Serum albumin in poorly differentiated, oral squamous cell carcinoma: a possible diagnostic marker of significance

Albumina sérica em carcinoma de células orais escamosas e pobremente diferenciadas: um possível marcador de diagnóstico de significância

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Abstract

Objectives: Serum albumin is considered to be the most potent and abundant extra-cellular anti-oxidant that might have a protective role in the ongoing process of transition of the various oral pre-cancerous lesions and conditions into frank malignant degenerations. The aim of this study was to check the reliability of serum albumin as one of the diagnostic anti-oxidant parameter. **Materials and methods**: The study consisted of sera analysis of albumin in the age and sex matched normal healthy adults and patients with histologically proven, poorly differentiated oral squamous cell carcinoma. The results were analyzed using Student's t-test and were averaged as mean ± standard deviation. In above test, p-values less than 0.05 were taken to be statistically significant. The normality of data was checked before the statistical analysis was performed. **Results**: The study revealed variations in sera levels of albumin to be statistically significant with the mean level of sera albumin to be 4.956 ± 1.0579 in controls as against 3.6933 ± 1.2177 in patients with histologically proven, poorly differentiated, oral squamous cell carcinoma. **Conclusions**: The results of the study emphasize the need for more studies with larger sample sizes to be conducted before a conclusive role could be drawn in favor of sera levels of albumin as diagnostic markers of significance in oral squamous cell carcinoma.

Keywords: Oral squamous cell carcinoma. Reactive oxygen species. Free radicals. Transformation. Pre-cancerous. Serum albumin.

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Resumo

Objetivos: A albumina sérica é considerada o antioxidante extracelular mais poderoso e mais abundante, que pode exercer um papel protetor no processo de transição das várias lesões e circunstâncias pré-cancerígenas orais em degenerações malignas. O objetivo deste estudo foi verificar a confiabilidade da albumina sérica como um dos parâmetros de diagnóstico antioxidante. **Materiais e métodos**: A análise consistiu no estudo da albumina sérica em pacientes adultos saudáveis normais, separados por idade e sexo, e em pacientes com carcinoma oral de células escamosas pobremente diferenciadas com evidência histológica comprovada. Os resultados foram analisados usando o teste t de Student, e as médias foram calculadas com ± desvio-padrão. No teste citado, os valores de p menores que 0,05 foram considerados estatisticamente significantes. A normalidade dos dados foi verificada antes da realização da análise estatística. **Resultados**: O estudo revelou diferenças estatisticamente significantes nos níveis de albumina sérica com o nível médio de 4.956 ± 1.0579 nos controles em contraste com 3.6933 ± 1.2177 nos pacientes com carcinoma oral de células escamosas pobremente diferenciadas. **Conclusões**: Os resultados do estudo enfatizaram a necessidade de mais estudos com tamanhos de amostra maiores antes que um papel conclusivo possa ser atribuído, em favor dos níveis de albumina sérica, como marcador diagnóstico para o carcinoma oral de células escamosas.

Palavras-chave: Carcinoma oral de células escamosas. Espécies de oxigênio reativo. Radicais livres. Transformação pré-cancerígena. Albumina sérica.

Introduction

Oral squamous cell carcinoma has a much higher prevalence in the elderly age groups as compared with the younger population. This higher prevalence among the elderly population might result from an age related increase in the magnitude of the attack of free radicals including the so-called reactive oxygen and nitrogen species-ROS and RNS-causing various DNA mutations and aberrations. It might also result from an age related reduction in the body's antioxidant defenses and/or, both (1, 2).

In fact, it has been found that whereas reactive oxygen and nitrogen species are involved in the initiation and promotion of the multi-step carcinogenesis, both are inhibited by the antioxidant defenses present in the saliva and the serum of the patient (1). This is only when this equilibrium is disturbed that damage to the DNA is brought about and cancer evolves (3, 4).

Serum proteins have long been implicated to have anti-oxidant properties owing to their rich concentration of free thiol groups. Amongst them, albumin is seen as the most potent and abundant extra-cellular anti-oxidant (5-11).

Moreover, the histopathological grading of oral squamous cell carcinoma, the gold standard, so far, in the diagnosis of oral squamous cell carcinoma, is a subjective method, depending on the area of the tumour sampled and the individual pathologist's criteria for evaluation. Also, clinical staging seems to correlate much better with the prognosis than the microscopic grading of the tumour (12).

