Highly Sensitive C reactive Protein (hsCRP) as marker in Oral & Oropharyngeal malignancy

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Introduction. Cancer is emerging as a major public health problem in India. It is estimated that the number of new cancer cases are likely to go up from ~979,786 cases in the year 2010 to ~1,148,757 cases by the year 2020. Majority of the cancer cases are of head and neck type. Approximately 1,75,791 cases of head and neck cancers occur every year in India. Out of the head and neck cancers the Oral and Oropharyngeal cancers are among the most prevalent. Oral and pharyngeal cancer is the sixth most common malignancy reported worldwide and one with high mortality ratios among all malignancies. The total number of new cases worldwide is estimated at 405,318 about 2/3 of these cases arising in developing countries [14].

The role of inflammation in cancer is not well known. Some organs of the body show greater risk of cancer when they are chronically inflamed [7]. Some studies have shown that when anti-inflammatory drugs are given the risk of developing cancer is reduced [3].

C-reactive protein (a pro-inflammatory cytokine) is a marker of inflammation. Recently highly sensitive C Reactive Protein (hsCRP) has evolved as a predictor of myocardial infarction etc., recent studies have shown that having CRP in the high normal range may also be associated with other diseases such as colon cancer, complications of diabetes, and obesity. The high-sensitivity C-reactive protein (hsCRP) assay is a quantitative analysis test of very low levels of C-reactive protein (CRP) in the blood. During an inflammatory process the levels of CRP are known to rise dramatically [11, 13].

Various tumor has been linked with inflammation since 1863, when Rudolf Virchow discovered leucocytes in neoplastic tissues and made the first connection between inflammation and cancer [6]. Since then, chronic inflammation has been identified as a risk factor for cancer. It has been demonstrated that patients with colon cancer have statistically significant higher serum CRP concentration. These findings concur with previous studies which indicate that anti-inflammatory drugs could lower colon cancer risk [3, 5].

Rationale for the study. C-reactive protein (CRP), being a pro-inflammatory marker is elevated in Inflammation. Therefore, elevated CRP levels in cancer cases indicate that inflammation can be linked with cancer. Certain international studies have indicated that the serum hs CRP values are elevated significantly in different types of cancer cases compared to controls. CRP values are known to be significantly elevated only in certain types of cancer.
cases depending upon the type of cancer and the demographic region. Oral and Oropharyngeal cancers are the most common forms of cancer in India. In this study we evaluate to anlayze if there is any relation between hsCRP in Head & Neck cancer patients compared to age matched controls.

Objectives of the study. To compare the serum hs-CRP levels in subjects with Head & Neck cancer and normal healthy subjects. To assess if there is any correlation between primary tumour size and serum hsCRP levels.

Materials and methods. This prospective cross sectional case control study was conducted as per ICH GCP guideline and Ethics committee approval. Informed consent was obtained prior to the study. The study subjects consisted of age and sex matched, Study Group (Cases): 36 subjects with proved Head & Neck cancer and Control Group (Controls): 36 normal healthy volunteer subjects.

Sample Size selection: In the absence of any literature providing hs CRP levels in the Head & Neck cancer, values of colorectal cancer has been employed for estimating the sample size. Erlinger et al [8], indicated that the mean CRP levels amongst cases of colorectal cancer and matched normal control subjects were around 2.44 and 1.94 respectively. In order to detect statistically significant difference in the mean hsCRP levels between the two groups, it was estimated that at least 36 subjects in each group need to be included.

Inclusion criteria: Age group: 18 to 80 years (Both sexes), In the Study group: newly diagnosed Oral and Oropharyngeal cancer confirmed by biopsy, Evaluated clinically by CT Scan. Leucocytes count within normal range. Exclusion criteria was: Subjects with inflammatory diseases, Subjects with Infections in the recent past (< 3mth), Subjects with tissue injury in the recent past (<6 mth, Subject on Corticosteroids, Statins, NSAIDS, Potassium humate. Subjects were enrolled as per the inclusion and exclusion criteria.

