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Prognostic Roles of SCC Antigen, CRP and CYFRA 21-1 in Oral Cavity Squamous Cell Carcinoma

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Abstract. *Background/Aim: Tumor-related and inflammation-related markers were reported to be prognostic in cancer patients. In this study, we evaluated squamous cell carcinoma antigen (SCC-Ag), cytokeratin 19 fragment (CYFRA 21-1) and C-reactive protein (CRP) simultaneously in oral cavity squamous cell carcinoma (OSCC) patients. Patients and Methods: Two hundred and forty-six newly diagnosed OSCC patients were retrospectively recruited between December 2010 and December 2016. Results: The elevation of CRP levels (≥5.0 mg/l) and SCC-Ag levels (≥2.0 ng/ml) were significantly related with tumor invasion parameters and metastatic factors. In contrast, the elevation of CYFRA 21-1 levels (≥3.3 ng/ml) was related with extranodal extension alone. For patients with all three markers being elevated before surgery, their overall survival and disease-free survival were significantly worse than others. Conclusion: Concurrent elevation of preoperative SCC-Ag, CYFRA 21-1 and CRP serum levels can be correlated with worse survival rates in OSCC.*

Oral cavity squamous cell carcinoma (OSCC) is the most common cancer of the oral cavity and the fifth most common cancer in Taiwan (1). The development of OSCC has a wide geographical variety due to the consumption of tobacco, alcohol and betel nut (2). Prognosis of OSCC patients depends on the stage of the disease and response to therapy. Multimodality treatment for OSCC includes radical excision with or without adjuvant chemoradiotherapy. However, despite recent advances in treatments for OSCC, survival of patients has not been significantly improved (3). The clinical staging does not always properly reflect the tumor behavior and disease outcome (4). For this reason, numerous studies evaluating clinical and histological parameters have been performed to demonstrate why some less advanced cancers present with early recurrence, while other, more advanced-stage cancers have a longer disease-free interval (DFS) (5-7). These motivated the search for significant biomarkers that can be used before surgery to provide estimates regarding the likelihood of regional metastasis, postoperative adjuvant therapy response and prognosis in OSCC patients (8, 9).

Clinically, detection of tumor markers in the blood is easy and non-invasive. The blood tumor markers can be classified into two categories: one includes the tumor-related markers

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that are secreted or released from the tumor; the second includes the immune reaction-related markers. The tumor-related markers in OSCCs ranged from squamous cell carcinoma antigen (SCC-Ag), cytokeratins and to the recently identified molecular markers. Immune reaction-related markers include C-reactive protein (CRP), tumor necrosis factor, interleukins, and others. We have previously investigated the tumor related markers SCC-Ag and CYFRA 21-1, and the inflammation related marker, CRP, separately. We found that pre-operative SCC-Ag serum levels are a biomarker associated with advanced tumor stage, lymph node metastasis, an increased rate of distant metastasis, and poor survival in OSCC patients (8, 10, 11). Elevated serum levels of CRP have been related with poor survival and tumor invasiveness in OSCC patients (9, 12). The measurement of cytokeratin 19 fragment (CYFRA 21-1) in head and neck squamous cell carcinoma (HNSCC) patients has also been established as a tumor marker and prognosticator (13-16). We have shown that elevated serum levels of CYFRA 21-1 predicted nodal metastases in OSCC patients (17).

In the literature, there are few articles investigating SCC-Ag, CYFRA 21-1 and CRP simultaneously in a specific cohort. The aim of this study was to evaluate the significance of these three markers and their relationship with clinicopathological variables, to predict prognosis in OSCC. The impact of these results on the treatment of OSCC was also investigated.

