), peptides, lipids, minerals, antioxidants, and other small molecules. Certain locally produced substances and other molecules derived from the systemic circulation, such as serum products, gingival crevicular fluid, electrolytes, microorganisms, and other foreign substances, are also present in saliva. Even markers for hormonal, infectious, immunological, and toxicological diseases can be determined in saliva.\[5\] An alternative tool for monitoring the oral and systemic health is the oral cavity. Therefore, saliva is often called the mirror of the body.

Several lines of evidence support the concept that oral squamous cell carcinoma arises from noninvasive lesions which encompass a histological continuum between the normal mucosa at one end and high-grade dysplasia/carcinoma in situ, at the other, establishing a model of neoplastic progression and are termed as premalignant or precancerous lesions or conditions. Oral submucous fibrosis (OSMF) is one of the examples of such lesions which cannot be cured permanently.

A detailed study of the role of the constituents of areca nut in the pathogenesis of OSMF has been rendered over few decades, and it has been proved that it leads to excessive deposition of collagen which is a protein and ultimately leads to hyalinization of subepithelial tissues causing restricted mouth opening.\[6,7\] In addition, protein levels in the serum of OSMF patients are also found to be altered compared to normal individuals, which could be an indicator of excessive collagen deposition in OSMF.

A tumor marker can be defined as substances present in, or produced by, a tumor itself or produced by host in response to a tumor that can be used to differentiate a tumor from normal tissue or to determine the presence of a tumor based on measurements in blood or secretions.

The idea of screening and following patients with malignancy by blood-based tests is appealing from several points of view including its ease, economic advantage, minimal invasiveness, and examination of a large sample size in short period of time and possibility of repeated sampling.\[8\]

Proteins are present in all body fluids and are the major components of all cell. Over 100 individual proteins have a physiological function in serum. The plasma proteins are actually a complex mixture that includes not only simple proteins but also conjugated proteins such as glycoproteins and various types of lipoproteins. Quantitatively, the single most important protein in the human body is albumin (alb) (3.5–5 g/dL), the other proteins are known collectively as globulins (2.5–3.5 g/dL).\[9] Hence, any changes in the concentration of these individual proteins will lead to a change in total serum protein. Salivary proteins may be originated as an ultra-filtrate of plasma proteins or through salivary and mucosal secretions, so any violation to the integrity of capillary bed or mucosal lining may be associated with an increase in salivary proteins, especially alb, which occur in oral neoplasia.

Serum proteins have long been implicated to have antioxidant properties owing to their rich concentration of free thiol groups. Among them, alb is seen as the most potent and abundant extracellular antioxidant.\[10\] The role of serum alb as plasma’s antioxidant defenses, which can be used as a reliable marker of oxidative stress in the body and could be taken up as an important diagnostic adjunct in assessing the diagnosis, response to treatment, periodic assessment of patient with the progress of treatment, chances of metastasis, and survival rates.\[11\]

The two major constituents of the human serum total proteins (TPs) are globulins (2.3–3.5 g/dL) and alb (3.2–4.5 g/dL). A major role in immunity and inflammations is played by the globulins. Although the alb and the total serum protein are directly measured, the alb to globulin ratio (A/G ratio) is calculated as A/G ratio = alb/(TP - alb).\[12\]

Thus, the present study was undertaken with an aim to estimate the level of salivary TPs, alb, globulin, and A/G ratio among healthy individuals and patients with oral cancer.

**MATERIALS AND METHODS**

This study had been carried out on patients attending the Hospital of Saveetha Dental College in Chennai city. Patients were evaluated by full medical history to exclude any existing of systemic disease or taking any drug that may affect the parameters to be examined. Three individuals were included in the present study; they were clinically and histologically diagnosed as patients with oral epithelial tumors (benign and malignant).
This was conducted as a pilot study to determine the sample size and feasibility of the research.

Samples of saliva were collected from two groups of subjects as follows:
• Group A condition of healthy people with no smoking and alcohol habits and people who have no precancerous lesions and no history of systemic diseases.
• Group B consists of subjects who have been diagnosed for carcinoma of buccal mucosa and currently undergoing treatment.