A complete general physical examination was performed. Subjects with Head & Neck cancer, the primary site, the size of the primary tumour & neck nodes was evaluated using clinically & with CT scan. Subjects were staged using UICC TNM staging system. Blood samples were taken for analysis of complete hemogram and hsCRP in all the study subjects (both cases and controls). Collection of blood samples: 4 ml blood was collected from both cases and controls and was allowed to clot. The samples were then centrifuged and serum was separated using a micropipette and stored at -20°C. Lab analysis: hsCRP values were determined using Quantitative Immunoturbidimetric method. The procedure was carried out at a wavelength of 540 nm and at a temperature of 37°C with a Cuvette length of 1 cm on fresh serum samples using a spectrometer. Absorbances A1 and A2 were taken at times t = 0 and t = 4 minutes and compared with the known calibrator values. For readings with absorbance greater than 3.5, tests were conducted after diluting the samples by 1:2 or 1:5 with normal saline (0.9% NaCl Solution). Statistical analysis was carried out on all the quantitative data such as serum hs CRP, age, tumour size, etc.

Statistical analysis: All the quantitative data, such as hsCRP, age, tumour size, etc., were summarized in terms of mean and median. Standard deviation was calculated as a measure of variation. Differences in the mean serum hsCRP between cancer cases and controls were tested through student’s T test/ appropriate non parametric tests. Relationship between the size of the tumour and hsCRP was estimated through correlation analysis. The Correlation coefficient was tested through student’s T test.

Results. Over a period of 6 months 72 subjects were recruited for the study. Number of Cases (Cancer subjects) was 36 and age and sex matched control were 36. All the subjects data were considered evaluable. Mean age of the Study subject was 59.55 yrs (SD + 9.9) and the control group 59.06 yrs (SD + 9.7). Male: Female was 3:1 in study group compared to 2.2:1 in controls.

In the study group 22 (61.11 %) had Oral Cancers and 14 (38.89 %) had Cancer of the Oropharynx. Overall in the Stage was Tumour T2 (2-4 cms) = 8
(22.2 %), T3 (<4 cms) = 8 (22.2 %) & T4 (>4 cms) = 20 (55.6 %). Nodes Size: –
N0 = 14 (38.9 %), N1 (<3 cms) = 5 (13.9 %), N2 (3-6 cms) = 14 (38.9 %) N3
(>6 cms) = 3 (8.3 %) [Fig. 1A & 1B].

The hs CRP (mg/L) of Control group v/s Case group was 3.039 ± 2.617 v/s
19.236 ± 19.003 respectively (P < 0.0001) [Fig. 2]. In the Study subject with
cancer the mean hsCRP levels with tumour size T2 was 19.362 ± 7.0104, T3 was
21.325 ± 23.2744, T4 was 18.350 ± 21.0915 and P value was 0.878. The mean
hs CRP levels of individuals with Node size N0 was 20.3 ± 35.07, N1 was 13.8
± 5.37, N2 was 21.6 ± 25.0 2, N3 was 12 .1 ± 1.7. A trend towards increasing
hsCRP with increasing tumour size and nodal size was seen [Fig. 3A & 3B].

Fig. 1A. (Tumour Size). T2 is Tumour less than 2 cms,
T3 is >2 cms, T4 involving adjacent structure and bone.

Fig. 1B. (Nodal Size): N0 - negative lymph node metastasis,
N1 Lymph node size <3 cms, N2 multiple lymph nodes, N3 is Nodes size >6 cms.
Fig. 2. hs CRP levels in normal subjects compared to subjects with oral & Oropharyngeal cancer.

Fig. 3A. An increasing trend in hsCRP is seen as the Tumour Size increases.