Patients and Methods

Patients. Two-hundred and forty-six OSCC patients between December 2010 and December 2016 were recruited at the Chang Gung Memorial Hospital, Linkou, Taiwan, ROC. Pathology other than squamous cell carcinoma, such as verrucous carcinoma or salivary gland tumor was excluded from our study. Patients with distant metastasis were also excluded from our study. All patients received thorough pre-operative imaging including a head and neck CT/MRI, abdominal sonography, whole-body scan or positron emission tomography (PET). The tumor stage used in this study was based on the 7th edition of the American Joint Committee on Cancer (AJCC)– TNM staging system for clinical staging (4). All the stages described in this study were pathological tumor stages.

Treatment of OSCC. After preoperative tumor survey, 246 patients undergone radical tumor excision and clinical stage–dependent neck dissection. Excision of tumor was performed with 1 cm safety margin. After surgery, the completeness of removal was evaluated by assessing frozen sections of the surgical margins. All margins were found negative. Neck dissection was done at the time of radical surgery. The extent of neck dissection depended on the clinical nodal staging. For nodal negative patients, ipsilateral supraomohyoid neck dissection (level I to III) was performed. For nodal positive patients, ipsilateral level I to V neck dissection was done. If the tumor invaded deeply and crossed the mid-line, bilateral neck dissection was performed. Adjuvant chemo/radiotherapies were given to patients with invasive tumor factors according to the National Comprehensive Cancer Network guidelines (18) such as peri-neural invasion, tumor depth greater than 10 mm, surgical margin less than 4 mm, bone marrow invasion, lymph node ENE, or poor tumor differentiation (17, 19). A cisplatin-based regimen was administered in most of the patients.

Table I. *Characteristics of 246 patients with OSCC.*

Characteristics	N (%)
Age	
Mean	53
Range	29-85
Gender	
Male	225 (91.5%)
Female	21 (8.5%)
Subsite	
Tongue	106 (43.1%)
Mouth of floor	9 (3.7%)
Lip	9 (3.7%)
Buccal mucosa	83 (33.7%)
Alveolar ridge	26 (10.6%)
Hard palate	1 (0.4%)
Retromolar trigone	12 (4.9%)
Pathologic t status	
T1	72 (29.3%)
T2	82 (33.3%)
T3	22 (8.9%)
T4a	57 (23.2%)
T4b	13 (5.3%)
Pathologic n status	
N0	155 (63%)
N1	38 (15.4%)
N2a	0
N2b	44 (17.9%)
N2c	9 (3.7%)
N3	0
Pathologic stage	
I	63 (25.6%)
II	51 (20.7%)
III	35 (14.2%)
IV	97 (39.4 %)
Treatment	
Surgery	161 (65.4%)
Surgery + RT	17 (6.9%)
Surgery + CCRT	68 (27.7%)

RT: Radiation therapy; CCRT: concurrent chemo-radiation therapy.

Measurement of SCC-Ag, CRP and CYFRA 21-1 levels. The serum levels of SCC-Ag CYFRA 21-1 and CRP were measured at the time of diagnosis, before any surgical intervention or medications. The methods of detection for the three serum markers followed the protocol described in the literature (8, 9, 17). The cut-off levels for SCC-Ag, CYFRA 21-1, and CRP were 2.0 ng/ml, 3.3 ng/ml and 5.0 mg/l, respectively.

Ethics. The study was approved by the institutional review board of Chang Gung Medical Foundation, Taiwan (IRB No.: 201701236B0, date of approval: Aug 24, 2017). All patients signed an informed consent for participation in this study.

Table II. *Associations between preoperative Biomarkers and clinicopathologic parameters (n=246).*

