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Clinical significance of preoperative serum C-reactive protein in oral squamous cell carcinoma

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Abstract. C-reactive protein (CRP) is an index of systemic inflammation. However, CRP is not usually assessed preoperatively. Hence the study intended to evaluate the preoperative serum CRP levels in oral squamous cell carcinoma (OSCC) patients and to analyse its relationship with the clinicopathologic characteristics. CRP values for 60 OSCCs and 30 healthy controls were evaluated using a CRP assessment kit and spectrophotometer. The Mann–Whitney U test, χ^2 test, receiver operating characteristic (ROC) curve analysis, and logistic regression were applied. The CRP ranged from 0.3 to 86 mg/L in OSCCs. CRP was significantly higher in OSCCs than in controls. A raised CRP was seen in 70% of OSCCs. CRP in OSCCs was associated with clinical nodal status and lymph node metastasis (LNM) ($P < 0.05$). CRP was significantly higher in the metastatic than in the non-metastatic group. The area under the ROC was 0.819. The best cut-off value for predicting LNM was 8.65 mg/L for the CRP with 0.767 sensitivity and 0.767 specificity ($P < 0.05$). The cut-off revealed a significant association with LNM. Raised CRP may predict LNM. The CRP levels regressed significantly in relation to LNM. CRP could offer prognostic information beyond staging and histology. Hence, CRP can be added as an extension to known clinicopathologic parameters to predict the prognosis in OSCCs.

Key words: oral squamous cell carcinoma; C-reactive protein; clinicopathologic parameters; systematic inflammatory response.

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Prognostic stratification in oral squamous cell carcinoma (OSCC) has been conventionally guided by the pathological staging of the tumour. Stage, nodal status, lymphovascular and perineural invasion, and the status of the margin are essential in the assessment of survival in OSCC[1]. In the last decade, it has become increasingly apparent that cancer-associated inflammation is a key determinant of disease progression and survival in cancers[2]. It has become evident that the cancer-associated systemic inflammatory response (SIR) has an indispensable influence on disease-related outcomes for many cancer sites[3]. Recently, there as been increasing substantiation that a SIR is of prognostic value in various cancers[4]. The SIR is

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Table 1. Comparison of CRP between healthy controls and OSCC patients.

Parameter	Statistics	Healthy control group ($n = 30$)	OSCC Group ($n = 60$)	*P*-value
CRP	Mean ± standard deviation	2.68 ± 1.32	14.267 ± 17.63	$P = 0.000$*
	Standard error	.24149	2.27687	
	95% confidence interval for mean	2.18, 3.17	9.71, 18.82	
	Median	2.60	8.65	$P = 0.000$*
	Interquartile range	2.11	9.02	

* Mann–Whitney U test.

deemed to reflect both disease activity as well as the host's innate response towards the tumour[5]. Recently, studies have investigated the most commonly used measures of the SIR and their potential use in stratifying cancer patients[2]. A number of measures of SIR have been used such as C-reactive protein (CRP), albumin, and white blood cell counts, and ratios have been reported to have prognostic value[1,3].

CRP is an acute-phase protein and is synthesized in hepatocytes[6]. CRP levels amplify in infections, immuno-inflammatory diseases, trauma, myocardial infarction, surgery, and many cancers[7]. Preoperative elevation of serum CRP has been reported to be a prognostic indicator in oesophageal, gastric, ovarian, and colorectal carcinomas[6]. Prevalent case–control studies have reported higher levels of CRP in patients with cancer than in controls, but results from prospective studies are conflicting, with some studies suggesting that CRP is not merely a marker of prevalent cancer but is also associated with incident cancer. It is still unclear whether, and to which extent, CRP levels are associated with incident cancer[8]. However, there is increasing evidence that chronic inflammation, of which CRP is a marker, is a causal factor in several malignancies[9].

Two hypotheses have been proposed to explain the relationship between elevated CRP levels and cancer. The first hypothesis states that elevated CRP levels are a result of an underlying cancer or a premalignant state, whereas the second hypothesis states that chronic inflammation and

Table 2. Relationship between CRP levels with clinicopathologic features in OSCC patients.

