CIRCULATING IMMUNE COMPLEXES (CIC) AS MARKER FOR DISEASE PROGRESS IN ORAL CANCER

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ABSTRACT
Oral Cancer is one of the five leading sites of cancer in Indian population. The circulating immune complexes were investigated in 100 serum samples of 60 oral cancer patients having different grades of the disease and 40 patients with precancerous lesions obtained from Nair Hospital Dental College, Mumbai. The results obtained were compared with those of group of 40 healthy blood donors. Elevated levels of Circulating Immune Complexes were observed in oral cancer patients and patients with oral precancerous lesions. 92% positive samples were observed in well differentiated squamous cell carcinoma whereas 100% positive samples were observed in both moderately and poorly differentiated squamous cell carcinoma. Oral leukoplakia and oral submucous fibrosis showed 15% and 90% positivity respectively. Increased level of Circulating Immune complexes in high grade tumor suggest that Circulating Immune complexes is likely to contribute in evaluating the degree of malignancy, but follow up study is needed to draw any conclusion regarding it’s prognostic role.

KEY WORDS
Oral cancer, Circulating Immune complexes, Squamous cell carcinoma, Oral leukoplakia, Oral submucous fibrosis.

INTRODUCTION
Oral cancer is an insidious devastating malignancy and is one of the five leading sites of cancer in India (1). Less than half of those who suffer from this disease survive its onslaught and/or the therapy necessary for its eradication. Among the oral tumors, 90% of them are squamous cell carcinomas (SCC) (2), which arise from the mucosal lining. Inspite of significant advances in surgery and radiation therapy, the five-year survival has remained at about 52% for the past few decades (3). The survival rate must be improved and the most significant method to accomplish this improvement is early detection. Several epidemiological and laboratory studies clearly suggest that tobacco (in different forms) is the single greatest risk factor for all oral malignancies in India (4). The most vulnerable area is the buccal mucosa (cheek) and this is made so by a higher concentration of carcinogenic exposure and by failure to clean the carcinogens from the mucosal surfaces. A variety of tobacco habits are prevalent in India and they differ from region to region (5). In Mumbai, many people use smokeless tobacco in the form of nass, naswar, khaini, pan masala, gutkha and betel-quad. These various forms are chewed, sucked or applied to teeth and gums (5). There are about 2500 different compounds present in tobacco and about 300 of these are considered carcinogenic (6). In India, oral cancer is almost always preceded by benign lesions or conditions for a varying length of time. Interestingly, the benign lesions also share the same risk factors as oral cancer, particularly the use of tobacco and exhibits the same site and habit relationships. Many of these have the potential to become cancers, and are termed as “precancerous lesions” or “premalignant lesions”. A precancerous lesion is defined as morphologically altered tissue in which cancer is more likely to occur than in its apparently normal counterpart. Oral Leukoplakia (OL) is defined clinically as a white keratotic plaque that cannot be removed by manual manipulations. Despite the increased risk associated with having leukoplakia, many people with this condition never get oral cancer (7). Oral submucous fibrosis (OSMF) is a chronic mucosal condition with mucosal rigidity of varying intensity due to fibroelastic transformation of the...
juxta epithelial connective tissue layer. Unlike leukoplakia, Oral Submucous Fibrosis (OSMF) does not show regression, even with cessation of the tobacco chewing habit (8).

Elevated levels of circulating immune complexes (CIC) in the sera of oral cancer patients have been reported by several workers (9-11). Oral cancer is prevalent cancer in Indian population. However, study on circulating immune complexes in oral cancer is unfortunately was not carried out after Ramani et al (10). No previous attempts have been made to study the levels of CIC in sera with premalignant lesions as well as different grades of oral cancer. With this aim, we have evaluated the levels of CICs in oral precancerous and cancerous lesions of varying grades.

MATERIALS AND METHODS

Patients with cancerous lesions – The present study included 60 patients (36 males and 24 females) with primary oral squamous cell carcinoma of the buccal mucosa ranging in age from 30-75 years (median age 52.5 years). Histopathologically the tumors were categorized as well differentiated squamous cell carcinoma (WDSCC), moderately differentiated squamous cell carcinoma (MDSCC), Poorly differentiated squamous cell carcinoma (PDSCC) with 20 cases in each category.