**Discussion.** Erlinger et al [4] indicated that plasma CRP concentrations were higher among all colorectal cases combined than controls (median CRP, 2.44 vs 1.94 mg/L; \( P = 0.01 \)). McSorley et al [9] indicated that C-reactive protein levels were significantly higher in the women who later developed ovarian cancer compared to normal individuals. The risk of developing ovarian cancer among women in the highest third of the distribution of CRP compared with those in the lowest third was 1.72 (95% confidence interval 1.06-2.77), with evidence of an increasing risk with increasing concentration of CRP (\( P\)-trend = 0.02). Otake et al [10] indicated that inflammation is linked to the growth of colorectal adenomas. The multivariate-adjusted odds ratios of large adenomas for the
lowest to highest categories of CRP were 1.00 (referent), 1.81 (95 % confidence interval 1.17-2.80), 1.61 (95 % confidence interval 1.03-2.52), and 2.21 (95 % confidence interval 1.28–3.84), respectively (P-trend = 0.01). Aleksandrova et al [11] indicated that elevated CRP concentrations are related to a higher risk of colon cancer, predominantly among men. Multivariable-adjusted relative risks for CRP concentrations of > or = 3.0 mg/L versus <1.0 mg/L were 1.36 (95 % confidence interval (CI): 1.00, 1.85; P-trend = 0.01) for colon cancer and 1.02 (95 % CI: 0.67, 1.57; P-trend = 0.65) for rectal cancer. Colon cancer risk was significantly increased in men (relative risk = 1.74, 95 % CI: 1.11, 2.73; P-trend = 0.01) but not in women (relative risk = 1.06, 95 % CI: 0.67, 1.68; P-trend = 0.13) [1].

Fig. 3B. An increasing trend in hsCRP is seen as the Nodal Size increases).

Certain other studies have indicated that the serum hs CRP values are not elevated significantly in different types of cancer cases compared to controls. Zhang et al indicated that C-reactive protein levels are not associated with increased risk for colorectal cancer in women, and low grade inflammation was seen in the cases (P-trend = 0.09) [15]. Ito et al indicated that high serum CRP levels did not appear to increase the risk of colorectal cancer. The OR of the highest serum CRP levels was 1.18 (95 % CI: 0.68-2.06) for colorectal cancer and 1.42 (95 % CI: 0.73–2.74) for colon cancer, compared to subjects with lowest serum levels. The OR for incidence of colorectal cancer showed a similar trend, but the difference was not significant [8]. Erlinger et al [4] and Aleksandrova et al [1] have also indicated that serum hs CRP levels were not significantly elevated in rectal cancer cases compared to matched controls. In our study serum hsCRP levels were markedly elevated in the subjects with cancers against age and sex matched healthy subjects 3.039 ± 2.617 v/s 19.236 ± 19.003 respectively (P < 0.0001) [Fig. 3].

Study by Erlinger et al [4] demonstrated that plasma CRP concentrations were higher among colorectal cancers subjects compared to controls (median CRP, 2.44 vs 1.94 mg/L; P = 0.01) [8]. McSorley et al [9] indicated that the
risk of developing ovarian cancer among women in the highest third of the distribution of CRP compared with those in the lowest third was 1.72 (95% confidence interval 1.06-2.77), with evidence of an increasing risk with increasing concentration of CRP (P-trend = 0.02) [9].

From our study we infer that subjects with Oral and Oropharyngeal cancers had higher hsCRP levels than ovarian cancer subjects (literature data). The association of tumour inflammation and higher hsCRP levels is clearly demonstrated.

In another study carried out by Otake et al [10] indicated that the multivariate-adjusted odds ratios of large adenomas for the lowest to highest categories of CRP were 1.00 (referent), 1.81 (95% confidence interval 1.17-2.80), 1.61 (95% confidence interval 1.03-2.52), and 2.21 (95% confidence interval 1.28-3.84), respectively (P-trend = 0.01) [10].

The study carried out by Aleksandrova et al [1] indicated that the Multivariate-adjusted relative risks for CRP concentrations of > or = 3.0 mg/L versus <1.0 mg/L were 1.36 (95% confidence interval (CI): 1.00, 1.85; P-trend = 0.01) for colon cancer and 1.02 (95% CI: 0.67, 1.57; P-trend = 0.65) for rectal cancer. Colon cancer risk was significantly increased in men (relative risk = 1.74, 95% CI: 1.11, 2.73; P-trend = 0.01) but not in women (relative risk = 1.06, 95% CI: 0.67, 1.68; P-trend = 0.13) [1].

hsCRP levels may not be markedly elevated in all types of cancers but they are significantly elevated in Oral and Oropharyngeal cancers. In our study serum hsCRP levels did not increase significantly with increase in tumour or neck node size. Mean serum hsCRP levels in tumour size T2 was 19.362 ± 7.0104, T3 was 21.325 ± 23.2744, T4 was 18.350 ± 21.0915 and P value was 0.878.

