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Oral Squamous Cell Carcinoma and C - Reactive Protein Levels as Systemic Biomarker-A Clinicopathological Prognostic Correlation For Local Inflammation And Carcinogenesis.

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ABSTRACT

This study was undertaken to observe association between serum C reactive protein levels and carcinogenesis in patients with history of tobacco and presenting with potentially malignant disorders and oral Squamous cell carcinoma. The incidence of oral squamous cell carcinoma has increased by four folds in this century in India. There is a requirement for a prognostic indicator in detecting in earlier stages. Materials and Methods: 90 patients with tobacco history reporting to department of oral pathology were selected. These 90 patients were divided into Group-I, Group-II each group comprising of 30 patients. Group I had patients with history of tobacco chewing of more than three years with no visible lesion. Group II had patients with history of tobacco chewing more than three years and diagnosed with potentially malignant disorders. Group III had patients with history of tobacco chewing with diagnosed oral squamous cell carcinoma. The values were compared and were statistically analyzed by independent sample 't' test. Results: On comparison, Group I and Group II showed no statistical significance for OHI- S, but Group III showed statistically significant in Group III. DMFT index was significant high in Group II and maximum in Group III. The local factors seemed to be increased in Group II and Group III. The systemic factors and CRP qualitative analysis shows there is no significant change in the Group I and Group II as seen Table II. The significant values were seen in Group III where the elevated CRP levels were seen in the established OSCC Group III. Conclusion: Carcinomas of the oral cavity have been a major challenge for treating surgeons. Serum CRP certainly will be useful as a prognostic indicator as a clinicopathologic indicator for oral squamous cell carcinoma. Prevention is better than cure. The patients who have a bad oral hygiene need to be counselled for maintaining the inflammatory components in the human body so that the vicious cycle of carcinogenesis is not activated.

Keywords: Serum C reactive protein, oral squamous cell carcinoma

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INTRODUCTION

The clinicopathologic parameters have been implicated in prognosis, recurrence and survival following oral squamous cell carcinoma (OSCC). Pre-treatment laboratory prognostic index (LPI) based on laboratory results-extension to clinicopathologic parameters for prognosis and treatment in patients with OSCC. The presence of systemic inflammatory response indicates poor prognosis in OSCC.

Virchow in 1863 declared that the origin of cancer at sites of chronic inflammation irritants, together with tissue injury and ensuing inflammation, enhance cell proliferation [1]. C-reactive protein (CRP), an acute-phase reactant, was discovered in the serum of patients with pneumonia by Tillett and Francis in 1930 [2]. Synthesis of APP in hepatocyte is regulated by three main inflammatory cytokines such as I-L1, IL6 and tumor necrosis factor produced by immunocompetent cells [3].

C reactive protein (CRP) annular, pentameric protein and acute phase protein found in blood plasma. The levels of which rise in response to inflammation. It is widely used systemic biomarker for diagnosing acute and chronic inflammation. The clinical use has been for diagnosing cardiovascular diseases and malignancies. Serum CRP is elevated in malignancies, implying close linkages in inflammation and malignancy. There is a direct positive correlation between the concentrations of APPs, especially of CRP and the severity of inflammation [4]. inconsistent. Oral cancer is the eleventh most common cancer globally [5]. There is variation in the incidence of oral cancer, with approximately two-thirds of patients in the developing countries of Southeast Asia, Eastern Europe and Latin America [6]. In India, the gingival-buccal complex (alveolar ridge, gingival-buccal sulcus, buccal mucosa) forms the most common subsite for cancer of the oral cavity, in contrast to cancer of the tongue that is more common in the western world [7]. India has one of the highest incidences of oral cancer, with men:women ratio of 2:1 and accounts for about 30% of all new cases annually [8]. In India oral cancer as the leading cause of mortality in men and responsible for 22.9% of cancer-related deaths [9].

Some authors have observed an association between elevated serum CRP levels in some cancers, like colorectal and lung cancer. There have been limited research proving the association of CRP to OSCC. The higher risk of developing cancer in subjects with elevated serum CRP. Thus this study was designed in order to determine the association of increased qualitative levels of CRP with oral hygiene status of the patient with or without potentially malignant disorders (PMD) and its correlation with oral squamous cell carcinoma.

Aim

To determine and correlate oral hygiene status of patient as local infection factors with C reactive protein as systemic inflammatory indicator.

METHODOLOGY

The groups were divided into three groups. Group I had patients with history of tobacco chewing of more than three years with no visible lesion. Group II had patients with history of tobacco chewing more than three years and diagnosed with PMD. Group III had patients with history of tobacco chewing with diagnosed oral squamous cell carcinoma. The total of 90 patients fulfilling the inclusion criteria listed below were included in the study. A pilot study was conducted with 12 patients 4 in each group with 95% confidence level and hence the sample size of 90 was derived.

