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Relationships between squamous cell carcinoma antigen and cytokeratin 19 fragment values and renal function in oral cancer patients

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Abstract

Squamous cell carcinoma antigen (SCC-Ag) and cytokeratin 19 fragment (CYFRA) are used for screening and monitoring of oral cancer patients. Recent studies have reported that tumour markers elevate as renal function decreases, regardless of tumour progression. A retrospective study was performed of 423 oral cancer patients who underwent blood testing for these tumour markers and other blood analytes during a 10-year period. The values of SCC-Ag and CYFRA increased significantly with decreasing renal function ($P < 0.01$), and the values were abnormal at a median 2.6 ng/ml for SCC-Ag and 4.7 ng/ml for CYFRA in the group with estimated glomerular filtration rate (eGFR) values of less than 30 ml/min/1.73m^2. The factors that were related to the variation in tumour markers were albumin and creatinine. The cut-off values of eGFR were 59.7 ml/min/1.73m^2 for SCC-Ag and 63.6 ml/min/1.73m^2 for CYFRA, and the cut-off age when the tumour markers might rise due to the effect of renal function were 72 years for SCC-Ag and 73 years for CYFRA. In conclusion, decreased renal function should be taken into account when evaluating tumour markers in oral cancer. In addition, tumour markers are likely to be overestimated in patients over the age of 72–73 years.

Keywords: oral cancer, tumour marker, squamous cell carcinoma antigen, CYFRA cytokeratin fragment, renal function

Introduction

Oral cancer is among the malignant tumours of the head and neck region. These oral malignant tumours occur in the tongue, upper or lower gingiva, buccal mucosa, hard palate, or floor of the mouth. Much of the oral cavity is covered with mucosa

consisting of squamous epithelium, and squamous cell carcinoma (SCC) arising from squamous epithelium accounts for more than 90% of oral cancer cases[1]. Oral cancer accounts for 2.0% of all malignancies, and the number of patients with oral cancer has been increasing in recent years with the aging of society; the number of new cases worldwide annually is expected to reach 1.08 million by 2030[2,3].

The diagnosis of oral cancer involves a medical interview, visual inspection, palpation, imaging tests, and biopsy. In addition, the measurement of tumour markers such as squamous cell carcinoma antigen (SCC-Ag) and cytokeratin 19 fragment (CYFRA) are used to support the diagnosis, determine the treatment efficacy, and evaluate the prognosis of oral cancer patients[4,5].

Tumour markers are molecules produced by tumours. The first tumour marker was discovered in 1848[6]. A large number of molecules such as hormones, metabolites, enzymes, immunoglobulins, tumour-associated antigens, and oncogenes have since been identified as tumour markers[7]. The levels of tumour markers in healthy people are low or zero, while tumour marker levels increase as tumours develop and grow[8].

SCC-Ag and CYFRA values have been reported to be useful in many studies. The levels of these markers are significantly higher in stage 3 and 4 advanced cancer than in stage 1 and 2 early-stage cancer[4,5,9]. Moreover, the values decrease significantly with the treatment of oral cancer[5]. Furthermore, the value of SCC-Ag has been shown to correlate with tumour invasion and lymph node metastasis factors, and the value of CYFRA to correlate with the presence of extranodal invasion[10]. Levels of these markers have also been correlated with overall survival (OS) and disease-free survival (DFS)[9,11].

However, recent studies have reported that some tumour markers are elevated when renal function is decreased and are not associated with tumour growth or

progression[8,12]. Elderly people often have decreased renal function due to aging. Since oral cancer is more common in the elderly, it is possible that some tumour markers are affected by renal function, but no report of a study on this possibility was found. The present study was conducted to investigate whether there is any relationship between renal function and tumour markers in patients with oral cancer.

Materials and methods

Study setting and patients

This single-centre, retrospective, observational study was undertaken using the electronic medical records of patients who attended the Department of Oral and Maxillofacial Surgery, Okayama University Hospital (Okayama, Japan) during the 10-year period from April 1, 2011 to March 31, 2021. The study protocol was approved by the Okayama University Ethics Committee (K2203-004).

Patients with oral cancer who had their serum level of the tumour markers SCC-Ag and CYFRA measured were included in this study. Measurement data were extracted from the electronic medical records. The normal reference values for these two tumour markers are SCC-Ag <1.5 ng/ml and CYFRA <3.5 ng/ml.