All patients were verbally explained the nature of the study and an informed written consent was obtained before the study. The study protocol was approved by the Ethical Committee of the Institution.

Sample Collection
About 10 ml of unstimulated (resting) whole saliva were collected, under resting conditions. Patients and healthy individuals were asked to rinse their mouth with water and to generate saliva in their mouth and to spit into a wide test tube.

This collected sample was then taken immediately to the laboratory for evaluation of salivary TPs, alb, globulin, and A/G ratio.

TP Determination
TP concentration of all samples (serum and saliva) was determined using the direct biuret method with a colorimetric determination of TP based on the principle of the biuret reaction (copper salt in an alkaline medium). Protein in plasma or serum sample forms a blue colored complex when treated with cupric ions in alkaline solution. The intensity of the blue color is proportional to the protein concentration. Protein concentrations of serum and saliva were expressed in g/dl.

Alb Determination
Serum and saliva alb were determined by dye-binding method using kit manufactured by agappe. The measurement of alb is based on its quantitative binding at pH 4.2 with bromocresol green to form a blue-green complex.

Globulins Determination
The concentration of the globulins in the saliva samples of healthy and oral tumor patient groups was calculated, using the following equation: Concentration of globulin (g/dl.) = concentration of TP-concentration of alb.

RESULTS
Analyses of the acquired detail were done and the mean values of the three samples from both control group [Table 1] and oral cancer patient [Table 2] were obtained. The mean value of alb in oral cancer patient (2.03 g/dL) was higher when compared to the alb of the control group (0.86 g/dL) as seen in Figure 1. The mean value of TP oral cancer patient (3.47 g/dL) was higher than the mean value of TP among the control group (2.19 g/dL) as depicted in Figure 2. A/G ratio’s mean value was higher among oral cancer patient (2.49 g/dL) as represented in Figure 3. The difference among the globulin levels in oral cancer patient and control group was not clearly distinguishing its values [Tables 1 and 2].

DISCUSSION
Comprehensive analysis and identification of the proteins in the collected saliva samples is a necessary first step toward the discovery of protein change for human disease detection.\textsuperscript{13}

In the current study, the TP in sera samples of patient groups shown in Table 1 was found to be markedly increased, when compared with that of the control group. This result is in accordant with several studies referred to the presence of significant increase in TP concentration in patients with different types of cancer.\textsuperscript{14,15} Such increase in TP concentration can be explained as follows: the whole body of cancer patient is engaged in synthesis of variable forms of proteins,

<table>
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<tr>
<th>Sample</th>
<th>Alb (g/dL)</th>
<th>Total protein (g/dL)</th>
<th>Globulin (g/dL)</th>
<th>A/G ratio (g/dL)</th>
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<td>3</td>
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Alb: Albumin

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Alb: Albumin
such as globulins, immunoglobulin, enzymes,[16] and other proteinous material. This increase may be explained on the basis that saliva in general contains arrays of proteins that have distinct biological function, most of them have antioxidant, antibacterial, antimicrobial and antibodies properties[17] defend the oral environment against any noxious agents, and such proteins were reported to increase in case of inflammation and tumors. Furthermore, it has been reported that many serum-derived proteins transferred to the saliva during inflammation.[18]

Salivary alb concentration showed a significant increase [Table 2] in patient groups in comparison to that of the control group. This increase is in agreement with other studies on oral squamous cell carcinoma patients where an increase in salivary alb concentration was also recorded.[19] In the oral cavity, alb in saliva was reported to be blood-derived protein, and it may diffuse into the mucosal secretions, so the marked increase level of alb 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 alb to extracellular spaces (interstitial) and through cervicular fluid to saliva causing increase in salivary alb.[20,21] It has been shown that salivary alb has increased in medically compromised patients whose general condition gets worse. It may be hypothesized that salivary alb can be used to assess the integrity of mucosal function in the mouth. Since an increase in alb concentration in whole saliva has always been detected before the clinical appearance of stomatitis, it can be said that alb in the whole saliva might be a marker and predictor of stomatitis.

REFERENCES


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