Parameters	Category	Total *n* ()	OSCC CRP level (mg/L) (mean ± SD)	*P* value	Significance
Age, years	<45	22 (37)	20.97 ± 21.93	0.031	S
	≥45	38 (63)	10.38 ± 13.44		
Gender	Male	51 (85)	14.34 ± 17.98	0.909	NS
	Female	9 (15)	13.84 ± 16.52		
Site	BM, RMT	31 (52)	14.57 ± 16.85	0.300	NS
	Others	29 (48)	13.94 ± 18.73		
Habits	PTC	42 (70)	15.23 ± 20.79	0.239	NS
	CH	18 (30)	12.00 ± 7.37		
Frequency of habits	≤5	34 (57)	15.00 ± 18.32	0.595	NS
	>5	26 (43)	13.30 ± 17.00		
Duration of habits	≤15 years	29 (48)	19.71 ± 22.24	0.007	S
	>15 years	31 (52)	9.17 ± 9.70		
Tumour size	T1 + 2	26 (43)	9.56 ± 7.26	0.235	NS
	T3 + 4	34 (57)	17.86 ± 22.03		
Clinical nodal status	N0	25 (42)	7.69 ± 6.86	0.001	S
	N+	35 (58)	18.96 ± 21.26		
Tumour stage	Early	17 (28)	7.76 ± 4.68	0.074	NS
	Advanced	43 (72)	16.84 ± 20.11		
Lymph node metastasis	N0	30 (50)	7.24 ± 6.24	0.000	S
	N+	30 (50)	21.29 ± 22.17		
Broders grade	Well	29 (48)	10.65 ± 12.76	0.051	NS*
	Moderate	16 (27)	9.55 ± 7.03		
	Poor	15 (25)	26.28 ± 26.92		
IFG (TMS)	4–8	8 (13)	6.20 ± 3.42	0.064	NS*
	9–12	30 (50)	10.09 ± 6.24		
	13–16	22 (37)	22.89 ± 26.24		
Tumour stromal border	Infiltrative	34 (57)	16.83 ± 19.69	0.121	NS*
	Pushing	18 (30)	10.94 ± 15.80		
	Mixed	8 (13)	10.84 ± 10.59		
Stromal response	Desmoplastic	13 (22)	14.37 ± 19.48	0.652	NS*
	Myxoid	15 (25)	11.96 ± 7.55		
	Inflammatory	32 (53)	15.30 ± 20.38		
Perineural invasion	Present	14 (23)	11.31 ± 5.11	0.421	NS
	Absent	46 (77)	15.16 ± 19.91		
Tumour budding	Present	40 (67)	15.09 ± 18.11	0.122	NS
	Absent	20 (33)	12.61 ± 16.96		
Tumour depth	<15 mm	27 (45)	9.99 ± 10.41	0.115	NS
	≥15 mm	33 (55)	17.76 ± 21.38		

PTC, paan tobacco chewers; CH, combination of habits; BM, buccal mucosa; RMT, retromolar trigone; others, includes lip, tongue, and floor of mouth; T1 + 2 < 4 cm; T3 + 4 > 4 cm; OPMDS, oral potentially malignant disorders; N_0, negative; N_+, positive; IFG, invasive front grading; TMS, total malignancy score. Mann–Whitney U Test; *Kruskal–Wallis test.

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elevated CRP might have a causal role in carcinogenesis[10].

There is a paucity of data with regard to the prognostic influence of SIR in OSCC[3]. Hence the aim of the study was to investigate the prognostic significance of CRP as a pretreament marker of the SIR in OSCC patients. CRP is an index of systemic inflammation. However, CRP is not routinely measured preoperatively[4,11]. Hence, the study intended to evaluate the preoperative serum CRP levels in OSCC patients and to analyse its relationship with the clinicopathologic characteristics.

Materials and methods

A total of 90 patients ranging in age from 25 to 75 years were included in this case–control study. Ethical clearance was obtained from the institutional review board (IRB no. 2014/OP/23) of our institution. After obtaining written informed consent from the participating patients, a thorough medical history, clinical details, and habit history regarding type, form, frequency, and duration of tobacco/areca nut/alcohol use were recorded.

Group I consisted of 60 untreated OSCC patients, reporting to the craniofacial unit of the institution for treatment (excision of the lesion along with neck dissection) from 2014 to 2016.

The patients were diagnosed with OSCC based on clinical and histopathological examinations. Staging was done according to the Union for International Cancer Control classification. Along with the complete blood investigations performed routinely prior to surgery, the patient's preoperative serum CRP was also evaluated. At the time of routine preoperative complete blood investigations which are done prior to treatment (surgery), OSCC patients were free from any form tobacco/areca nut preparations and alcohol use.

Clinicopathologic data

Clinical details of the patients were collected during clinical examination. Imaging and gross specimen details were obtained from the investigations and gross examination of the surgically excised specimen. Paraffin sections for the analysis of histopathological features were obtained from the surgical excised tissue specimen.

