Serum Biomarkers: Evaluation of Serum Albumin & Serum Albumin: Globulin Ratio in Oral Leukoplakia and Oral Squamous Cell Carcinoma

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Abstract

Introduction

Squamous cell carcinoma is the most common cancer in the oral cavity. There are 222,000 new cases (5% of all cancers) of oral cancer diagnosed in men each year globally and 90,000 new cases (2% of all cancers) diagnosed in women. The 5-year survival rate is estimated to be about 50% [1].

It is the most common cancer in the males and the third most common cancer in the females in India. In India, about 60,000 new cases of oral cancer are reported to occur every year. Tobacco consumption is considered as the single most important risk factor for the development of oral cancer [2].

Leukoplakia is the most common potentially malignant disorder of the oral mucosa. The frequency of dysplastic or malignant change varies from 15.6-39.2% in different studies. The risk of developing malignancy at the lesional site is 5 times greater in patients with leukoplakia than in patients without them. Hence, it is necessary for early diagnosis of both leukoplakia and Oral squamous cell carcinoma [3].

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 [4].

Extensive biochemical studies have been carried out on tumour tissue and peripheral blood to explore the etiology of cancers and to establish tumour markers as an adjunct for establishing the diagnosis and prognosis of disease. Biochemical changes in the tissue provide a better understanding of the chemical processes responsible for malignancy. Although pre-treatment A/G ratio has been established as an independent, significant predictor of long-term mortality in various extra-oral cancers e.g. breast cancer patients, such studies are lacking in context of oral carcinoma.

Aims and Objectives: The objective of the following study is to estimate & compare Serum Albumin & Serum A/G ratio in normal healthy adults, oral leukoplakia and oral cancer and thus to evaluate their possible role as a biochemical parameter in the diagnosis of oral cancer.

Materials and Methods: This study included 100 subjects categorized as healthy (Group I = 20), oral leukoplakia (Group II = 40) & Oral squamous cell carcinoma (Group III = 40).

Statistical Analysis used: The normality of data was checked using Shapiro-Wilk test. For statistical analysis, Kruskal Wallis test & Mann-Whitney U test were applied.

Results: Serum albumin levels showed gradual decrease from control to leukoplakia patients and then further in oral cancer patients. Also, Serum A/G ratio was significantly lower in oral cancer patients as compared to leukoplakia patients.

Conclusions: The present study demonstrated the significant decrease in serum Albumin levels and serum A/G ratio in oral carcinoma; these results support the possibility of their use in the diagnosis of oral cancer as adjunctive serum tumor markers.

Keywords: Serum albumin, Serum albumin: Globulin ratio, Oral leukoplakia, Oral squamous cell carcinoma, Tumor markers

Background: Extensive biochemical studies have been carried out on tumour tissue and peripheral blood to explore the etiology of cancers and to establish tumour markers as an adjunct for establishing the diagnosis and prognosis of disease. Biochemical changes in the tissue provide a better understanding of the chemical processes responsible for malignancy. Although pre-treatment A/G ratio has been established as an independent, significant predictor of long-term mortality in various extra-oral cancers e.g. breast cancer patients, such studies are lacking in context of oral carcinoma.

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peripheral blood to explore the etiology of cancers and to establish tumour markers as an adjunct for establishing the diagnosis and prognosis of disease.

Biochemical changes in the tissue provides a better understanding of the chemical processes responsible for malignancy [5].

Early diagnosis of oral cancer as well as detection of dysplastic changes in oral precancer can significantly decrease the mortality rate.

The present clinical approach for cancer diagnosis and management involves relatively invasive, expensive and complex procedures. Therefore, a simple and minimally-invasive tumor marker is required for early diagnosis as well as for monitoring progress during the treatment [6,7].

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, examination of a large sample size in short periods of time and possibility of repeated sampling [8].

Oral squamous cell carcinoma has a much higher prevalence among the elderly population. This might result from a 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 abbreviations. It might also result from an age related reduction in the body’s antioxidant defenses and / or both [9,10].

In fact, it has been found that although reactive oxygen & nitrogen species are involved in the initiation and promotion of multistage carcinogenesis, both are inhibited by the antioxidant defenses present in the saliva & serum of the patients [9]. This is only when this equilibrium is disturbed, the damage to the DNA is brought about and cancer evolves [11].

Serum proteins have long been implicated to have antioxidant properties owing to their rich concentration of free thiol groups. Amongst them, Albumin is seen as the most potent and abundant extracellular anti-oxidant [12].

The role of serum albumin as plasma’s antioxidant defenses, if comes out be convincing enough to be used as a reliable marker of oxidative stress in the body, 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 [12].

