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Comparison of Serum C - Reactive protein level in Oral potentially Malignant disorders and in healthy individuals.

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ABSTRACT

C-reactive protein (CRP) is an acute-phase protein and a nonspecific marker for inflammation. Liver is the site of its synthesis & normally present as trace constituent of serum of plasma at levels less than 0.3 mg/dl_3. Synthesis of CRP in the hepatocytes is regulated by pro-inflammatory cytokines like IL-1, IL-6, and TNF which have been linked with malignancies. To investigate pretreatment serum C-reactive protein (CRP) levels in oral potentially malignant disorders (oral leukoplakia and Oral submucous fibrosis) & evaluate their usefulness as prognostic markers. The study sample consisted of 20 control groups and 20 oral potentially malignant disorders patients (oral Leukoplakia and Oral submucous fibrosis) with no other systemic disease. All the samples were subjected to CRP analysis. Leukoplakia patient showed more CRP positivity than OSMF patients. CRP the classical marker of acute phase response is an indicator of a variety of pathological processes including infection, tissue damage, and chronic inflammatory disease. CRP is a nonspecific marker of systemic diseases and inflammation and can contribute toward the management of these diseases. CRP values can be interpreted at the bedside, in full knowledge of all other clinical and pathological results. We need to do further studies with large sample to assess regarding CRP levels and PMD.

Keywords: Introduction, aims and objectives, material and methods, statistical procedure, results, discussion, conclusion, reference.

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INTRODUCTION

The incidence and death rate due to cancer have shown a sharp acceleration since the last two decades, more intense efforts are required to fight against this life threatening disease [1]. The risk of malignant transformation of patients with PMD increases day by day and hence it is very important to assess the prognosis of these patients at an early stage. If identified, the incidence of death rates due to OSCC can be reduced considerably [2]. Many investigators have been searching for a specific, reliable and easily identifiable biomarker, which can differentiate cancer patients from healthy individuals and also to find out patients with precancerous lesions who have high risk of developing cancer [1]. One such biomarker that can be used is the C-reactive protein (CRP). It has been identified that CRP increases during both acute and chronic inflammatory conditions. As it is well-known fact that inflammation and cancer are linked, the role of this protein has to be identified in PMD and OSCC [2].

C-reactive protein, a member of the pentaxin protein family was first identified by Tilet & Francis (1930) in the plasma of patient with pneumonia. It is an alpha globulin with a molecular weight of 110,000 to 140,000 Daltons. CRP, named for its capacity to precipitate the somatic C-polysaccharide of *Streptococcus pneumonia* [1]. Liver is the site of its synthesis & normally present as trace constituent of serum of plasma at levels less than 0.3 mg/dl. The synthesis of CRP in the hepatocytes may be regulated by pro-inflammatory cytokines like interleukin-1 (IL-1), interleukin-6 (IL-6), and tumor-necrosis factor (TNF), which have been linked with inflammatory disorders. Therefore, these pro-inflammatory cytokines are currently the subject of intense studies as influencing factors in various types of inflammatory disorders [3].

CRP and other acute-phase molecules are usually present at relatively low levels in plasma, but may be raised dramatically within 72 h of tissue injury, or with infection. The advantages of CRP over other acute phase proteins is levels appear after the onset of disease and levels increase within 4-6 h after an acute tissue injury, whereas serum levels of all the other acute phase reactants increase 12-24 h from injury. CRP is consistently found in bacterial infection, acute rheumatic fever, and malignant diseases, viral infections, tuberculosis, and also in patients following surgical operations and blood transfusions [4].

CRP has the ability to prevent the adhesion of neutrophils to endothelial cells by decreasing the surface expression of L selectin, inhibit the generation of superoxide by neutrophils, and stimulate the synthesis of interleukin-1 (IL-1) receptor antagonist by mononuclear cells. CRP has also been reported to stimulate tissue factor production by human peripheral blood monocytes and has a procoagulant effect. It has also been reported that CRP recruits monocytes by receptor-mediated chemotaxis into the arterial wall. It colocalizes with foam cells in atherosclerotic lesions [5].

There are few studies where they have found an association between elevated serum CRP levels and cancers of the colorectal region and lung. On the other hand, there are studies that have revealed that CRP can be used as a prognostic marker [2].

There are less of studies in the literature where there is an association between serum CRP and PMD and head and neck cancers. So we made an attempt to study the CRP levels with oral PMD as a useful prognostic marker.