The results of blood chemistry tests performed at the same time as the tumour marker measurements were also extracted from the electronic medical records. Measurements of the following blood analytes were recorded: albumin (Alb), alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN), calcium, chloride, creatinine (Cr), estimated glomerular filtration rate (eGFR), haemoglobin (Hb), haematocrit (Ht), potassium (K), leucine aminopeptidase (LAP),

sodium, platelet count (PLT), red blood cell count (RBC), gamma-glutamyltransferase (GGT), total protein (TP), uric acid (UA), and white blood cell count (WBC). All blood samples had been obtained by a nurse in the central blood collection room and then analysed in the Division of Clinical Laboratory, Okayama University Hospital. In addition, age and gender were also extracted from the electronic medical records.

Classification of renal function

The study patients were classified into three groups according to the clinical practice guidelines for chronic kidney disease (CKD), using their eGFR values: a control group (eGFR ≥60), a moderate CKD group (eGFR ≥30, <60), and a severe CKD group (eGFR <30). The eGFR was calculated using the Modification of Diet in Renal Disease (MDRD) formula for Japanese people: eGFR (ml/min/1.73m^2) = $194 \times \mathrm{Cr} - 1.094 \times \mathrm{age} - 0.287$ (×0.739 for women).

Data analysis

Continuous variables are presented as the median with interquartile range (IQR). Categorical variables are presented as numbers and percentages. Fisher's exact probability test was used to compare categorical variables. The Mann–Whitney *U*-test was used to compare continuous variables between two groups. The Kruskal–Wallis test was used to compare continuous variables between three groups. A regression analysis was performed, with the log-transformation of the SCC-Ag and CYFRA values.

A multiple regression analysis was used to identify blood analyte values related to renal dysfunction, with eGFR as the dependent variable and the analytes that differed significantly among the three groups (control, moderate, and severe) as independent variables. The patients were divided into two groups according to whether the SCC-Ag or CYFRA values were greater than the reference value or not, and the cut-off values of eGFR against the SCC-Ag and CYFRA values were calculated using the receiver operating characteristic (ROC) curve. The patients were also divided into two groups according to whether eGFR values were greater than the cut-off values of eGFR against the SCC-Ag and CYFRA values, and the age at which the tumour marker levels might rise due to the effect of renal function was calculated.

Probability values <0.05 were considered significant. The package R ver. 4.0.5 (R Core Team, Vienna, Austria) and Excel (Microsoft, Redmond, WA, USA) were used for the statistical analyses.

Results

Clinical characteristics of the control, moderate, and severe groups

A total of 423 patients with oral cancer met the study inclusion criteria. The median age of the patients was 71 years (IQR 61–80 years); 214 were female and 209 were male. The median blood analyte values were within the normal range set by the hospital. Each median blood analyte values for the total patient population were within the normal range set by the hospital. According to their eGFR, 311 patients were assigned to the control group, 104 to the moderate group, and eight to the severe group. The median patient age was significantly higher in the moderate and severe

groups compared to the control group (both $P < 0.01$). The sex distribution also varied across the groups, with increased proportions of female patients in the moderate and severe groups. The median blood analyte values for the control and moderate groups were within the normal range, and for the severe group were higher than the reference range for BUN and creatinine and lower for Hb. There were also significant differences in TP, Alb, ALT, LAP, UA, BUN, Cr, eGFR, K, RBC, Hb, and Ht among the three groups (Table 1).

[Table 1 here]

Factors related to renal function

A logistic regression analysis was conducted with the normal, moderate, and severe groups set as dependent variables and using the blood analyte values that differed significantly between the groups as independent variables, to investigate which values were related to renal dysfunction. The following were identified as significant factors for renal dysfunction: Alb (odds ratio (OR) 1.045, 95% confidence interval (CI) 1.012–1.080, $P = 0.006$), BUN (OR 0.396, 95% CI 0.304–0.516, $P < 0.001$), Cr (OR 0.814, 95% CI 0.787–0.843, $P < 0.001$), K (OR 0.019, 95% CI 0.009–0.366, $P = 0.011$), and RBC (OR 1.351, 95% CI 1.076–1.697, $P = 0.016$).