The role of serum albumin as plasma's potent antioxidant defense, if comes out to convincing enough to be used as a reliable marker of oxidative stress in the body, could be helpful as an important diagnostic adjunct in the identification of oral squamous cell carcinoma as against the other subjective, invasive and not so cost-effective procedures of diagnosis.

Hence, the present study is being planned to assess the role of serum albumin as a potential diagnostic marker of significance in oral squamous cell carcinoma by comparing the sera levels of albumin in the normal healthy adults as against the patients with clinically diagnosed and histologically proven, poorly differentiated, oral squamous cell carcinoma.

Materials and methods

The study was conducted in the Dept. of Oral Medicine and Radiology, Govt. Dental College and Research Institute, Bangalore over a period of three months from January 2010 to March 2010. The study consisted of 30 new cases of clinically diagnosed carcinoma buccal mucosa that were histologically proven cases of poorly differentiated, oral squamous cell carcinoma,

and 30 age and sex matched normal healthy adults taken from the same institute. Before the start of the study, based on the selection criteria, patients enrolled in the study were explained the protocol of the study and their written informed consents were obtained. The ethical clearance was obtained by the ethical committee of the institution with the protocol approval number GDCRI/ACM/PG/Ph.D./1/2009-2010.

Oral squamous cell carcinoma arises from dysplastic surface epithelium and is characterised histopathologically by invasive islands and cords of malignant squamous epithelial cells. When the tumour is sampled fortuitously at the earliest moment of invasion, the adjectives superficially invasive or microinvasive often are used with the invasion being represented by irregular extension of lesional epithelium through the basement membrane and into subepithelial connective tissue (12).

Invading cells and cell masses may extend deeply into underlying adipose tissue, muscle or bone, destroying the original tissue as they progress. Lesional cells may also be seen surrounding and destroying blood vessels and may invade into the lumina of veins or lymphatics. There is often a strong inflammatory or immune cell response to invading epithelium and focal areas of necrosis may be present. The lesional epithelium is also capable of inducing the formation of new small blood vessels (angiogenesis) and occasionally, dense fibrosis (desmoplasia or scirrhous change) (12).

Histopathologic evaluation of the degree to which these tumours resemble their parent tissue (squamous epithelium) and produce their normal product (keratin) is called grading. Lesions are generally graded on a 3-point (grades I to III) or a 4-point (grades I to IV) scale. The less differentiated tumours receive the higher numerals (12).

The histopathologic grade of a tumour is related somewhat to its biologic behaviour. In other words, the one that is mature enough to closely resemble its tissue of origin seems to grow at a slightly slower pace and to metastasise later in its course. Such a tumour is called low grade, or grade I or well differentiated squamous cell carcinoma. In contrast, a tumor with much cellular or nuclear polymorphism and with little or no keratin production may be so immature that it becomes difficult to identify the tissue of origin. Such a tumour often enlarges rapidly, metastasises early in its course and is termed high grade, grades III/IV, poorly differentiated or

anaplastic. A tumour with a microscopic appearance somewhere between these two extremes is labelled a moderately differentiated carcinoma (12).

The histopathological slides stained with haematoxylin and eosin were reviewed according to the above criteria to include the cases of clinically diagnosed carcinoma buccal mucosa that were histologically proven as poorly differentiated, oral squamous cell carcinoma as per the biopsy results from the Dept. of Oral Pathology, Government Dental College and Research Institute, Bangalore.

The histopathological grading of the tumor was further re-confirmed by the Dept. of General Pathology, Bangalore Medical College and Research Institute and Associated Hospitals, Bangalore.

The controls as well as the patients who were chronic alcoholics were excluded from the study to rule out the probability of derangement of liver functions that could have contributed towards the variations in sera levels of albumin.

Also, none of the patients were on any therapeutic modality prior to the inclusion in the study or, suffering from any systemic condition, especially hepatic or, renal disorders that could have affected the sera albumin levels.

Biochemical analysis of sera levels of albumin was done in the Dept. of Clinical Biochemistry, Bangalore Medical College and Research Institute and Associated Hospitals, Bangalore.