This study was done to assess the serum CRP levels in PMD and to evaluate their significant role as a prognostic marker.

Aims and objective:

To investigate pretreatment serum C-reactive protein (CRP) levels in oral potentially malignant disorders (oral leukoplakia and Oral submucous fibrosis), periodontal diseases & evaluate their usefulness as prognostic markers.

MATERIALS AND METHODS

The study samples were selected & prior consent has been taken from all the participants. Subjects with clinical diagnosis of leukoplakia, Oral submucous fibrosis (OSMF) were included in the study group. The present study was conducted after approval by local ethical committee.

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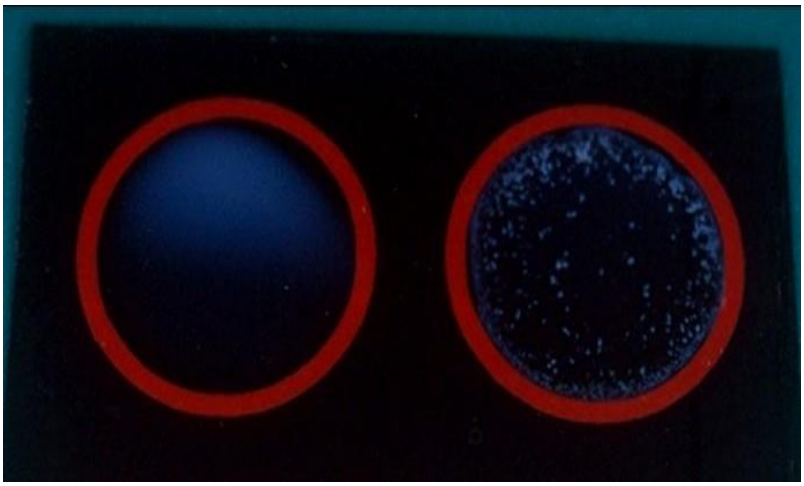
A total of 40 subjects, both male and female visiting the Department of Oral Medicine and Radiology, Rajarajeswari Dental College and Hospital, Bangalore was recruited for the study.

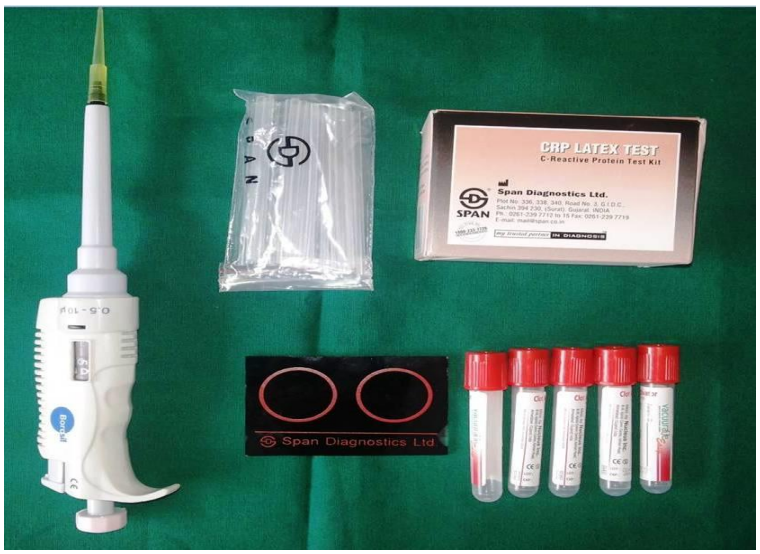
- **Group 1-** Positive controls
- **Group 2-** Negative controls
- **Group 3-** Oral Leukoplakia
- **Group 4-** OSMF

Patients with periodontal disease were taken as positive controls (group 1) with no other underlying diseases and patients who are healthy without any underlying diseases are taken as negative control.

Materials used in the study are: CRP kit (Span Diagnostics), micropipette, blood sample collection tube.



We can see the agglutination on the right circled area of the slide



Immunologic reaction between CRP as an antigen and latex particles have been coated with monospecific anti-human CRP and sensitized to detect levels greater than 6µg/ml CRP.