Characteristics	SCC Ag (n, %)			CRP (n, %)			CYFRA 21-1 (n, %)		
	Negative	Positive	*p*-Value	Negative	Positive	*p*-Value	Negative	Positive	*p*-Value
Pathologic t status									
Early (T1-T2) (n=154)	136 (88.3)	18 (11.7)	0.000	134 (87)	20 (13)	0.000	129 (83.8)	25 (16.2)	0.651
Advanced (T3-T4) (n=92)	51 (55.4)	41 (44.6)		51 (55.4)	41 (44.6)		75 (81.5)	17 (18.5)	
Pathologic nodal status									
N0 (n=155)	128 (82.6)	27 (17.4)	0.002	127 (81.9)	28 (18.1)	0.006	132 (85.2)	23 (14.8)	0.040
N1 (n=38)	28 (73.7)	10 (26.3)		25 (65.8)	13 (34.2)		34 (89.5)	4 (10.5)	
N2 (n=53)	31 (58.5)	22 (41.5)		33 (62.3)	20 (37.7)		38 (71.7)	15 (28.3)	
N3 (n=0)									
Nodal status									
(–) MET (–) ENE (N=155)	128 (82.6)	27 (17.4)	0.004	127 (81.9)	28 (18.1)	0.003	132 (85.2)	23 (14.8)	0.120
(+) MET (–) ENE (N=43)	30 (69.8)	13 (30.2)		30 (69.8)	13 (30.2)		37 (86)	6 (14)	
(+) MET (+) ENE (N=48)	29 (60.4)	19 (39.6)		28 (58.3)	20 (41.7)		35 (72.9)	13 (27.1)	
Differentiation[a]									
Well (n=66)	53 (80.3)	13 (19.7)	0.453	47 (71.2)	19 (28.8)	0.176	60 (90.9)	6 (9.1)	0.150
Moderate (n=150)	109 (72.7)	41 (27.3)		119 (79.3)	31 (20.7)		118 (78.7)	32 (21.3)	
Poor (n=29)	24 (82.8)	5 (17.2)		18 (62.1)	11(37.9)		25 (86.2)	4 (13.8)	
Tumor stage									
Early (I-II) (n=114)	102 (89.5)	12 (10.5)	0.000	101 (88.6)	13 (11.4)	0.000	94 (82.5)	20 (17.5)	0.855
Advanced (III-IV) (n=132)	85 (64.4)	47 (35.6)		84 (63.6)	48 (36.4)		110 (83.3)	22 (16.7)	
Perineural invasion[a]									
Yes (n=91)	63 (69.2)	28 (30.8)	0.145	57 (62.6)	34 (37.4)	0.002	73 (80.2)	18 (19.8)	0.632
No (n=154)	123 (79.9)	31 (20.1)		127 (82.5)	27 (17.5)		130 (84.4)	24 (15.6)	
Skin invasion[a]									
Yes (n=20)	9 (45)	11 (55)	0.003	9 (45)	11 (55)	0.004	14 (70)	6 (30)	0.253
No (n=225)	177 (78.7)	48 (21.3)		175 (77.8)	50 (22.2)		189 (84)	36 (16)	
Bone invasion[a]									
Yes (n=47)	22 (46.8)	25 (53.2)	0.000	26 (55.3)	21 (44.7)	0.002	38 (80.9)	9 (19.1)	0.830
No (n=198)	164 (82.8)	34 (17.2)		158 (79.8)	40 (20.2)		165 (83.3)	33 (16.7)	
Tumor depth ≥10 mm									
Yes (n=125)	77 (61.6)	48 (38.4)	0.000	76 (60.8)	49 (39.2)	0.000	102 (81.6)	23 (18.4)	0.574
No (n=121)	110 (90.9)	11 (9.1)		109 (90.1)	12 (9.9)		102 (84.3)	19 (15.7)	

[a]In 1 case, differentiation could not be determined. SCC-Ag: Squamous cell carcinoma antigen; CRP: C-reactive protein; CYFRA 21-1: cytokeratin 19 fragment; MET: lymph node metastasis; ENE: extranodal extension.

Statistical analysis. The relationships between serum markers and clinicopathologic parameters were analyzed by x^2 test. The univariate survival difference was calculated by log-rank test. The hazard ratio of each parameter was analyzed by Cox's proportional hazard model after multivariate adjustment. A two-sided *p*-value of <0.05 was considered statistically significant. The statistics were analyzed by the SPSS software, version 19.0 (SPSS, Chicago, IL, USA).