The institutional review board with ethical clearance was taken before the study started. The inclusion criteria were patients with adults of both genders belonging to age group of 20-70 years with no known chronic inflammatory disorders. Patient with chronic inflammatory or immunological conditions such as arthritis, chronic obstructive airway diseases, type 1 and type 2 diabetic, pregnant and lactating women, patient receiving antibiotic therapy from past 6 months. A thorough clinical history was recorded followed by detailed clinical examination. All the patients were evaluated for DMFT and OHI-S index in order to determine the oral hygiene status of the patient.

A venous blood sample of approximately 2 ml was collected to qualitatively assess the CRP levels. The blood sample was transferred to plain sterile glass test tubes and then centrifuged at 2000 rpm for three to 5

min. The top layer of clear serum in the tube was separated with the help of a micropipette. One drop of serum was mixed with CRP latex reagent on a glass slide. Elevated CRP concentration of above 6 mg/l leads to visible agglutination of the latex particles. The data was analysed with by using statistical computer software SPSS 11.0. The statistical analysis was done by student's t test.

RESULTS

Figure 1 shows student t-test and mean \pm standard deviation. On comparison, Group I and Group II showed no statistical significance for OHI-S, but Group III showed statistically significant in Group III. DMFT index was significant high in Group II and maximum in Group III. The local factors seemed to be increased in Group II and Group III.

The systemic factors and CRP qualitative analysis shows there is no significant change in the Group I and Group II as seen Figure 2. The significant values were seen in Group III where the elevated CRP levels were seen in the established OSCC Group III.

Local Factors - OHIS/DMFT

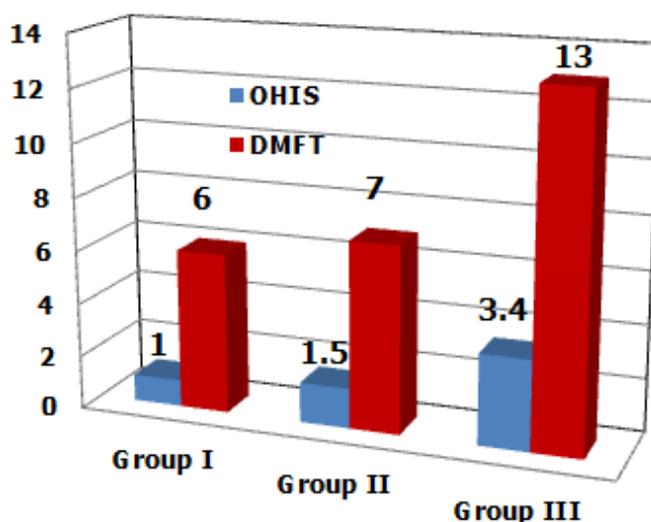


Figure 1: Local factors-OHIS/DMFT

Systemic Factor- Qualitative CRP

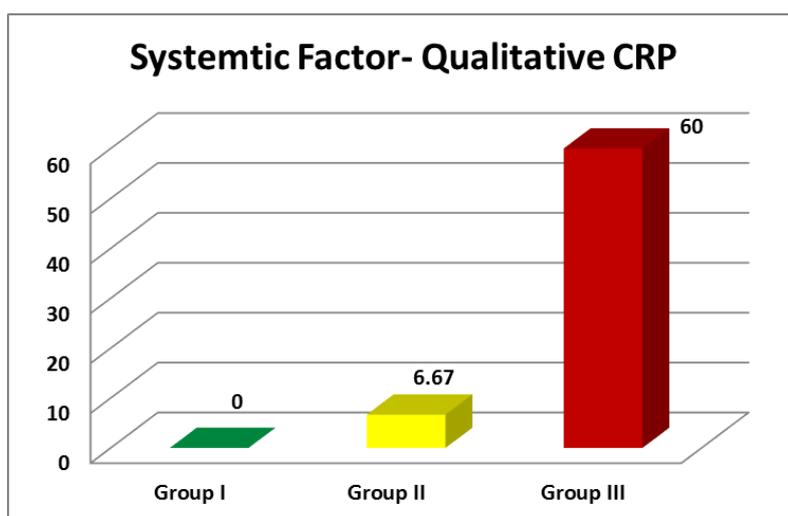


Figure 2: Systemic factor-Qualitative CRP

DISCUSSION

The positive association between preoperative CRP levels and prognosis of OSCC was well established in our study. The possible mechanisms to explain such association are

1. Tumour growth can cause tissue inflammation and hence increase CRP levels.
2. CRP as an indicator of immune response to tumour antigens
3. Evidence of cancer cells increasing the production of inflammatory proteins.
4. Some cancerous cell lines secrete interleukin -6(IL-6) and IL-8, which in turn induce the production of inflammatory proteins.
5. The oral hygiene could cause local inflammation and hence be the precipitating factor for increase in CRP levels.