Patients with precancerous lesions – Premalignant lesions consisting 20 oral leukoplakias (12 males and 8 females) and 20 oral submucous fibrosis (13 males and 7 females) ranging in age from 23-60 years (median age, 41.5 years) were studied.

Normal subjects- 40 Normal subjects(22 males & 18 females) ranging in age from 25 to 60, who were not having any major illness in the past were studied.

All the samples were obtained from the Department of Oral Medicine, Diagnosis and Radiology, Nair Hospital Dental College, Mumbai. Informed consent was taken from the patients as per the Ethics Committee rules. None of the patients had received any treatment prior to the study. All the subjects were screened clinically, biochemically and biophysically to exclude any infections and previous history of allergy and/or autoimmune diseases. All the patients were tobacco chewers for a minimum period of 10 years. For comparison of the results, 40 adult healthy normals were also included.

Approximately 10 ml blood was collected from each case in sterile dry glass tubes and allowed to clot at 37°C for 4 hrs. The serum was aspirated and clarified by centrifugation at 1500 g for 10 mins. Modified polyethylene glycol – 6000 (PEG) mediated precipitation technique was used to estimate the levels of CICs in serum (12). One part of the freshly obtained serum was mixed with two parts of 0.01M-borate buffer, pH 8.4. To this mixture 27 parts of 4.166% PEG was added (final 1:30 serum dilution and 3.75% PEG concentration). After incubation at room temperature for 60 mins, the turbidity developed was measured spectrophotometrically at 450 nm against control containing 1:30 diluted serum in borate buffer without PEG. The level of CIC in serum was expressed in terms of OD$_{450}$ measured at the end of 60 mins.

RESULTS

Table 1 brings to light the significant and obvious differences in the categories studied. The mean levels of CIC in categories of the patients were higher than that in the normal donors. Since all the samples from the normal donors showed variable levels of CIC, the turbidity value at (mean ± 2 S.D) i.e. 0.05 for the normal samples was chosen as the cut-off limit for positivity.

### Table 1: Levels of CIC in sera of normal volunteers and patients with OL, OSMF, WDSCC, MDSCC, and PDSCC

<table>
<thead>
<tr>
<th>Subjects</th>
<th>No. of samples</th>
<th>Range</th>
<th>Mean</th>
<th>Turbidity value at 450 nm SD</th>
<th>SE</th>
<th>% Positive Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>40</td>
<td>0.010-0.046</td>
<td>0.02315</td>
<td>0.013461</td>
<td>0.00246</td>
<td>-</td>
</tr>
<tr>
<td>OL</td>
<td>20</td>
<td>0.013-0.085</td>
<td>0.03817</td>
<td>0.016808</td>
<td>0.0376 *</td>
<td>15</td>
</tr>
<tr>
<td>OSMF</td>
<td>20</td>
<td>0.045-0.253</td>
<td>0.01871</td>
<td>0.054718</td>
<td>0.0173**</td>
<td>90</td>
</tr>
<tr>
<td>WDSCC</td>
<td>20</td>
<td>0.040-0.153</td>
<td>0.08912</td>
<td>0.032887</td>
<td>0.00735**</td>
<td>92</td>
</tr>
<tr>
<td>MDSCC</td>
<td>20</td>
<td>0.100-0.162</td>
<td>0.1129</td>
<td>0.016603</td>
<td>0.00371**</td>
<td>100</td>
</tr>
<tr>
<td>PDSCC</td>
<td>20</td>
<td>0.150-0.435</td>
<td>0.3051</td>
<td>0.090199</td>
<td>0.0202**</td>
<td>100</td>
</tr>
</tbody>
</table>

*In the subjects OL p<0.002 in comparison with normals.
**In the subjects OSMF WDSCC MDSCC and PDSCC; p< 0.001 in comparison with normals.