Comparison of tumour marker values

In the control group, the median values of the two tumour markers did not exceed the standard values: SCC-Ag = 0.9 ng/ml (reference value <1.5 ng/ml) and CYFRA =

1.7 ng/ml (reference value <3.5 ng/ml). However, the analyses revealed that as the patients' renal function decreased, the values of SCC-Ag and CYFRA increased significantly ($P < 0.01$), and the median values in the severe group were significantly higher than the reference values: SCC-Ag = 2.6 ng/ml and CYFRA = 4.7 ng/ml (Table 2).

[Table 2 here]

The percentages of patients who were positive for the tumour markers (marker level above the reference value) were 15.4% for SCC-Ag and 10.3% for CYFRA in the control group, while the corresponding positive rates were significantly increased to 37.5% and 27.9% in the moderate group and 100.0% and 75.0% in the severe group (Fig. 1).

[Figure 1 here]

Association between tumour marker levels and renal dysfunction

The results of the analyses demonstrated that certain factors were related to the variation in tumour markers. For SCC-Ag these were Alb (OR 0.98, 95% CI 0.97–0.99, $P < 0.01$) and Cr (OR 1.03, 95% CI 1.02–1.05, $P < 0.01$). For CYFRA they were Alb (OR 0.99, 95% CI 0.98–0.99, $P < 0.01$), Cr (OR 1.02, 95% CI 1.00–1.03, $P < 0.01$), and RBC (OR 0.55, 95% CI 0.29–1.05, $P = 0.07$). It is known that Alb decreases and Cr increases with decreasing renal function. Since no other factors were

calculated to be significant, it was concluded that there is a close association between each tumour marker in a patient with oral cancer and renal function.

The cut-off values of eGFR and age

The cut-off values for eGFR related to renal dysfunction were 59.7 for SCC-Ag and 63.6 for CYFRA. The cut-off values for age when the tumour markers might rise due to the effect of renal function were 73 years for SCC-Ag and 72 years for CYFRA (Fig. 2).

[Figure 2 here]

Discussion

The levels of some biomarkers are closely related to the proliferation and differentiation of malignant tumour cells and are useful for cancer screening, diagnostic support, treatment assistance, and prognostic evaluation[13,14]. Tumour markers are biomarkers found in the blood or urine and are widely used in clinical practice. SCC-Ag is a protein that was identified by Kato and Torigoe in 1977 in the serum of patients with SCC of the cervix, and it is now used as a tumour marker for various SCCs such as lung cancer, oesophageal cancer, and cervical cancer[15–18]. CYFRA is a fragment of cytokeratin 19 that was identified by Wu and Rheinwald in 1981 in cells collected and cultured from SCC of the oral cavity[19]. CYFRA has been used as a tumour marker for SCC occurring in the oesophagus, lung, and bladder[17,20,21]. In gynaecology, CYFRA has been reported to be useful not only for

screening for malignant diseases, but also for benign diseases such as endometriosis and uterine fibroids[22,23].

The clinical correlation between SCC-Ag and oral cancer has been demonstrated in many clinical studies. According to the results of studies on the association between SCC-Ag and the TNM classification, an elevated SCC-Ag is correlated with clinical stage, tumour size, lymph node metastasis, tumour depth, and tumour invasiveness, with tumour size T3–4, positive lymph node metastasis N1–N3b, and clinical stage III–IV being particularly strong positive factors[9,24]. In addition, a study on the relationship between SCC-Ag and C-reactive protein (CRP) revealed that SCC-Ag >2.0 ng/ml and CRP >5.0 mg/l significantly shortened DFS and OS, which are strongly correlated with the prognosis[25].

A further study also investigating CYFRA demonstrated the following: (1) when SCC-Ag and CRP were both elevated before surgery, the DFS and OS were significantly shorter; (2) SCC-Ag and CRP were strongly associated with tumour invasiveness and metastasis; and (3) CYFRA was strongly associated with extranodal extension[10]. A significant correlation was observed between CYFRA and DFS and OS by Liu et al.[11], who also described high CYFRA levels as a poor prognostic factor. In a study by Yang and Chen[4], five serum biomarkers including SCC-Ag and CYFRA were compared. In the comparison of the five serum biomarkers, the specificity was higher for CYFRA than SCC-Ag, sensitivity was higher for SCC-Ag than CYFRA, and the accuracy of SCC-Ag was equal to that of CYFRA. As noted by Barak et al.[5], surgical therapy, chemotherapy, and radiation therapy for oral cancer significantly reduced the values of each tumour marker compared to the pre-treatment values.