Globulins (2.3 to 3.5 g/dL) along with albumin (3.2 to 4.5 g/dL) are the two major constituents of the human serum total proteins. The globulins play a major role in immunity and inflammation. Although the albumin and the total serum protein are directly measured, the albumin to globulin ratio (A/G ratio) is calculated as A/G ratio = albumin / (total protein – albumin) [13].

Although in spite of our efforts, we could not found any available literature regarding the estimation of A/G ratio in oral cancer, pretreatment A/G ratio has been established as an independent, significant predictor of long-term mortality in various extra-oral cancers e.g. breast cancer patients [14].

Hence, the present study was planned to assess the role of Serum Albumin and Serum A/G ratio as potential tumor markers of significance in diagnosis of leukoplakia and oral squamous cell carcinoma.

**Material and Methods**

Total of 100 patients were included in this study. 20 belonged to control (Group I), 40 belonged to oral leukoplakia (Group II) and 40 belonged to oral squamous cell carcinoma (Group III). After recording thorough case histories and informed consent, they were subjected to blood examination & incisional biopsies.

Histopathological confirmation of the clinical diagnosis was made. Leukoplakia cases were subdivided clinically according to LCP staging by Van der Waal, et al. (2000) [15] and histopathologically according to WHO criteria (2005) [16]. Oral squamous cell carcinomas were clinically grouped according to TNM staging, (2009) [16,17] and histopathologically scored on the basis of Bryne’s criteria, (1992) [18-20].

**Sample collection & biochemical analysis**

5 ml of blood sample of all the participants was taken and serum was separated by centrifugation. Then serum albumin & serum total protein levels were estimated using respective kits on a semi-automatic biochemical analyser (ROBONIK Prietest™) according to the manufacturer instructions. Although the albumin and the total serum protein levels were directly measured, the Albumin to Globulin ratio (A/G ratio) was calculated as A/G ratio = Albumin / (Total protein - Albumin).

**Statistical Analysis**

Data collected in this study was analysed statistically using IBM SPSS Statistics (version 20) software package. Data was expressed as means with Standard deviation (SD). The normality of data was checked using Shapiro-Wilk test (Table 1) for significance before the statistical analysis was performed. Since the data was statistically not normally distributed, therefore log method or alternative tests such as for ANOVA, Kruskal Wallis test & for Independent t test, Mann-Whitney U test was applied. Kruskal Wallis test was applied.

Kruskal Wallis test (Table 2) was applied to compare mean ranks of serum Albumin, serum Total Protein & serum A/G ratio in control, leukoplakia and OSCC groups. Mann-Whitney U test (Tables 3-5) was done to compare mean ranks of serum Albumin, serum Total Protein & serum A/G ratio between any two groups, i.e. between group I & II, group I & III and group II & III. The results were considered statistically significant whenever \( p < 0.05 \).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group</th>
<th>Statistics</th>
<th>df</th>
<th>( p )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum Albumin</td>
<td>Control</td>
<td>0.407</td>
<td>20</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>Leukoplakia</td>
<td>0.921</td>
<td>40</td>
<td>0.008</td>
</tr>
<tr>
<td></td>
<td>OSCC</td>
<td>0.941</td>
<td>40</td>
<td>0.038</td>
</tr>
<tr>
<td>Serum Total Protein</td>
<td>Control</td>
<td>0.785</td>
<td>20</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td>Leukoplakia</td>
<td>0.980</td>
<td>40</td>
<td>0.689</td>
</tr>
<tr>
<td></td>
<td>OSCC</td>
<td>0.976</td>
<td>40</td>
<td>0.544</td>
</tr>
<tr>
<td>Serum A/G ratio</td>
<td>Control</td>
<td>0.873</td>
<td>20</td>
<td>0.013</td>
</tr>
<tr>
<td></td>
<td>Leukoplakia</td>
<td>0.797</td>
<td>40</td>
<td>0.000</td>
</tr>
<tr>
<td></td>
<td>OSCC</td>
<td>0.821</td>
<td>40</td>
<td>0.000</td>
</tr>
</tbody>
</table>

Statistically significant \( p < 0.05 \)

**Table 1:** Table depicting tests of normality of data
Results

In group I (control), mean serum Albumin levels were 4.66 ± 0.97 g/dl, mean serum Total Protein were 8.12 ± 1.06 g/dl and mean serum A/G ratio were 1.17 ± 0.20.

In group II (OL), mean serum albumin levels were 4.12 ± 0.29 g/dl, mean serum total protein were 7.47 ± 0.80 g/dl and mean serum A/G ratio were 1.33 ± 0.49.

In group III (OSCC), mean serum albumin levels were 3.58 ± 0.55 g/dl, mean serum total protein were 7.08 ± 0.48 g/dl and mean serum A/G ratio were 1.10 ± 0.43.