The prognostic significance of serum CRP has been demonstrated in a variety of patients with primary malignancies, including oesophageal, esophagogastric, colorectal, hepatocellular, pancreatic, prostate, urinary bladder, ovarian and cervical cancers, melanoma, and thymoma [10-12] and in the advanced stage of cancers including inoperable nonsmall cell lung cancer, unresectable pancreatic cancer, biliary tract cancer, and metastatic brain diseases [13,14]. Elevated CRP has been associated with progressive disease and worse survival for patients with these malignancies.

Serum C-reactive protein (CRP) is a very sensitive indicator of current disease activity for inflammation.

It has been most widely used for the clinical diagnosis of acute or chronic inflammation. The introduction of a high sensitivity technique enables identification of the group of patients with chronic inflammation that manifested by a minor elevation of CRP [15].

In tumour tissues, cancer cells are embedded in a microenvironment resembling chronic inflammation. In addition to tumour cells, the microenvironment contains leucocytes, lymphocytes and macrophages with cytokines and chemokine's acting as mediators, reflecting a persistent inflammatory state. This microenvironment may contribute to carcinogenesis through induction of genomic instability, epi-genetic alterations, and subsequent inappropriate gene expression, leading to enhanced proliferation, resistance to apoptosis, neovascularization, and spread of cancer cells [16]. These cancer cells influence the microenvironment through the up regulation of inflammatory pathways by producing proinflammatory mediators such as cytokines, chemokine's, cyclooxygenase-2 (COX-2), prostaglandins, inducible nitric oxide synthase, and nitric oxide [4].

Serum CRP measurements are simple, cheap, and available in daily practice. CRP-lowering agents may have promising roles for the prevention and therapy of malignancies in the future. Serum CRP level is a sensitive marker of inflammation that is elevated in response to tissue damage or infection and has been shown to be a prognostic factor in OSCC. [17, 18]

During the past decade, serum CRP has been re-emphasized by extending its clinical use to the prediction or diagnosis of cardiovascular diseases and other conditions, particularly malignancies. Serum CRP has also been found to be elevated in patients with many malignancies, implying a close linkage between inflammation and malignancy [4]. In our study the serum CRP has proven to determine the correlation between the local factors such as OHIS and DMFT index is on the higher range in patients with potentially malignant disorder patient group II and Group III with diagnosed OSCC. Salzberg et al [19]. reported that patients with aggressive periodontitis had increased serum concentrations of CRP.

In the study conducted by Anita et al [20] CRP levels were increased three- fold in condition with both cardiovascular diseases(CVD) and chronic periodontitis and was increased by two- fold with chronic periodontitis without CVD. This was proven even in our case were periodontitis was closely related to periodontitis and gingivitis in Group II , Group III in comparison to Group I.

Two hypotheses could be associated with increased CRP levels as a sign of chronic inflammation. First, the induction hypothesis: chronic inflammation results in excessive cell proliferation and activation of a

cascade of cellular actions, leading to induction of irreversible DNA damage [21]. Second, the response hypothesis: the immune response of the host as a consequence of tumour growth itself could be the reason for the elevation in CRP levels [22].

In the study done by Kruse et al [23] to assess CRP as a prognostic marker, the mean value of serum CRP was 7.36mg/L; the serum level was raised in 85 patients (30.6%);the increase was mostly moderate (Fig. 1); and a high increase of more than 50 mg/L, seen in infectious disease, was found in 6 patients. The patients in our study also showed similar results.

Elevated CRP levels were detected in 36% (range, 13-72%) of patients with cancers, which was significantly higher than that in healthy control subjects in study conducted by Wang et al .The prospective study by Hou Chen et al [25] demonstrated that the combined measurement of Squamous cell carcinoma antigen and CRP levels served as a marker of clinical status in Pharyngolaryngeal carcinoma and may represent a biomarker capable of predicting prognosis. This was concluded in our study where proven cases of OSCC had increased CRP levels indicating as a systemic marker for prognosis.

CONCLUSION

Carcinomas of the other organs exhibit an immense increase in CRP levels. In our study we did find an increase of CRP levels in OSCC patients. The raise in CRP levels could be an association of multiple factors such as tobacco, arecanut along with bad oral hygiene. Therefore we have come to a conclusion that multitude of factors could play a vital role in carcinogenesis.

Future Scope

Patients diagnosed with PMD would be subjected to thorough oral prophylaxis along with counselling for discontinuation of habit. This would reduce the incidence of oral cancer by reducing bacterial load and local inflammation.

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