Tumour markers are useful for screening and monitoring malignancies, but they are modified by a variety of factors. In particular, they are greatly influenced by

renal function. The reason for this is that when renal function deteriorates, small molecules with a molecular weight <25–30 kDa are not eliminated and remain in the bloodstream[12]. Prostate-specific antigen (PSA), a tumour marker for prostate cancer, exists in serum in the forms of free PSA with a molecular weight of ~28 kDa and complex PSA with a molecular weight of ~90 kDa. Although no relationship between the complex PSA level and eGFR values was found in the study by Bruun et al., the free PSA level increased significantly with decreasing eGFR. It has also been reported that the free PSA level as a percentage of PSA is greatly affected in individuals with CKD and should be evaluated with caution[26].

SCC-Ag and CYFRA, which have low molecular weights (similarly to PSA), are believed to be greatly affected by renal function. Chen et al.[27] compared the serum levels of SCC-Ag and CYFRA in lung cancer patients with diabetic nephropathy and those with normal renal function. They reported that increased urinary albumin excretion was observed with decreased renal function, which was accompanied by higher levels of each of these tumour markers[27]. The present study analyses revealed that SCC-Ag and CYFRA increased significantly with decreasing renal function in oral cancer patients, and the positivity rate increased. The common factors for elevated tumour markers and decreased renal function were albumin and creatinine. The age when SCC-Ag or CYFRA might rise due to the effect on renal function was 72–73 years. In short, patients over 72–73 years of age may have physiologically decreased renal function and thus elevated values of tumour markers, even if they do not have a history of renal disease. It should be kept in mind that tumour markers tend to be elevated not only in patients with decreased renal function and in patients in whom various environmental factors are present, but also in individuals aged >60 years, which coincides with the peak age of oral cancer development[28].

This study has several limitations. The patients were treated at a single centre and the results might not be generalizable to other facilities. The patients' eGFR values were calculated using a formula designed for the standard weight of Japanese people, and the results may vary depending on ethnicity and body size. Finally, only blood chemistry test values were investigated, and environmental factors and the degree of tumour progression were not taken into account.

In conclusion, in the 423 patients with oral cancer included in this study, the levels of tumour markers SCC-Ag and CYFRA increased significantly with decreasing renal function. Tumour markers cannot be used alone in the evaluation of oral cancer, and various tests should be used in combination to reach a multifaceted diagnosis.

Funding

This study was not funded.

Competing interests

None.

Ethical approval

The study protocol for this research project was approved by the Okayama University Ethics Committee (K2203-004) and it conforms to the provisions of the Declaration of Helsinki.

Patient consent

Informed consent was waived.

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Figure captions

Fig. 1. Relationship between the percentages of patients positive for the tumour markers (marker level above the reference value) and renal dysfunction.

Fig. 2. Relationship between eGFR of each tumour marker and age; data shown are the cut-off age value (specificity, sensitivity)

Table 1. Clinical characteristics of the patients overall and by study group, and the results of the univariate analysis; median (IQR values).