The values for serum Albumin & Serum Total Protein were found to be statistically significant between group I (control) & group II (leukoplakia), between group I (control) & group III (OSCC). The values were also found to be statistically significant between group II (leukoplakia) & group III (OSCC) (Tables 6 & 7).

Discussion

Cancer, a disease characterized by uncontrolled growth and spread of abnormal cells is one of the major causes of death in humans. Recent studies indicate that increased production of reactive oxygen species (ROS) can promote development of malignancy. Over production of ROS within the tissue can damage DNA, proteins, lipids and carbohydrates [21,22].
Serum Albumin (g/dl)

<table>
<thead>
<tr>
<th></th>
<th>Control (group I) (n=20)</th>
<th>Leukoplakia (group II) (n=40)</th>
<th>Oral Squamous cell carcinoma (group III) (n=40)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>4.65855</td>
<td>4.12035</td>
<td>3.58667</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>SD</td>
<td>0.97793</td>
<td>0.29501</td>
<td>0.55218</td>
<td></td>
</tr>
<tr>
<td>p1</td>
<td>&lt;0.001</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>p2</td>
<td>-</td>
<td>&lt;0.001</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>p3</td>
<td>-</td>
<td>-</td>
<td>&lt;0.001</td>
<td></td>
</tr>
</tbody>
</table>

Statistically significant p< 0.05
p1: Comparison between Control (group I) & Leukoplakia (group II).
p2: Comparison between control (group I) and Oral squamous cell carcinoma (group III).
p3: Comparison between leukoplakia (group II) and Oral squamous cell carcinoma (group III).

Table 6: Table depicting mean Serum Albumin levels in study groups along with standard deviation and p-values.

Serum A/G ratio

<table>
<thead>
<tr>
<th></th>
<th>Control (group I) (n=20)</th>
<th>Leukoplakia (group II) (n=40)</th>
<th>Oral Squamous cell carcinoma (group III) (n=40)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>1.1735</td>
<td>1.33735</td>
<td>1.40987</td>
<td>0.007</td>
</tr>
<tr>
<td>SD</td>
<td>0.20358</td>
<td>1.30438</td>
<td>0.43706</td>
<td></td>
</tr>
<tr>
<td>p1</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>p2</td>
<td>0.092</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>p3</td>
<td>-</td>
<td>0.003</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

Statistically significant p< 0.05
p1: Comparison between Control (group I) & Leukoplakia (group II).
p2: Comparison between control (group I) and Oral squamous cell carcinoma (group III).
p3: Comparison between leukoplakia (group II) and Oral squamous cell carcinoma (group III).

Table 7: Table depicting mean serum A/G ratio levels in study groups along with standard deviation and p-values.

producing oxidized products like advanced oxidation protein products (AOPP), malondialdehyde (MDA), advanced glycation end products etc. [23]. It is widely recognized that oxidation of proteins play an essential role in pathogenesis of cancer [24]. Knock-out of various antioxidant defence enzymes promotes cancer development in animals. Oxidative events have remarkable importance in development of oral cancer [25].

Prior studies have demonstrated that low serum albumin is an independent predictor of long-term mortality in several malignancies including gastric [26], colorectal [27], pancreatic [28], lung [29], ovarian [30], and breast [31]. Also A/G ratio has been established as an independent, significant predictor of long-term mortality in breast cancer [14] & colorectal cancer [32].

Hypo-albuminemia is one of the most common features of various cancers, hence this study was planned to evaluate serum Albumin and serum Albumin: Globulin ratio (A/G ratio) in oral leukoplakia and oral cancer patients.

Serum albumin

In present study the cause of the observed reduced albumin level in sera of group II 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. 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 [33].

Reactive oxygen species (ROS), implicated in multistage carcinogenesis are generated in substantial amount in oral cavity due to tobacco usage. Serum albumin contains one reduced cysteine residue (Cys34) which, due to the large amount of albumin in plasma, constitutes the largest pool of thiol in the circulation [34]. In healthy adults, about 70-80% of the Cys34 in albumin contains a free sulphhydril group; the rest forms a disulfide with several compounds like cysteine, homocysteine, or glutathione [35]. Through the reduced Cys34, albumin is able to scavenge hydroxyl radicals.

Reactive nitrogen species (RNS) constitute nitrogen-centred species analogous to ROS. Some RNS, such as nitric oxide (NO), contribute to various biological processes. Other RNS, such as per-oxynitrite (ONOO-), constitute powerful oxidants and nitrating species [36]. The -SH group of albumin represents an important antioxidant against per-oxynitrite as thiol group was shown to be oxidized to a sulfinic acid (HSA-SOH) [37].

As part of the systemic inflammatory response to the tumor, pro-inflammatory cytokines and growth factors are released [38,39]. Interleukin-6, produced by the tumor or surrounding cells, stimulates liver production of acute-phase reaction proteins (such as C-reactive protein (CRP) and fibrinogen) [40].