5-10 ml venous blood was collected into sterile tube/vial without anticoagulant. Serum was separated by centrifugation. Test serum are used undiluted, using the disposable plastic dropper, one drop of test serum was placed within the circled area on the special slide provided o the kit. One drop of latex CRP reagent was added to the above serum drop and mixed well with a disposable applicator stick. Rocking of the slide to and fro for 2 minutes was done and examined for macroscopic agglutination under direct light source. Highest

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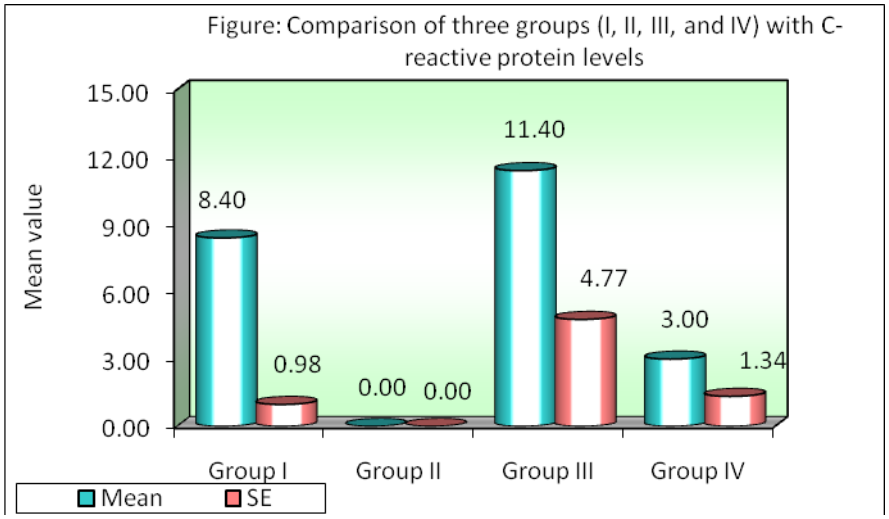
dilutions which show agglutination is taken as CRP titre of the test serum. The procedure followed was semi-quantitative slide test.

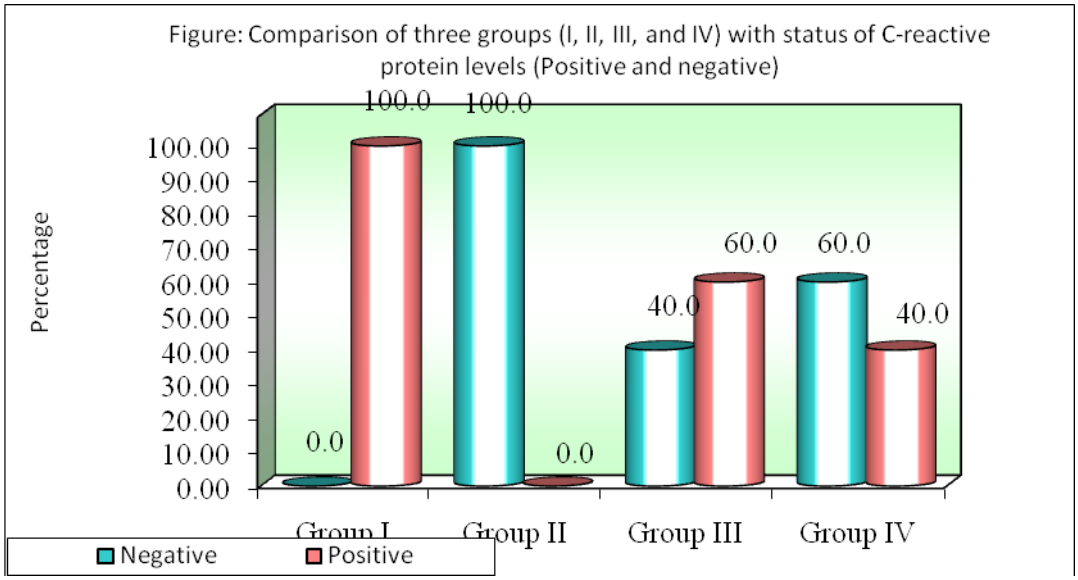
Statistical procedures:

The statistical analyses were carried out to obtain the prevalence & comparison of CRP levels in precancer & control group. The results were statistically analyzed using Kruskal Wallis ANOVA and Mann-Whitney U test.

RESULTS

In our study, Pair wise comparison of 4 groups with C reactive protein levels by Mann Whitney U test showed significance between Group I and II , Group I and IV and Group II and III with p values being 0.0002, 0.0126and 0.0233 respectively (*p<0.05). Comparison of all 4 groups with status of C reactive protein levels by Kruskal Wallis ANNOVA showed significance between Group I and II, Group I and IV and Group II and III with p values being 0.0001, 0.0233 and 0.0233 respectively (*p<0.05) .