	All	Control (eGFR ≥60)	Moderate (eGFR ≥30, <60)	Severe (eGFR <30)	*P*-value
Number of patients	423	311	104	8	
Age, years	71 (61–80)	67 (57–76)	81 (76–87)	83 (79–88)	<0.01**
Sex, *n* (%)					0.04*
Female	214 (50.6%)	146 (46.9%)	63 (60.6%)	5 (62.5%)	
Male	209 (49.4%)	165 (53.1%)	41 (39.4%)	3 (37.5%)	
Total protein (g/dl)	7.2 (6.9–7.5)	7.2 (6.9–7.5)	7.1 (6.8–7.4)	6.7 (6.6–6.9)	<0.01**
Albumin (g/dl)	4.3 (4.0–4.5)	4.3 (4.1–4.5)	4.3 (4.1–5.1)	4.0 (3.4–4.1)	<0.01**
Aspartate aminotransferase (U/l)	21 (18–27)	21 (18–27)	22 (18–26)	18 (12–21)	0.08
Alanine aminotransferase (U/l)	16 (12–23)	17 (13–24)	15 (11–22)	11 (7–15)	<0.01**
Gamma-glutamyl transpeptidase (U/l)	24 (16–40)	26 (16–47)	22 (16–31)	23 (17–29)	0.09
Leucine aminopeptidase (U/l)	52 (46–57)	52 (46–60)	50 (45–55)	48 (42–52)	<0.01**
Uric acid (mg/dl)	5.1 (4.1–6.0)	5.0 (4.0–5.9)	5.5 (4.5–6.4)	5.7 (4.4–6.6)	<0.01**
Blood urea nitrogen (mg/dl)	14.9 (12.1–18.0)	13.9 (11.6–16.5)	18.1 (15.3–21.4)	30.6 (29.1–34.8)	<0.01**
Creatinine (mg/dl)	0.76 (0.64–0.89)	0.70 (0.62–0.82)	0.96 (0.78–1.11)	2.11 (1.88–4.07)	<0.01**
eGFR (ml/min/1.73m^2)	68.2 (59.3–79.7)	74.6 (66.1–82.8)	51.3 (42.6–55.7)	20.2 (11.3–24.6)	<0.01**
Sodium (mmol/l)	140 (139–141)	140 (139–141)	140 (138–141)	139 (136–140)	0.21
Potassium (mmol/l)	4.3 (4.0–4.5)	4.3 (4.0–4.5)	4.3 (4.1–4.5)	4.3 (4.0–4.5)	0.04*
Chloride (mmol/l)	105 (104–107)	105 (104–106)	105 (103–107)	106 (101–110)	0.91

Calcium (mg/dl)	9.3 (9.1–9.5)	9.3 (9.1–9.5)	9.3 (9.0–9.6)	9.0 (8.7–9.2)	0.09
Total cholesterol (mg/dl)	195 (174–223)	198 (176–225)	189 (165–213)	183 (165–223)	0.12
White blood cell count ($\times 10^9$/l)	5.91 (4.94–7.02)	5.84 (5.00–6.96)	6.01 (5.00–7.48)	6.34 (5.01–7.64)	0.60
Red blood cell count ($\times 10^{12}$/l)	4.33 (3.98–4.66)	4.39 (4.08–4.72)	4.13 (3.71–4.50)	3.68 (3.49–3.88)	<0.01**
Haemoglobin (g/dl)	13.7 (12.6–14.8)	13.9 (13.1–15.0)	12.9 (11.5–14.0)	11.3 (10.6–12.2)	<0.01**
Haematocrit (%)	41.2 (38.2–44.3)	42.0 (38.9–44.8)	39.1 (35.3–42.8)	36.1 (33.5–37.8)	<0.01**
Platelet count ($\times 10^6$/l)	221 (187–266)	226 (190–272)	210 (176–254)	226 (200–254)	0.07

eGFR, estimated glomerular filtration rate; IQR, interquartile range.

Table 2. Inter-group comparisons of tumour marker values in the control, moderate, and severe groups; median (IQR values).

Parameter	All	Control (eGFR ≥60)	Moderate (eGFR ≥30, <60)	Severe (eGFR <30)	Control vs Moderate	Moderate vs Severe	Control vs Severe
SCC-Ag (ng/ml)	1.0 (0.7–1.5)	0.9 (0.7–1.3)	1.3 (1.0–1.9)	2.6 (2.1–5.6)	<0.01**	<0.01**	<0.01**
CYFRA (ng/ml)	1.9 (1.4–2.7)	1.7 (1.2–2.3)	2.4 (1.7–3.3)	4.7 (4.4–5.3)	<0.01**	<0.01**	<0.01**
eGFR (ml/min/1.73m^2)	68.2 (59.3–79.7)	74.6 (66.1–82.8)	51.3 (42.6–55.7)	20.2 (11.3–24.6)	<0.01**	<0.01**	<0.01**

CYFRA, cytokeratin 19 fragment; eGFR, estimated glomerular filtration rate; IQR, interquartile range; SCC-Ag, squamous cell carcinoma antigen.