Group II included 30 age- and sex-matched healthy individuals registered for a routine dental check-up and treatment in our institution. These subjects were without any oral lesions and had no history of tobacco/areca nut products and alcohol use. These subjects were selected randomly from people who had visited the same hospital during the same time period. All of the selected control subjects had a similar socioeconomic background and diet to OSCC patients.

Exclusion criteria

Patients suffering from systemic conditions such as cardiovascular disease, anaemia, liver, kidney and pancreatic diseases, blood dyscrasias, stroke, muscular dystrophy, autoimmune diseases, other inflammatory conditions, concurrent acute inflammatory disease, and other mucosal lesions were excluded. Patients with cancer at other sites, OSCC patients with preoperative chemotherapy or radiotherapy, patients with only local resection without neck dissection, and patients with recurrence were not included.

Method for CRP estimation

Under all aseptic precautions, approximately 5 mL of fasting venous blood was collected from the antecubital vein of study and control groups into a plain sterile bulb. The sample was then allowed to clot at room temperature and then centrifuged at 3,000 rpm for 10 min to separate the serum. Unhemolysed serum samples were collected. CRP levels were measured using a biochemistry analyser (Spectrophotometer EM 360; Erba Diagnostics, Mannheim, Germany) with cell holder and thermo-stable at 37 °C, along with a commercially available CRP assessment kit. CRP-Turbilatex (***SPINREACT***, S.A. Ctra.Santa Coloma, 7 E-17176 SANT ESTEVE DE BAS (GI) SPAIN) is a quantitative turbidimetric test for the measurement of CRP in human serum. Latex particles coated with specific anti-human CRP are agglutinated when mixed with a sample containing CRP. The agglutination causes an absorbance

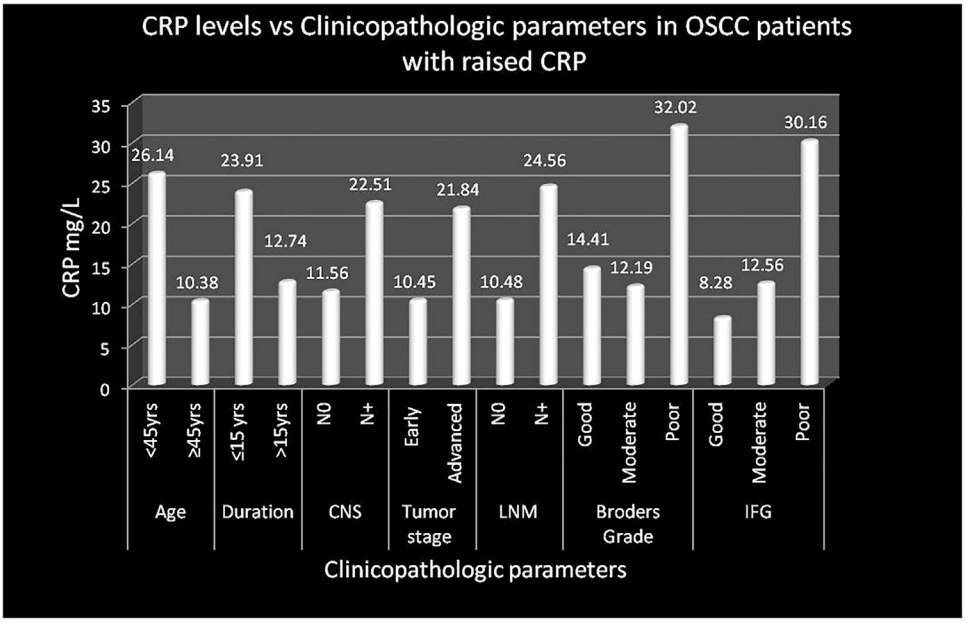

Fig. 1. Difference in the CRP across the categories of various clinicopathologic parameters of OSCC patients with elevated CRP ($n = 42$).