Therefore, hsCRP levels in the serum may not relate to locoregional cancers burden. The probable explanation for this is the etiologic association of Human papillomavirus (HPV) infection in many oral and oropharyngeal squamous cell carcinoma [6, 12]. The number of cancer cases is increasing day by day. According to some studies anti-inflammatory drugs decrease the risk of developing cancer [3]. Therefore; further studies need to be done, to evaluate the role of hsCRP as a biomarker for cancer, screening and risk evaluation.

Conclusions. Inflammatory responses play decisive roles at different stages of tumour development, initiation, promotion, malignant conversion, invasion, and metastasis. This study shows serum hsCRP levels were significantly elevated in Head & neck cancer cases compared to age matched normal control subjects. hsCRP Can be used as a surrogate marker of oral & Oropharyngeal cancer. Measuring and charting hsCRP values can prove useful in determining disease progress or the effectiveness of treatments. Further studies are contemplated.

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REFERENCES


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High-sensitivity C-reactive protein (hsCRP) an acute phase inflammatory reactant protein, it has been used to predict the risk of cardiovascular disease in healthy individuals and to monitor treatment responses. Epidemiological evidence also points to link between inflammation and development of cancer, i.e. long-term inflammation and dysplasia. Worldwide ~ 15 % of the cancer incidence is associated with microbial infection. NSAIDs have been used for cancer prevention in familial adenomatous polyposis. The objective of study is to evaluate the association between hs-CRP and head and neck cancer (SCCHN).

Methods. Prospective, cross sectional case-control study was under taken involving 36 SCCHN (cases) and 36 normal volunteers age matched (controls). Cases staged as per UICC TNM. The study subjects 4 ml clotted blood, centrifuged, serum stored at -20 °C. hsCRP (mg/L) determined using Quantitative Immunoturbidimetric method. Statistical analysis was performed using SPSS. Results: Evaluable subjects = 72, Cases (Cancer patients) = 36 & Control = 36. Age group range 18 to 80 yrs. Mean Age Cases 59.5 ± 9.9 v/s Control 59 ± 9.6 yrs. M: F Cases - 25:9 v/s Control 27:9. In Cases Oral Ca - 22 (61.1 %) & Oropharynx - 14 (38.9 %). T4 – 20 (55.6 %), T3 - 8 (22.2 %) & T2 - 8 (22.2 %). N3 - 3 (8.3 %), N2 - 14 (38.9 %), N1 - 5 (13.9 %) & N0 - 14 (38.9 %). The hsCRP of Control group v/s Cancer Case group = 3.03 ± 2.61 v/s19.23 ± 19.003 respectively (P < 0.0001). hsCRP for Oral Cancer - 15.07 + 7.66 & OPX - 25.77 + 28.31. In the case group, the mean hsCRP levels with tumour size T2 was 119.36 ± 7.0, T3 was 21.32 ± 23.27, T4 was 18.3350 ± 21.0915 and with Node size N0 - 20.3 ± 35.07, N1 - 13.8 ± 5.37, N2 - 21.6 ± 25.022, N3 - 122.1 ± 1.7.

Conclusions. Inflammatory responses play decisive roles at different stages of tumor development, initiation, promotion, malignant conversion, invasion, and metastasis. This study shows serum hsCRP levels were significantly elevated in SCCHN cases compared to age matched normal control subjects. hsCRP Can be used as a surrogate marker of SCCHN. Measuring and charting hsCRP values can prove useful in determining disease progress or the effectiveness of treatments, Furthers studies are contemplated.

Key words: hsCRP, CRP, Inflammation, Cancer, Head & Neck cancer, Oral cancer.