Results

The clinicopathological parameters are shown in Table I. A total of 246 OSCC patients were enrolled in this study. The mean age of the patients was 53 years old. Most of the patients were male (91.5%) and tongue (43.1%) was the most frequent tumor site followed by buccal mucosae (33.7%). The tumor stage was evenly distributed from stage I to stage IV. All patients were regularly followed up after surgery, in the clinic. All of them were followed up for more than 6 months and the median duration of follow-up was 24 months (range=6-72 months). In this study, the relationship between three serum markers and clinicopathologic factors was analyzed (Table II). SCC-Ag and CRP associated similarly with tumor invasiveness. They were both related to pathologic T status, pathologic N status, positive nodal metastasis with ENE, tumor stage, skin invasion, bone invasion and tumor depth ≥10 mm (Table II). The increase in CRP levels was additionally related to perineural invasion (p=0.002). The third marker, CYFRA 21-1, was only related to the pathologic nodal status (p=0.004). It is the blood marker that is the least related to clinicopathological parameters. The variability of the three markers in every patient was also analyzed. The extent of variation in these serum markers was higher in CRP serum levels [standard deviation (S.D.)=11.68] and smaller in CYFRA 21-1 (S.D.=1.540) (Figure 1).

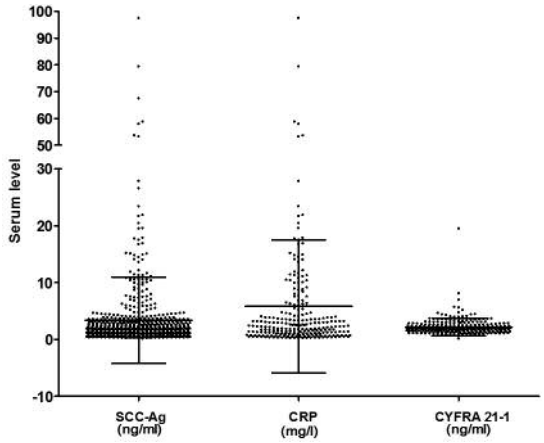

Figure 1. *The scatter plot of SCC-Ag, CRP and CYFRA 21-1 in all OSCC patients. (The lines in each group stand for mean±standard deviation).*

The relationships between the three markers were analyzed: SCC-Ag and CRP (R=0.287, $p<0.001$), CYFRA 21-1 and CRP (R=0.131, $p=0.040$); SCC-Ag and CYFRA 21-1 (R=0.062, $p<0.001$). The highest correlation was found between SCC-Ag and CRP. The combined results from Table I, suggest that CRP is the more sensitive marker to tumor reaction and SCC-Ag is the marker for tumor invasiveness.

We further examined the correlation of the number of positivity in the 3 markers and the clinicopathological features of the patients (Table III). The number of positivity in three serum markers (SCC Ag ≥2.0 ng/ml, CRP ≥5.0 mg/l, CYFRA 21-1 ≥3.3 ng/ml) was statistically related with pathologic T status ($p<0.001$), pathologic nodal status ($p=0.001$), positive nodal metastasis with ENE ($p=0.002$), tumor stage ($p<0.001$), skin invasion ($p<0.001$), bone invasion ($p<0.001$) and tumor depth ≥10 mm ($p<0.001$) (Table III).

The prognostic relationship for these three serum markers were analyzed by log-rank test. SCC-Ag and CRP were significantly related to DFS [$p=0.049$, hazard ratio (HR) 1.671, 95 % confidence interval (CI)=1.003-2.782; $p=0.039$, HR=1.714, 95%CI=1.029-2.854, respectively]. CYFRA 21-1 was not related to DFS ($p=0.172$, HR=1.493, 95%CI=0.840-2.652). For overall survival (OS), CRP was also significantly related to poor prognosis ($p=0.057$, HR=1.879, 95%CI=0.981-3.599). SCC-Ag and CYFRA 21-1 were not related to OS ($p=0.104$, HR=1.715, 95%CI=0.896-3.285; $p=0.157$, HR=1.677, 95%CI=0.820-3.432). While we combined the three markers, patients positive for all 3 markers had the worse prognosis in terms of DFS and OS ($p<0.001$, HR: 6.215, 95%CI=2.717-14.216; $p<0.001$, HR=6.372, 95%CI=2.354-17.247) (Figure 2).