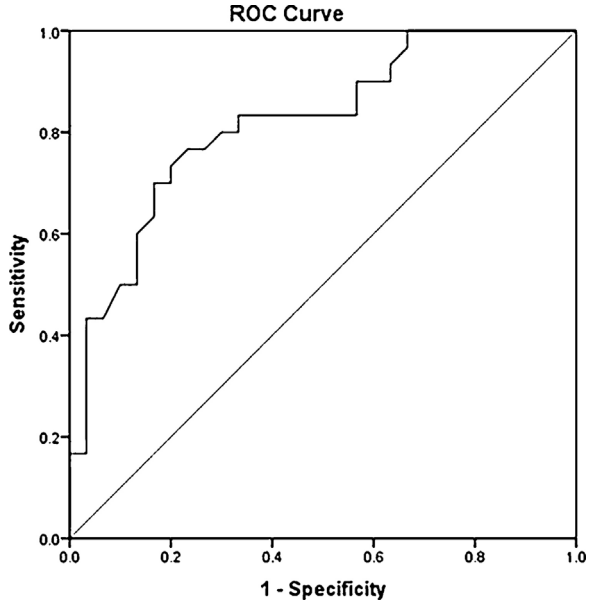

Fig. 2. ROC curve for the CRP used to make the clinical decision regarding LNM. The area under the ROC is 0.819.

change, measured at 540 nm, dependent upon the CRP contents of the patient sample that can be quantified by comparison from a calibrator of the known CRP concentration (reference value: normal value up to 6 mg/L)[6].

The patients were divided into two groups according to the preoperative measure of CRP concentration: those with CRP values in the normal range (≤5.0 mg/L), and those with elevated CRP levels (>5.0 mg/L) according to Khandavilli et al.[5] and Komai et al.[12]. The cut-off point for serum was set at 5.0 mg/L, which is internationally adopted for inflammation[7].

Statistical analysis

The data were analysed using SPSS 11.0 (SPSS Inc., Chicago, IL, USA). The data are presented as mean ± standard deviations, median, and percentages. The Mann–Whitney U test, Kruskal–Wallis test, χ^2 test, receiver operating characteristics (ROC) curves analysis and logistic regression were used. A P value of <0.05 was considered to indicate statistical significance.

Results

Patient characteristics

The age of the OSCC patients ranged from 27 to 72 years with mean age of 47.9 years. The buccal mucosa and retromolar trigone ($N = 31$, 52%) were the most common primary tumour sites. The tumour stage distribution was 3 (5%) in stage I, 14 (23%) in stage II, 16 (27%) in stage III, and 27 (45%) in stage IV. All the patients were followed up fort at least 8–12 months, except for those who died. All patients received radical surgeries, with adjuvant therapies in 43 (72%) patients. Of the 60 patients enrolled in this study, surgical margins were positive in five cases. Of the 60 OSCC cases, 63% of patients were ≥45 years, the majority were male (85%), 70% had a history of chronic paan tobacco chewing, 52% had practised the habits identified above for > 15 years; clinical tumours > 4 cm were noted in 57%, and nearly 72% were at an advanced stage; 50% showed LNM, and nearly 57% showed an infiltrative type of tumour stromal border; 53% revealed an inflammatory type stromal response, and tumour budding was observed in 67%; infiltration ≥15 mm was noted in 55% tumours.

Quantitative estimation of CRP

The preoperative serum CRP levels ranged from 0.3 to 86 mg/L with a mean of 14.26 ± 17.63 mg/mL in OSCC patients. Out of 60 OSCC patients, 42 patients had elevated preoperative CRP levels (>5 mg/L) and 18 had a normal CRP value (≤5 mg/L). A raised CRP was seen in 70% (42/60) of OSCC patients. Mean and median CRP levels were significantly higher in OSCC patients than controls. There was a significant difference between the OSCC group and the control group with regard to CRP (Table 1) The median activity value of CRP in the control group was 2.6 mg/L and 232% higher in the cancer patients.

When compared with all the clinicopathologic parameters, CRP levels in OSCC patients were associated with clinical nodal status and LNM ($P < 0.05$). CRP was significantly higher in OSCC patients with clinical nodal involvement (N_+) than in patients without (N_0) ($P = 0.001$). CRP was significantly higher in the metastatic (N_+) than in the non-metastatic group (N_0) ($P = 0.000$).

CRP was significantly higher in OSCC patients <45 years of age than in OSCC patients ≥45 years ($P = 0.031$). CRP was significantly higher in OSCC patients who had tobacco/areca nut/alcohol habits for ≤15 years than in patients who had habits >15 years ($P = 0.007$) CRP significantly differed in parameters such as age (<45 vs. >45 years), duration of habits (<15 vs. >15 years), clinical nodal status (N_0 vs. N_+), and LNM (N_0 vs. N_+) in the OSCC group studied (60 OSCC patients) (Table 2).

Among the 42 OSCC patients with raised CRP (>5 mg/L) the CRP significantly differed in the parameters such as age (<45 vs. >45 years), duration of habits (<15 vs. >15 years), clinical nodal status (N_0 vs. N_+), tumour stage (early vs. advanced), LNM (N_0 vs. N_+), Broder's grading, and invasive front grading (Fig. 1).

