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

Cynthia Jane, A.V.Nerurkar and F.R. Karjodkar*

Department of Biochemistry, T.N.Medical College, B.Y.L.Nair Hospital, Mumbai

*Department of Oral Medicine, Diagnosis and Radiology, Nair Hospital Dental, College, Mumbai

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 collage, 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. In spite 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, paan masala, gutkha and betel-quid. 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 "pre-malignant 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

Address for Correspondence:

Dr. A. V. Nerurkar

Dept of Biochemistry, T.N Medical college,
B.Y.L.Nair Hospital, Mumbai 400008.

Email: alka_nerurkar@rediffmail.com

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 OD₄₅₀ 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 $\pm$ 2 S.D) i.e. 0.05 for the normal samples was chosen as the cut-off limit for

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

Subjects	No. of samples	Range	Mean	Turbidity value at 450 nm SD	SE	% Positive Samples
Normal	40	0.010-0.046	0.02315	0.013461	0.00246	-
OL	20	0.013-0.085	0.03817	0.016808	0.00376 *	15
OSMF	20	0.045-0.253	0.01871	0.054718	0.0173**	90
WDSCC	20	0.040-0.153	0.08912	0.032887	0.00735**	92
MDSCC	20	0.100-0.162	0.1129	0.016603	0.00371**	100
PDSCC	20	0.150-0.435	0.3051	0.090199	0.0202**	100

*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.

discriminating between positive and negative samples. All the samples giving turbidity values above this level were considered to be positive. Using this guideline, the frequency of positive serum samples was found to be very high in untreated OSCC.

The statistical evaluation using unpaired t test of these results show that the mean levels of CIC in each category of patients differ significantly from the mean level of the normal subjects. In the subjects OL, $p < 0.002$ in comparison with normals and in the subjects OSMF, WDSCC, MDSCC and PDSCC $p < 0.001$ in comparison with normals. Thus elevated CIC levels were found in all the different grades of OSCC. The frequency of positive serum samples was found to be 92% in WDSCC and 100% each in MDSCC and PDSCC. This clearly depicts that as the disease progresses, there is a significant increase in the levels of CIC. In case of serum samples obtained from precancerous lesions i.e. OL ($n=20$) and OSMF ($n=20$), the mean level of CIC was found to be 0.03817 in OL and 0.1871 in OSMF. Only 15% of the OL samples had elevated levels of CIC whereas 90% of the OSMF samples showed elevated levels of CIC.

DISCUSSION

The occurrence of CIC is an event in any normal immune response. The half-life of such CIC is transitory in nature. Continued presence of CIC over extended periods, however, is a cause of consequence of some pathological condition or infection. Elevated levels of CIC have been found in a variety of diseases including neoplasia. The suggestion that CIC might compromise in the host-tumor relationship on the immunological front, has led to extensive studies aiming at unraveling the correlation between CIC levels in serum and clinical course of neoplastic diseases (13,14). Presuming the involved antigens to be of malignancy-specific, serum CIC levels in cancer patients have been used for early diagnosis, metastatic spread, tumor burden, degree of aggressiveness, therapeutic response as well as prognosis. (9,13,15,16)

Several factors become clear from the values given in Table 1. The mean levels of CIC in each category of patients differ significantly ($p < 0.001$) from the mean level in the normal subjects. Since these complexes represent the host defense against tumour antigen by production of antibodies the results obtained were obvious. Rajendran et al (17) has reported a depression in the cell mediated and humoral immunity in premalignant lesions of the oral cavity. The results of the present study however clearly show that there is an elevation in the CIC in patients with OSMF whereas minimal

changes were observed in OL patients. This may be attributed to the fact that OSMF carries a high relative risk for malignant transformation (18). These results are congruent with the results reported by Remani *et al* (10). It is thus clear that the level of CIC may be of help in predicting the malignant transformation of OSMF.

Elevated levels of CIC were found in all the different grades of OSCC. In the present study it was found that as the disease progresses from well differentiated to poorly differentiated form, there is a significant increase in the CIC levels thus it is associated with poor prognosis. These results are in consistent with those obtained by other workers (10,11). In most cancers, the antigenic part of the circulating immune complexes have been shown to originate from the disease affected tissues (19,20). As the disease progresses the shredding of the antigen by these tissues also increases and it is thought to increase the level of CICs. Observations in the present investigation are congruent with these reports. Although there are record on relationship of CIC levels to the stages of malignancies (15) no detailed study correlating CIC levels with histological grades has been reported so far. We had obtained significant correlation between CIC levels and histopathological grades of oral cancer. Increased level of CIC in high grade tumor suggest that CIC is likely to contribute in evaluating the degree of malignancy. The above investigation may also indicate the potential of CIC estimation in predicting the malignant transformation of premalignant lesions like OSMF but follow up study is needed in order to draw any conclusion. Detection of antigen specific CIC in sera of cancer patients may provide an approach for a more precise test to predict prognosis of the disease. Further study of biochemical analysis of CIC is awaited which will enable us to detect the antigenic component of CIC and its relationship with the tumour.

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