In multivariate analysis, nodal status had significant influence on survival outcomes. The presence of positive lymph nodes with ENE significantly influenced DFS ($p=0.001$, HR=3.046, 95%CI=1.581-5.870) and OS ($p=0.000$, HR=6.304, 95%CI=2.725-14.580) (Table IV).

Discussion

Different pathologic variables have been evaluated in the different editions of the AJCC staging manual on cancer to improve prognostic stratification and treatment strategies (20). However, survival outcomes for patients with OSCC just improved 5% in the last two decades despite the different and aggressive treatment modalities (1). For this reason, serum tumor biomarkers arise as prognostic factors to complement the stage systems on cancer and improve prognosis of survival outcomes. Therefore, these biomarkers are very important for the early detection of OSCC, and for predicting recurrence and response to treatment (21).

SCC-Ag, a tumor associated protein, was isolated four decades ago from SCC tissues of the cervix (22). SCC-Ag is a serine proteinase inhibitor that protects the tumor cells by blocking proteinase-mediated damage. It also promotes cancer cell survival by inhibiting apoptosis (23). In the effect of SCC-Ag on tumor invasiveness and metastasis could be mediated by epidermal growth factor, matrix metalloprotease-9, NF-kB protein complex and interlukine-6 (IL-6) signaling (23-25). Different studies have shown that preoperative elevated serum levels of SCC-Ag in OSCC patients were correlated with advanced tumor stage, distant metastasis, and poor survival outcomes (8, 26, 27). This study also demonstrated the correlation between elevated SCC-Ag serum levels with pathologic tumor status, tumor stage and the presence of lymph node metastasis with ENE ($p<0.001$, $p<0.001$, and $p=0.004$, respectively) (Table II).

Rudolf Virchow in 1863, postulated the hypothesis that cancer originates in sites exposed to chronic inflammation (28). An argument to prove this hypothesis is derived from the reduction in the risk of colorectal cancer in patients with long-term use of nonsteroidal anti-inflammatory drugs (29). CRP is an acute-phase reactant and an inflammation marker whose synthesis is regulated by cytokines (30). The elevated CRP serum levels are the results of the tumor-host interaction in the tumor microenvironment. The host immune response releases different mediators that induce chronic inflammation, cell proliferation and activation of different biochemical pathways to induce irreversible DNA damage (28, 31). The elevation of CRP levels was nonspecific. It was not only related to the response to the tumor microenvironment reactions, but also to the response to local tissue damage (9, 12, 32, 33). We demonstrated in our previous study that elevated CRP serum levels are associated with advanced tumor status, bone invasion, skin invasion and have a statistically significant relationship with worse survival outcomes (9). In this study, we expanded the study