Collection of blood and serum separation: For this, following an overnight fasting period, 5 mL of venous blood was taken from selected patients from the antecubital vein using a sterile disposable syringe in the sitting position between 8 a.m. and 10 a.m. The samples were allowed to clot and serum was immediately separated by ultracentrifugation taking full precautions to prevent hemolysis as an inappropriate storage or, prolonged storage as well as an early centrifugation before the settling of the formed elements of the blood and the separation of the serum after the clot formation, would have led to hemolysis on centrifugation. The supernatant was discarded and the rest of the sample was stored at $-20\,°C$.

Assay of albumin in sera

Assay of sera levels of albumin was done with the help of Biuret method (2, 3). Serum albumin was expressed as g/dL.

Method of statistical analysis

The results were analyzed using Student's t-test and were averaged as mean ± standard deviation. In above test, p values less than 0.05 were taken to be statistically significant. The normality of data was checked using Kolmogorov-Smirnov and Shapiro-Wilk tests for significance before the statistical analysis was performed.

Results

Sera levels of albumin came out to be statistically significant (p < 0.001) with levels falling from a minimum of 3.00 g/dL in controls to as low as 1.7 g/dL in poorly differentiated, oral squamous cell carcinoma (Table 1). The mean level of sera albumin was found to be 4.956 ± 1.0579 in controls as against 3.6933 ± 1.2177 in patients with histologically proven, poorly differentiated, oral squamous cell carcinoma (Table 1). The standard error between the study groups was rated to be 0.3.

Discussion

The role of oxygen free radicals in the initiation, promotion and progression of carcinogenesis and the protective role of anti-oxidant defenses has been the subject of much speculation in the recent past with conflicting reports in the literature (4). In recent years, increasing experimental and clinical data have provided compelling evidence for the

involvement of oxidative stress in a large number of pathological states, including cancers (13).

Plasma is known to contain a wide range of important antioxidants including albumin, ascorbic and uric acids. In plasma, free thiol groups are quantitatively the most important scavengers of the various free radicals and are known to be located largely on the various serum proteins, one amongst them being albumin. While ascorbate is an important extra-cellular antioxidant, albumin via its thiol groups, provides quantitatively almost ten folds greater antioxidant protection against the various reactive oxygen and nitrogen species held responsible for the genetic and molecular damage eventually transforming into frank malignant degenerations (1, 3-7, 14).

In humans, albumin is the most abundant plasma protein accounting for about 55%-60% of the measured serum proteins. It consists of a single polypeptide chain of 585 amino acids with a molecular weight of around 66,500 Da (13). Albumin synthesis takes place only in the liver and secreted into the portal circulation. In healthy young adults, the rate of synthesis is about 12-25 g of albumin/day, varying with various nutritional and disease states (8, 13, 15, 16). Amongst the numerous plasma proteins that possess anti-oxidant properties owing to their rich concentrations of free thiol groups, albumin is unusual in having a free sulfhydryl group in addition. The usual half-life of albumin is 20 days (9, 10, 13, 17).

The sera levels of albumin are largely a function of its rate of synthesis and degradation and its distribution between the intra-(42%) and extra-vascular compartments. With normal concentrations lying between 3.5-5.5 g/dL, only a small number of factors are known to result in variation in serum albumin. Besides analbuminaemia, a rare congenital disease, the main pathological situation known to lower albumin concentration, is the nephrotic syndrome, which is the subject of most studies (10, 11, 13, 17).

Several lines of evidence suggest strongly that a reduced serum albumin concentration, although within the normal range, is associated with increased mortality risk (13). From studies performed with healthy subjects and patients, it has been reported that the estimated increase in the odds of death ranges from 24% to 56% for each 2.5 g/L decrement in serum albumin concentration. The serum albumin level thus appears to be an independent

Table 1 - Serum albumin in study groups along with the mean, standard deviation, minimum and maximum values and p-value; wherein

Control [n = 30]		Poorly differentiated, oral squamous cell carcinoma [n = 30]		p-value
Mean [Min. 3 g/dL], [Max. 7.8 g/dL]	SD	Mean [Min. 1.7 g/dL], [Max. 7.2 g/dL]	SD	
4.956	1.0579	3.6933	1.2177	< 0.001

Note: * = p < 0.05.
Source: Research data.

predictor of mortality risk with a direct protective effect of the albumin molecule being suggested by the persistence of the association after adjustment for other risk factors. Albumin may thus represent quantitatively the most important component that plays a role in the efficient antioxidant defense; organisms have developed to protect against the various oxidant mediated disease processes including cancers (7, 10, 13).