ROC curve analysis was used to evaluate the cut-off, sensitivity, and specificity values. The ROC was used to determine the best CRP value that yielded the optimal predictive values for determining LNM. Figure 2 shows the ROC curve

Table 3. Area under the curve and cut-off values obtained by ROC curve analysis.

Variable	Cut-off value (mg/L)	Sensitivity	Specificity	AUC	Standard error	95% confidence interval for AUC	*P*-value
CRP	8.65	0.767	0.767	0.819	0.54	0.713, 0.925	0.000

AUC, area under the curve; CRP, C-reactive protein.

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Table 4. Patient characteristics according to CRP cut-off values.

Characteristics	Category	CRP $\leq$ 8.65 (n = 30)	CRP >8.65 (n = 30)	P-value
Lymph node metastasis	N_+	7 (23%)	23 (77%)	0.000
	N_0	23 (77%)	7 (23%)	

χ^2 test

for CRP used to make the clinical decision regarding LNM. The area under the ROC was 0.819 (95% CI for AUC 0.713–0.925; standard error 0.54). The best cut-off value for predicting LNM was 8.65 mg/L for CRP with 0.767 sensitivity and specificity ($P < 0.05$) (Table 3). The CRP cut-off shows a significant association with the LNM. The group having CRP > 8.65 had a higher number of cases with LNM (N_+) than in the group with CRP $\leq$ 8.65 (77% vs. 23%) ($P = 0.000$) (Table 4). Based on these findings, it appears that preoperative CRP can be considered as a parameter to predict LNM in OSCC patients.

A logistic regression analysis was performed using CRP as the dependent variable against all the other 21 independent variables. Of the 18 regression models of this analysis, CRP regressed with habits, LNM, tumour depth, and stromal response with maximal dependency on the LNM in the final model (Table 5). The analysis suggests an overall predictability of CRP levels with 80% accuracy and a change in CRP of up to 44.5% in the final model with the aforementioned variables.

Discussion

CRP is an extensively useful systemic biomarker for diagnosing acute and chronic inflammation. During the past decade, the role of CRP has been re-emphasized by widening its clinical use for the prediction or diagnosis of cardiovascular diseases[13] and other conditions, particularly malignancies. Serum CRP has also been found to be elevated in patients with many malignancies, implying a close connection between inflammation and malignancy. CRP is an acute-phase reactant in the inflammatory process that is up-regulated by pro-inflammatory cytokines such as IL-6, IL-8, and TNF. An abundance of pro-inflammatory cytokines in a tumour microenvironment can lead to a level of inflammation that potentiates angiogenesis, thus favouring neoplastic growth[14].

The role of CRP in cancers is more controversial. First, the exact mechanism by which CRP is involved in tumorigenesis remains unclear. Two theories exist to explain elevated CRP levels in human cancers. 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. 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[15,16].

The second theory has some support because the tumour microenvironment and pro-inflammatory cytokines lead to inflammation and angiogenesis, which then up-regulates the acute-phase reactant CRP. IL-6 indirectly helps CRP bind to tumour cells, which may lead to tumour lysis. Thus CRP is not only a response to the tumour microenviroment but may also be a reflection of tumour cell killing and local tissue damage[16]. However, it is still unclear whether CRP levels are elevated before the biological onset of cancer or if an elevated CRP level is also a risk factor for the development of cancer[15].

Tumour growth can cause tissue inflammation. There is evidence that cancer cells can increase the production of inflammatory proteins, which could explain the high CRP concentrations in cancer patients. Some cancerous cells have been shown to express CRP and cancer cell lines have been shown to secrete IL-6 and IL-8, which in turn induce the production of CRP[6,16].

CRP is produced in hepatocytes as a systemic response to cytokines (particularly IL-6) in the blood stream that are released from leucocytes within the tumour microenvironment. IL-6 also indirectly helps CRP bind to tumour cells, which may lead to tumour cell lysis. Thus, CRP is not only a response to the tumour microenvironment but also a reflection of tumour cell killing as part of the host's immune reaction[14]. CRP could be an indicator of an immune response to tumour antigens[6,16].

Local bone destruction by tumours or LNM by tumour foci could induce an inflammatory reaction, which is further

Table 5. Depiction of variables in the regression equation and the best logistic classification obtained.