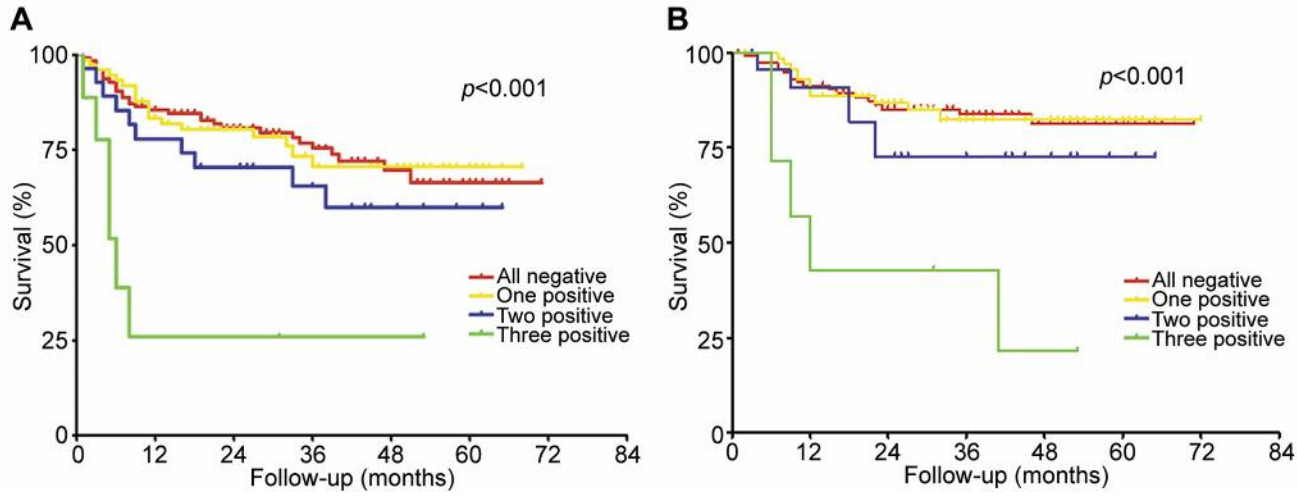

Figure 2. *The survival curves according to the number of positivity of SCC-Ag, CRP and CYFRA 21-1. (A) Disease-free survival (log-rank test: p<0.001), (B) overall survival (log-rank test: p<0.001).*

Table III. *Associations between preoperative biomarkers and clinicopathologic parameters (n=246).*

Characteristics	Serum markers (n, %)				
	Three negatives	One positive	Two positives	Three positives	*p*-Value
Pathologic t status					
Early (T1-T2) (n=154)	102 (66.2)	42 (27.3)	9 (5.8)	1 (0.6)	<0.001
Advanced (T3-T4) (n=92)	30 (32.6)	34 (37)	19 (20.7)	9 (9.8)	
Pathologic nodal status					
N0 (n=155)	95 (61.3)	44 (28.4)	14 (9.0)	2 (1.3)	0.001
N1 (n=38)	18 (47.4)	14 (36.8)	5 (13.2)	1 (2.6)	
N2 (n=53)	19 (35.8)	18 (34)	9 (17)	7 (13.2)	
N3 (n=0)					
Nodal status					
(–) MET (–) ENE (N=155)	95 (61.3)	44 (28.4)	14 (9.0)	2 (1.3)	0.002
(+) MET (–) ENE (N=43)	20 (46.5)	16 (37.2)	5 (11.6)	2 (4.7)	
(+) MET (+) ENE (N=48)	17 (35.4)	16 (33.3)	9 (18.8)	6 (12.5)	
Differentiation[a]					
Well (n=66)	38 (57.6)	20 (30.3)	6 (9.1)	2 (3.0)	0.949
Moderate (n=150)	78 (52)	46 (30.7)	20 (13.3)	6 (4.0)	
Poor (n=29)	15 (51.7)	10 (34.5)	2 (6.9)	2 (6.9)	
Tumor stage					
Early (I-II) (n=114)	75 (65.8)	33 (28.9)	6 (5.3)	0 (0)	<0.001
Advanced (III-IV) (n=132)	57 (43.2)	43 (32.6)	22 (16.7)	10 (7.6)	
Perineural invasion[a]					
Yes (n=91)	41 (45.1)	28 (30.8)	14 (15.4)	8 (8.8)	0.049
No (n=154)	90 (58.4)	48 (31.2)	14 (9.1)	2 (1.3)	
Skin invasion[a]					
Yes (n=20)	6 (30)	4 (20)	6 (30)	4 (20)	<0.001
No (n=225)	125 (55.6)	72 (32)	22 (9.8)	6 (2.7)	
Bone invasion[a]					
Yes (n=47)	14 (29.8)	14 (29.8)	16 (34)	3 (6.4)	<0.001
No (n=198)	117 (59.1)	62 (31.3)	12 (6.1)	7 (3.5)	
Tumor depth ≥10 mm					
Yes (n=125)	48 (38.4)	44(35.2)	23 (18.4)	10 (8)	<0.001
No (n=121)	84 (69.4)	32 (26.4)	5 (4.1)	0 (0)	