DISCUSSION

Acute phase proteins are defined as proteins whose concentration is altered at least 25% in response to inflammation and include proteins of the complement, coagulation and fibrinolytic systems, antiproteases,

transport proteins, inflammatory mediators and others [1]. C-reactive protein, serum amyloid A, fibrinogen, albumin and transferring are few acute phase proteins. C-reactive protein, serum amyloid A and fibrinogen concentration increases with inflammation where as albumin and transferrin concentration decreases with inflammation.

C-reactive protein is a type I acute phase protein, which can increase upto 1000 fold after the onset of a stimulus. Due to its opsonising abilities and its capability to activate human complement, CRP plays an important role in the innate host defense against different microorganisms, such as bacteria and fungi. Its level gets raised in connective tissue diseases, cardiovascular diseases, infections, lupus erythematosus, periodontal diseases and in cancer patients [1].

CRP is protein synthesized in the liver and the major protein of plasma. Its half-life is approximately 6-4 hours. The serum levels of this protein increase rapidly within 24 to 72 hours in conditions of inflammation or tissue damage and will subside after the removal of inflammation or infection.

Two hypotheses could be associated with increased CRP levels as a sign of chronic inflammation. First, the induction hypothesis by Rudolf Virchow (1863) states that chronic inflammation results in excessive cell proliferation and activation of a cascade of cellular actions that can lead to induction of irreversible DNA damage. Persistent irritation and inflammation subsequently promote these initiated cells, resulting in tumor growth, progression of metastatic disease, and immunosuppression. Second, Response hypothesis the immune response of the host is studied as a consequence of tumor growth itself. In both hypotheses, products of inflammatory processes are believed to be biomarkers [7].

One of the new approaches for control of this cancer is early detection of leukoplakia—considered as the most common premalignant oral lesion of the oral cavity and present in 60% of patients diagnosed with oral squamous cell carcinoma [8]. Epithelial dysplasia in OSMF tissues appeared to vary from 7 to 26% depending on the study population. Malignant transformation rate of OSMF was found to be in the range of 7–13% [9]. Serum elevation of CRP has been reported to be an indicator of the unfavorable outcome in patients with some malignant tumors (Hirasaki et al.) [10]. Some of the research papers have observed an association between serum CRP levels and different types of malignancies such as esophageal, colorectal, renal, and prostate cancers.

Our study shows increase in the level of CRP in both leukoplakia and OSMF. Kumar and Bhateja[1], Srilalitha Kaja, Sashi Kiran Sanapala Venkata Naga et al [11] , studied CRP levels in oral precancer and cancer in which they observed that CRP level was elevated in PMD. In study done by Kaja, Sashi Kiran Sanapala Venkata Naga et al [2] OSMF patient showed prominent CRP elevation where as in our study Leukoplakia patient showed more elevation.

Several investigations regarding the relationship between salivary CRP levels and periodontal disease have also been done. Among these researches, one can refer to the study of Giannobile et al. in 2009 who showed that the saliva and serum CRP levels were elevated in patients with chronic and aggressive periodontitis. In studies done by Pitiphat et al. 2008, Wohlfeil et al. 2011 and Haba et al. 2011, an increase of serum CRP levels were approved in patients with periodontitis.

In a study done by Claire Sieme et al., he found high baseline values of CRP and a particular Single nucleotide pleomorphism in the CRP gene were both associated consistently with an increased risk of lung cancer [7]. In another study done by Ines Gockel, he found preoperative CRP is an easily determined and independent prognostic marker in patients with squamous cell carcinoma and adenocarcinoma of the oesophagus [13]. Many studies in the past have shown that CRP levels increase as cancer progresses.

In our study, we have assessed the levels of CRP in PMD such as leukoplakia and OSMF and in periodontitis. Leukoplakia patients showed prominent elevation of CRP than OSMF. Periodontal patients showed more positivity than oral leukoplakia. The results obtained in our study could also be due to the small sample size.

CONCLUSION

CRP, the classical marker of acute phase response, is an indicator of a variety of pathological processes including infection, tissue damage, and chronic inflammatory disease [5]. The serum CRP level is a simple, reliable, cheap, non invasive and reproducible method, the interpretation of which is reliable [12]. CRP is a nonspecific marker of systemic diseases and inflammation and can contribute toward the management of these diseases. CRP values can be interpreted at the bedside, in full knowledge of all other clinical and pathological results. We need to do further studies with large sample to assess regarding CRP levels and PMD.

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