The logistic regression model							
		B	SE	Wald	df	Sig.	Exp(B)
Step 1	Age	0.092	1.134	0.007	1	0.935	1.097
	Sex	−1.007	1.527	0.435	1	0.510	0.365
	Site	2.213	1.926	1.319	1	0.251	9.141
	Extension	2.799	1.914	2.138	1	0.144	16.422
	Habits	4.503	2.437	3.414	1	0.065	90.314
	Frequency	−1.986	1.650	1.449	1	0.229	0.137
	Duration	−2.471	1.878	1.731	1	0.188	0.085
	Size	−3.864	2.780	1.932	1	0.165	0.021
	Nodes	−2.863	2.562	1.249	1	0.264	0.057
	Stage	3.490	3.067	1.295	1	0.255	32.793
	LNM	2.769	1.884	2.160	1	0.142	15.949
	Tumour_Depth	1.082	1.297	0.696	1	0.404	2.950
	Type_of_lesion	−1.736	2.030	0.731	1	0.392	0.176
	Bone Involvement	−2.020	1.672	1.459	1	0.227	0.133
	Broders_Grade	−1.031	1.194	0.746	1	0.388	0.356
	IFG_TMS	1.690	1.509	1.254	1	0.263	5.417
	Eosinophils	−1.729	1.952	0.784	1	0.376	0.178
	Tumour_stromal_border	−2.379	1.335	3.177	1	0.075	0.093
	Stromal_response	1.244	0.893	1.944	1	0.163	3.471
	PNI	−0.146	1.500	0.010	1	0.922	0.864
	TB	−0.282	1.552	0.033	1	0.856	0.754
	Constant	0.521	6.142	0.007	1	0.932	1.683
Step 18	Habits	1.949	1.015	3.684	1	0.055	7.023
	LNM	2.428	0.877	7.663	1	0.006	11.332
	Tumour_Depth	1.545	0.764	4.088	1	0.043	4.690
	Stromal_response	1.136	0.474	5.737	1	0.017	3.113
	Constant	−5.581	2.071	7.265	1	0.007	0.004

Using the results in step 18, interpretation is as follows:
log(neg/pos) = −6.681 + 1.949*Habits + 2.428*LNM + 1.545*Tumour_Depth + 1.138*Stromal_ response.
Thus log odds ratio heavily depends on LNM then Habits etc.
This equation predicts correctly in 80% of cases. Its R^2 value is 0.445.

Table 6. Head and neck squamous cell carcinoma (HNSCC) and preoperative CRP levels: A brief literature update.

Authors	Sample size (*n*)	Result and conclusion
Gallo et al. (1995)[27]	18 HNSCC	Significance of CRP and IL-6 in regard to tumour stage
Jablonska et al. (1997)[12]	42 OSCC	CRP, IL-1b, IL-6, TNF- serum levels related to clinical stage
Tartour et al. (1997)[25]	85 HNSCC	A high lymph node classification was significantly associated with elevated IL-6 and CRP levels
Khandavilli et al. (2009)[5]	60 OSCC	No association between CRP level, tumour stage and LNM
Kruse et al. (2010)[15]	278 OSCC	No significant correlation between preoperative CRP levels and development of recurrence and metastasis
Tariq et al. (2011)[24]	31 OSCC	Higher values of CRP corresponded with higher TNM staging and poor overall 5-year survival
Chen et al. (2011)[14]	59 OSCC	Preoperative serum CRP levels are associated with advanced tumour stage, bone invasion, LNM, lymph node ECS and patients' survival
Huang et al. (2012)[18]	142 OSCC	Concurrent high levels of both preoperative SCC-Ag and CRP levels act as a predictor for LNM, advanced tumour stage and recurrence
Grimm and Lazariotou (2012)[16]	187 OSCC	Combination of inflammatory CRP, Hb, and WBC count as the most important independent prognostic factor in predicting disease recurrence in OSCC
Fang et al. (2013)[28]	226 OSCC	Elevated CRP is an independent prognostic factor in OSCC
Chang et al. (2013)[29]	151 OSCC	In patients with relapse, IL-6, CRP, and serum amyloid A remained at elevated levels Patients with CRP $\geq$ 2 mg/L at baseline had highest probability of relapse.
Peter (2013)[23]	261 HNSCC	The most frequent laboratory pathologies were elevated CRP value (66%), impaired liver enzymes (30–50%), decrease urea levels (33%), leucocytosis (20%) and anaemia (10%) in HNSCC patients CRP one of the several pretherapeutic laboratory values having prognostic relevance for overall survival in HNSCC patients
Chen et al. (2014)[30]	100 OSCC	Concurrent high levels of both SCC-Ag and CRP at the diagnosis of recurrence acts as a predictor of recurrent tumour status, recurrent advanced tumour stage, distant metastasis, and survival after the diagnosis of recurrence
Hsu et al. (2015)[26]	130 OSCC	CRP level predicts greater extent of tumour destruction including bone invasion, skin invasion, tumour status, and LNM Preoperative CYFRA 21-1 and CRP levels are probable candidates as biomarkers for risk stratification in OSCC
Metgud and Bajaj (2016)[31]	20 OSCC	CRP levels in OSCC patients were elevated and were associated with advanced tumour stages