The lower serum albumin concentration may be due to the production of cytokines such as IL-6, which modulate the production of albumin by hepatocytes [40]. Alternatively, tumor necrosis factor (TNF) may increase the permeability of the microvasculature, thus allowing an increased transcapillary passage of albumin. Presence of micro-metastatic tumor cells in liver may induce the kupffer cells to produce a variety of cytokines (IL-1β, IL-6 & TNF), which may modulate albumin synthesis by hepatocytes [40,41].

Thus there is slight or no hypoalbuminemia in early stages of cancer but as the disease progresses albumin levels drop significantly and serve as good indicators of prognosis of cancer.

Above explanation supports our findings that mean values of serum albumin was low in leukoplakia patients as compared to control group which further got reduced in oral cancer patients.

Studies in the past have established low serum albumin as an independent predictor of long-term mortality in malignancies of lung [14], gastric [26], colorectal [27], pancreatic [28], hepatocellular [14], ovarian [30] and breast [31].

Additionally, previous studies proposed several anticancer mechanisms of circulating albumin that include its antioxidant function of some carcinogens (eg, nitrosamine and aflatoxin), its role in hemostasis of calcium and steroid hormone, and its inhibitory effect on cancer cell lines, including human breast cancer cell lines [14].

Serum A/G ratio

The findings regarding Serum A/G ratio may be explained by the fact that low A/G ratio and high globulin measure the extent of activities related to chronic inflammation as serum globulins plays a major role in immunity & inflammation [13]. As chronic
inflammation is a critical contributor to the development of leukoplakia & oral cancer; hence the values of A/G ratio may be decreased in these conditions [14].

The results can be further described by discussing the role of inflammation in oral carcinogenesis. Tobacco, alcohol, environmental agents and inflammatory mediators have the capacity to activate transcription factors (e.g. STAT-3, AP-1 & NF-κB) which in turn activate oncoproteins regulating apoptosis, cell proliferation and angiogenesis.

Through these transcription factors, cancer cells concurrently induce both inflammation and uncontrolled proliferation. The inflammatory microenvironment in turn is conducive to tumor progression [42].

Jeng, et al. (2003) [43] in their in vitro study demonstrated that oral keratinocytes can produce IL-6 in response to factors known to increase oral cancer risk including tobacco, smoking and areca nut.

Gashe, et al. (2011) [44] performed a study to evaluate role of IL-6 in promoting tumorigenesis by altering DNA methylation in oral cancer cells. They showed that IL-6 can promote tumorigenesis by causing DNA hypomethylation.

Studies have also done in which A/G ratio was estimated in breast & lung cancer. Azab, et al. (2013) [14] conducted a retrospective study of 354 breast cancer patients who had documented total protein & albumin levels prior to chemotherapy. They found out that the patients with higher A/G ratio (>1.45) had a lower 5-year mortality rate compared with those in the middle range (1.21 to 1.45) and the lower A/G ratio (<1.21). After adjusting for confounding variables, A/G ratio remained a significant predictor of mortality in their study.

They concluded that pre-treatment A/G ratio was an independent, significant predictor of long-term mortality in breast cancer patients, even in patients with normal albumin levels.

Conclusions

Serum albumin levels showed gradual decrease from control to leukoplakia patients and then further in oral cancer patients. Also, Serum A/G ratio was significantly lower in oral cancer patients as compared to leukoplakia patients. Hence, serum albumin levels and serum A/G ratio could be used to find out possibility of malignant transformation in leukoplakia patients.

As serum albumin levels were significantly lower in oral cancer patients as compared to control group, hence serum albumin levels can be used as an adjunctive tumor marker in diagnosis of oral cancer.

As values for Serum A/G ratio were significantly lower in oral cancer patients as compared to leukoplakia patients, hence serum A/G ratio can be used as an adjunct in diagnosis of oral cancer.

Thus it can be concluded that both serum Albumin levels and serum A/G ratio can be used in diagnosis of oral cancer as adjunctive tumor markers.

Future Scope

The results obtained in the present study emphasize the need for more studies with large sample size to be conducted in this regard for the assessment of sera levels of Albumin and A/G ratio to accept their utility and to assess their role in the pathogenesis and their impact on the diagnosis and prognosis of oral cancers. These results can also play a role in providing a scientific background for the use of diverse chemo-preventive strategies in controlling damage at molecular levels to prevent the ongoing transition of various potentially malignant disorders into oral carcinoma.

Serum albumin has also been described as an independent prognosticator of survival in various cancers of extra oral sites. Also A/G ratio has been established as an independent, significant predictor of long term mortality in breast cancer & colorectal cancer. Such clinical investigations can also be planned for oral cancer.

References


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