[a]In 1 case, differentiation could not be determined. SCC-Ag: Squamous cell carcinoma antigen; CRP: C-reactive protein; CYFRA 21-1: cytokeratin 19 fragment; MET: lymph node metastasis; ENE: extranodal extension.

Table IV. *Multivariate cox regression model of prognostic covariates in 246 patients with OSCC regarding DFS and OS.*

Characteristics	DFS		OS	
	p-Value	HR (95%CI)	*p*-Value	HR (95%CI)
Pathologic t status				
Early (T1-T2)	0.146	1	0.795	1
Advanced (T3-T4)		1.558 (0.858-2.830)		1.107 (0.513-2.389)
Nodal status				
(–) MET (–) ENE	0.004	1	0.000	1
(+) MET (–) ENE	0.052	1.957 (0.995-3.850)	0.224	1.871 (0.682-5.131)
(+) MET (+) ENE	0.001	3.046 (1.581-5.870)	0.000	6.304 (2.725-14.580)
Differentiation[a]				
Well/moderate	0.583	1	0.959	1
Poor		1.212 (0.609-2.412)		0.978 (0.428-2.239)
Tumor depth				
<10 mm	0.964	1	0.555	1
≥10 mm		1.015 (0.530-1.945)		1.296 (0.548-3.068)
Markers*				
Three negatives	0.068	1	0.137	1
One positive	0.247	0.696 (0.377-1.285)	0.347	0.682 (0.307-1.514)
Two positives	0.905	0.954 (0.440-2.069)	0.788	0.871 (0.320-2.376)
Three positives	0.068	2.414 (0.936-6.225)	0.113	2.540 (0.802-8.042)

[a]In 1 case, differentiation could not be determined. SCC-Ag: Squamous cell carcinoma antigen; CRP: C-reactive protein; CYFRA 21-1: cytokeratin 19 fragment. MET: lymph node metastasis; ENE: extranodal extension. *Combining SCC-Ag, CRP and CYFRA21-1 serum markers. The cut-off of SCC-Ag was 2.0 ng/ml, 5.0 mg/ml for CRP and 3.0 ng/ml for CYFRA21-1.

population and demonstrated the correlation between elevated CRP serum levels and pathologic tumor status ($p<0.001$), tumor stage ($p<0.001$) and lymph node metastasis with ENE ($p=0.003$) (Table II).

CYFRA 21-1 was first described three decades ago as a serum soluble fragment of cytokeratin-19 from epithelial cells (15). Serum CYFRA 21-1is considered as a highly sensitive and specific tumor biomarker in non-small cell lung cancer, breast carcinoma and bladder cancer (34-36). In patients with HNSCC, elevated serum levels of CYFRA 21-1 were associated with advanced tumor stage and positive nodal status, demonstrating its correlation with tumor progression (13-15, 37, 38). In our previous study, elevated serum levels of CYFRA 21-1 were associated with positive lymph node metastasis demonstrating that CYFRA 21-1 is released into the bloodstream by metastatic OSCC cells (17). In this study, CYFRA21-1 was related to lymph node metastasis alone ($p=0.040$) (Table II).