reflected by elevated serum CRP levels. As tumour stage becomes more advanced, the increased volume usually causes necrosis in the central part of the tumour because of inadequate blood supply. Elevated CRP levels are most likely to be a secondary response to tumour necrosis, local tissue damage and associated inflammation in patients with malignancies[14].

OSCC is the primary lesion and may be ulcerative or ulceroproliferative with microbial colonization as a result of comprised oral hygiene status. A recent clinical cohort study showed the shifts in the abundance of operational taxonomic units (OTUs) in OSCC cancer and pre-cancer patients when compared with healthy subjects[17]. Poor oral hygiene status in most of patients with OSCC would superimpose infection in this situation and synergistically amplify the inflammation process[14]. These mechanisms imply that increased CRP is a response to the neoplastic process and that CRP concentrations could thus provide a marker for identifying people with cancer at an early stage when treatment might be more effective[7,18]. Almost 90% of oral cancers are OSCC, which have shown origin from oral potentially malignant disorders (OPMDs). Studies on OPMDSs with elevated serum CRP levels displayed highly significant differences with different grades of dysplasia and it has been suggested that high preoperative CRP levels are associated with the development of cancer[19].

OSCC presents different clinical aspects which are related to the location of the tumour, evolution time, precancerous lesions, and risk factors. There is evidence that chronic inflammation brought about by persistent chemical, bacterial, or viral agents is a risk factor for cancer[20]. Currently, approximately a quarter of worldwide malignancies have a microbial contribution[17]. Epidemiological studies suggest that there is an association betweendentogingival bacterial plaques and chronic periodontal disease on the one hand, and OSCC on the other hand[20].

The pathogenesis of cancers is in general conceptualized to be very complex, while the accumulated evidence collectively emphasizes the occurrence of highly complex intermicrobial interactions within the oral microbiome that can concurrently modulate and also be swayed by the host and environmental factors. All these interactions in return could lead to a local environment favourable to dysplastic transformation[17]. Indeed, the concept that persistent microbes that cannot be cleared from the host continue to participate in ongoing combat that damages host tissues and promotes malignancy. Cellular pathways activated by chronic inflammation brought about by chronic infections, by immune-mediated diseases, or by dysregulated wound healing at sites of repetitive tissue injury constitute risk factors for initial cell transformation and for cancer progression[20].

The link between cancer and inflammation is through specific transcription factors that once activated have the capacity to enhance expression of genes that are common to both the regulation and the

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production of mediators of inflammation, and also to the regulation of the survival and proliferation of cancer cells[20]. In established cancers, the cancer cells induce development of an exaggerated inflammatory state in the stroma, which in turn promotes cancer growth, invasion, and metastasis. CRP in the inflammatory process is up-regulated by pro-inflammatory cytokines such as TNF- and IL-6 produced by the malignant keratinocytes, and by stromal fibroblasts and macrophages in the established OSCC[20].

In most prevalent studies, CRP levels were found to be highly elevated in patients with cancers compared with healthy or with benign conditions. Elevated CRP was detected in 36% of patients with cancers, which was significantly higher than that in healthy subjects as reported by Wang and Sun[21]. Jablonska et al.[22] observed that OSCC patients had significantly higher CRP levels than controls. The increased CRP was found in 57% of cases.

Khandavilli et al.[5], in an investigation on serum CRP in OSCC observed that preoperative CRP levels ranged from 0.1 to 89.3 mg/L with a mean value of 5.7 mg/L. Eighteen patients (30%) had raised preoperative serum CRP (>5 mg/L)[6]. Kruse et al.[15], in a study on CRP in head and neck cancers patients found that out of 278 patients with a mean CRP of 7.36 mg/L, 193 (69.4%) patients had a preoperative CRP level $\leq$ 5 mg/L; 85 (30.6%) patients had a CRP level >5 mg/L. Chen et al.[14], in an investigation to analyse the relationship between preoperative CRP levels, clinicopathologic factors, and prognosis in OSCC patients observed that the mean CRP level (5.51 mg/L) in OSCCs was significantly higher than in leukoplakia patients (3.39 mg/L); 13 (22%) patients had CRP level >5 g/L. Peter[23], in a study to evaluate the prognostic impact of pretherapeutic laboratory values in a cohort of 261 HNSCC patients, found that the most frequent laboratory pathologies were elevated CRP value (66%), impaired liver enzymes (30–50%), d urea levels (33%), leucocytosis (20%), and anaemia (10%) in HNSCC patients. Tariq et al.[24] noted that preoperative levels of CRP ranged from 4 to 57.2 mg/L with the mean of 36.88 mg/L. Among the 31 OSCC patients, 24 patients (77%) had elevated levels of preoperative CRP.