Albumin in our study came out to be statistically significant with values as low as 1.7 g/dL in patients afflicted with clinically diagnosed and histologically proven poorly differentiated, oral squamous cell carcinoma to as high as 7.8 g/dL in the healthy controls (Table 1). The possible mechanism behind decreased sera levels of albumin could be related to the consumption of a major fraction of serum albumin in the quenching of free radicals that are supposed to be increased in this set of patients. This is in concordance with the observations of the several studies conducted in the past that laid emphasis on the protective role of albumin as one of the most abundant extracellular antioxidant available in the plasma (1, 3-7, 14).

There is however a lack of consistent studies in relation to the exact role serum albumin plays in patients of oral squamous cell carcinoma or, for that matter, patients suffering from the various oral precancerous lesions and conditions gradually progressing towards frank malignant degenerations. The exact role of albumin as a diagnostic marker of significance or, in the pathogenesis or, in assessing the prognosis is, therefore, warranted by larger, follow-up studies correlating the level of serum albumin in these groups of patients with the overall 5-year survival rates.

Since oral cancers are in an anatomic site that is easy to examine, the prognosis for treated lesions is supposed to be high. Although this is not usually the case as unfortunately, many health care providers are not aggressive in their clinical examination of the early, incipient lesions. That, coupled with the fact that oral cancers are usually asymptomatic, often delays the diagnosis, which adversely affects the prognosis (18).

Moreover, a poorly differentiated or anaplastic oral squamous cell carcinoma with much cellular or nuclear polymorphism and with little or no keratin production may be so immature that it becomes difficult to identify the tissue of origin. Such a tumour often enlarges rapidly and metastasises early in its course (12).

Over the past several decades, investigators have proposed various multiparameter histopathologic assessment systems in an attempt to provide more objective criteria that correlate with the diagnosis as well as in predicting the prognosis of the tumours (12). Serum albumin, in this aspect, if could be proven as a diagnostic marker of significance, could be taken up as an important diagnostic adjunct to the histopathological gradation of the lesions as an aid in assessing the diagnosis, response to treatment, their periodic assessment with the progress of treatment as well as the chances of metastasis and survival rates, the factors more common in consideration in cases of poorly differentiated, oral squamous cell carcinoma because of protracted durations of the treatment.

The intention behind selective inclusion of histologically proven cases of poorly differentiated, oral squamous cell carcinoma was to select cases wherein the sera levels of albumin, if, would have come to be as diagnostic markers of significance, would have provided an easy method of estimation of the response of the cases to the treatment as well as an adjunct in predicting the course, the chances to metastasize and survival rate of the patients as few studies conducted in the past have related low sera albumin levels with increased mortality of the patients in relation to general body cancers.

The purpose behind selective inclusion of poorly differentiated, oral squamous cell carcinoma was also to check the reliability of sera levels of albumin if they could be related to the chances of an early metastasis during the course of evolution of the malignancy.

This is a preliminary study; its results suggest the potential significance sera levels of albumin carry as important biochemical markers that can aid in the diagnosis of oral squamous cell carcinoma. Hence, this study gives a scope for further studies to be conducted to establish the role of this biochemical marker as a convincing enough evidence of the changes taking place in the body eventually turning out into frank oral cancers.

Conclusions

The study revealed variations in sera levels of albumin to be statistically significant emphasizing the need for more studies with larger sample sizes to be

conducted before a conclusive role could be drawn in favor of serum albumin as a potential diagnostic marker in the diagnosis of frank oral squamous cell carcinoma.

Acknowledgement

Ethical declaration

The study has been approved by the ethical committee appointed by the Government Dental College and Research Institute, Bangalore and Bangalore Medical College and Research Institute and Associated Hospitals, Bangalore and has therefore been performed in accordance with the ethical standards laid down in the 1975 declaration of Helsinki and its later amendments in 2000 after a written informed consent from the patients for their inclusion in the study. Details that might disclose the identity of the patient have been omitted.

Competing interests and other declarations: None.

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