Several studies have examined the relation between two or more different serum markers with OSCC which evaluated also their relevance as biomarkers for risk stratification and prognosis (14, 17, 19, 27). In our previous study, we have shown that concurrent elevated serum levels of SCC-Ag and CRP exhibited significant potential as predictive biomarkers for advanced tumor stage, lymph node metastasis, and tumor recurrence in OSCC patients (19). However, when we evaluated the value of combining elevated serum levels of CYFRA 21-1 and CRP, they predicted a higher risk of recurrence and distant metastasis (17). This is the first study that evaluated the relationships between serum levels of SCC-Ag, CRP and CYFRA 21-1 simultaneously as prognostic markers in OSCC. Preoperative elevated levels of these three serum markers were statistically significantly related with pathologic tumor status ($p<0.001$), tumor stage ($p<0.001$), nodal status ($p=0.001$), positive nodal metastasis with ENE ($p=0.002$), skin invasion ($p<0.001$), bone invasion ($p<0.001$) and tumor depth ≥10 mm ($p<0.001$) (Table III). When we compare the prognostic role of these three markers separately, CYFRA 21-1 was the least sensitive serum marker. It was only associated with lymph node metastasis. In addition, the range of CYFRA 21-1 was narrowest (0.20-19.51 ng/ml) compared with that of SCC-Ag (0.20-67.50 ng/ml) and CRP (0.27-87.47 mg/l) (Figure 1). This indicates that the expression or secretion of CYFRA 21-1 is less evident in OSCC than that in non-small cell lung cancer or esophageal cancer (39, 40).

In this study, the relationship between the three serum markers was investigated. The strongest association was found between SCC-Ag and CRP. Tumor-related serum marker, SCC-Ag, reflects a high tumor burden such as large tumor volume and lymph node metastasis. The induced host reaction subsequently elevates CRP levels. From our previous study, in the induced inflammatory

response, neutrophils increase compared to lymphocytes (41). These cascades change the tumor microenvironment and influence the patients' survival. The recruitment of tumor infiltrating leukocytes also provides clues to the recent advances in immunotherapy in head and neck cancers (42, 43).

According to the current results, the combination of the elevated levels of these three serum markers was significantly related to a worse 5-year DFS and OS in the univariate analysis ($p<0.001$ and $p<0.001$, respectively). The prognostic value of preoperative elevated serum levels of SCC-Ag, CRP and CYFRA 21-1 was confirmed in multivariate analysis, being significantly correlated with the presence of positive neck lymph nodes with ENE in OSCC patients with worse survival rates (DFS, $p=0.001$, HR=3.046, 95%CI=1.581-5.870, and OS, $p<0.001$, HR=6.304, 95%CI=2.725-14.580) (Table IV).

Tumor status, depth of tumor invasion, nodal status and ENE are important pathological risk factors used to guide the necessity of postoperative adjuvant therapy in OSCC patients after surgical treatment (6, 7, 20, 44-49). We suggest that preoperative elevated serum levels of SCC-Ag, CRP and CYFRA 21-1 would predict an unfavorable histopathological risk factor as ENE, which relates to an aggressive tumor behavior and poor survival outcomes. Although this study was limited by its small case number and the inherent bias associated with retrospective studies, it provides important clinical information so as to provide a more comprehensive and effective postoperative treatment planning.

Conclusion

OSCC patients with elevated preoperative SCC-Ag, CRP and CYFRA 21-1 serum levels had worse survival rates. Preoperative, concurrently elevated serum levels of these biomarkers were associated with positive lymph nodes and ENE. They can be used as tools to modify the treatment modality and elaborate an accurate adjuvant treatment improving the survival outcomes.

Conflicts of Interest

The Authors declare no competing financial interests.

Authors' Contributions

DD and SFH conceived the idea for the manuscript, conducted a literature search, and drafted the manuscript. CKY and SFH organized the manuscript and critically revised the manuscript. DD, CKY, HTC, CKT, CCF, KHF, CTL, HMW, CJK and JTCC collected the data. DD, CKY, HTC, and SFH analyzed the data. DD, CKY, HTC, CKT, CCF, KHF, CTL, HMW, CJK, JTCC and SFH read and approved the final manuscript.

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