In the present investigation the preoperative CRP ranged from 0.3 to 86 mg/L with a mean of 14.26 ± 17.63 mg/L in OSCCs. An appropriate control group was analysed to take into account possible causes other than OSSC for serum CRP elevation. Out of 60 OSCC patients, 42 patients had elevated preoperative serum CRP levels while 18 had a normal serum CRP value. Raised CRP was seen in 70% of OSCC patients. This could be attributed to the highly sensitive technique used to assess the CRP in this analysis. It may also be due to the greater number of patients reported in advanced stage disease 72% (43/60) and 77% of tumours >6 mm in thickness, supporting the assumption that larger tumours may secrete higher amounts of IL-6 leading to increased hepatic synthesis of CRP. Decreased physical activity, cessation of habits, weight loss, malnutrition or poor nutritional conditions, reduced liver function due to chronic alcoholism could be some reasons for 18 OSCC patients having serum CRP $\leq$ 5 mg/L.

CRP levels have been used to predict the risk of cancer, detect cancer recurrence, and in prognosis.[13] In the present investigation when compared with all the clinicopathologic parameters, CRP levels in OSCC patients were significantly associated with clinical nodal status and LNM. Similar findings were illustrated by Tartour et al.[25], Chen et al.[14], Huang et al.[18], and Hsu et al.[26]. Table 6 presents a brief literature update on the prognostic significance of preoperative CRP in HNSCC[6,14,15,22–31].

The levels of CRP alter on daily basis, increases with ageing, increased blood pressure, smoking, smokeless tobacco use, and alcohol use[17,32]. Smoking and alcohol abuse can also lead to chronic inflammation in the oral mucosa. Though all OSCC patients enrolled in the study had a history of the habits identified earlier, only 70% of OSCC patients have CRP >5 mg/L.

Cessation of habits after the diagnosis could be a reason for the above observation. With the development of an oral lesion and after the diagnosis of cancer based on the biopsy report most of them quit the habits. In the present investigation there was no significant difference in CRP with respect to type and frequency of habits. However, CRP was significantly higher in OSCC patients who had practised the habit for $\leq$15 years than in patients who had practised >15 years. CRP was significantly higher in OSCC patients <45 years of age than in OSCC patients $\geq$45 years; higher CRP in patients <45 years may point to better immune status, and lower CRP in patients $\geq$45 years could be due to decreased physical activity, malnutrition due to chronic substance abuse, and decreased immunity.

Limitations of the current study were the sample size and that CRP was measured at one point in time. Therefore, intra-individual variations were not considered. As this was a case–control cross-sectional study, obtaining data on survival and recurrence was not possible. Further prospective studies with longer follow-up will be needed to confirm its reliability. CRP is a non-specific marker of inflammation, and additional studies on specific cytokines that regulate the acute-phase response are necessary to elucidate the mechanisms by which inflammation influences the risk of cancer. Prospective studies focusing on checking for CRP in patients with severe dysplasia, carcinoma in situ, and early invasion will give a better perspective about CRP being used as a marker.

To conclude, the results of the study validate that CRP is raised in OSCC compared with the healthy group. Investigation confirms the association between raised CRP and the malignant potential of OSCC. The findings appear to support an association between the elevated preoperative CRP levels and LNM. Raised CRP may predict LNM. The result of regression analysis suggests an overall predictability of CRP levels with 80% accuracy, and the CRP concentration variation seems to be significantly dependent on LNM. CRP might provide prognostic information beyond that provided by cancer type, stage, and histology. Hence, CRP can be added as an extension to known clinicopathologic parameters to predict prognosis in OSCC patients. Combining CRP and clinical nodal status enhances the predictive power of CRP and may provide a useful, simple and reproducible clinical tool. These findings need authentication by a prospective study with a large sample size.

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Competing interests

None.

Ethical approval

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Patient consent

Not required.

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