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Analytical and clinical evaluation of CYFRA 21-1 by electrochemiluminescent immunoassay in head and neck squamous cell carcinoma

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Abstract

This paper attempts to evaluate the clinical usefulness of CYFRA 21-1 as a serum tumour marker in patients with head and neck squamous cell carcinoma (HNSCC).

The serum concentration of CYFRA 21-1 was measured utilizing a new electrochemiluminescent immunoassay (ECLIA) in 142 patients with HNSCC before and after treatment, 68 patients with benign tumours of the head and neck, and 50 healthy controls.

Serum levels of CYFRA 21-1 in patients with HNSCC were significantly higher than those of benign tumours and healthy controls ($p<0.001$). The diagnostic sensitivity and specificity of CYFRA 21-1 for HNSCC were 62 per cent and 100 per cent, respectively. The positive rates of CYFRA 21-1 increased with progression of HNSCC, serum CYFRA 21-1 levels were related to the tumour stage expressed by primary tumour (T) and nodal status (N) ($p<0.001$), but not related to patient age, gender, smoking and drinking habit, or histopathological grade ($p>0.05$). Post-treatment levels of CYFRA 21-1 in HNSCC decreased significantly ($p<0.001$). Among 38 patients with clinical or radiological evidence of a recurrence during follow-up, 78.9 per cent (30 of 38) showed an increase in CYFRA 21-1.

The analytical ECLIA performance for serum CYFRA 21-1 provides a new means of clinical assessment for HNSCC. The results of ECLIA suggest that the serum marker CYFRA 21-1 is valuable not only for diagnosis but also for close monitoring of patients with HNSCC.

Key words: Head and Neck Neoplasms; Carcinoma, Squamous Cell; Immunoassay

Introduction

Head and neck squamous cell carcinoma (HNSCC) is the sixth most common malignancy and is a major cause of cancer morbidity and mortality worldwide. The overall survival has not changed in the last several decades, whereas the management of HNSCC has improved.[1] This may partly depend on the fact that these tumours present symptoms late in the disease progression and are at an advanced stage when treatment is initiated. Lately, efforts have been made to find a successful means of surveillance for HNSCC. Tumour markers, which have been accepted as a valuable tool for diagnosis, prognosis, and treatment monitoring in recent years,[2–4] could be an easy and desirable means of achieving this purpose. Numerous tumour markers for HNSCC have been studied, including carcinoembryonic antigen (CEA), total sialic acid, lipid-associated sialic acid, glutathione S-transferase, polyamines, soluble immune complexes, oncogene products, and others.[4–9] However, clinical use of these markers has been restricted because of their low sensitivity for head and neck carcinoma. Therefore, it is important to develop more reliable tumour markers for HNSCC.

CYFRA 21-1, which recognizes soluble cytokeratin (CK) 19 fragments, has been considered as a useful tumour marker for several malignant tumours,[2,3,10] especially the squamous cell type. CYFRA 21-1 has been used for assessing head and neck tumours recently,[4] however, there was controversy about the clinical value of CYFRA 21-1 for HNSCC. In order to determine the clinical role of CYFRA 21-1, we performed an analytical evaluation of the new electrochemiluminescent immunoassay (ECLIA) for CYFRA 21-1 in the Elecsys 2010 immunoassay system. To our knowledge, no previous results have been reported regarding the ECLIA for CYFRA 21-1 in HNSCC. The data we obtained are the first to assess the clinical significance of CYFRA 21-1in patients with HNSCC using ECLIA.

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TABLE I
COMPARISON OF SERUM CYFRA 21-1 LEVELS BETWEEN HEALTHY CONTROLS AND PATIENTS WITH BENIGN TUMOUR OR WITH HNSCC

Status	No.	Serum CYFRA 21-1 (ng/ml)			p value*
		Range	Median	Mean ± SD	
Healthy control	50	0.4– 2.0	0.8	0.90 ± 0.40	–
Benign tumour	68	0.3– 2.8	1.0	1.09 ± 0.56	0.084
HNSCC	142	1.4–19.9	3.7	4.06 ± 1.94	<0.001
Recurrence	38	2.1–18.6	5.4	5.89 ± 3.13	<0.001

SD = Standard deviation; HNSCC = Head and neck squamous cell carcinoma before therapy;
* = Compared with healthy control using the Mann-Whitney U test.

Materials and methods

Patients

A total of 260 subjects were enrolled in this study. The subjects were divided into three groups: Group 1 comprised 50 healthy controls, 28 males and 22 females. The mean age ± the standard deviation (SD) was 41.63 ± 9.55 years. Group 2, included 68 patients with benign tumours of the head and neck (including papilloma, haemangioma, fibroma, and pigmented naevus). They consisted of 39 males and 29 females. The mean age ± SD was 38.86 ± 14.32 years. Group 3 was 142 patients with HNSCC, comprised of 102 males and 40 females. The mean age ± SD was 45.57 ± 10.58 years. The patients of Group 3 were staged according to the 1997 International Union Against Cancer (UICC) staging system. The clinical stage distribution was as follows: I, 27 patients; II, 40 patients; III, 46 patients; IV, 29 patients. In Group 3, 26 tumours, 83 tumours, and 33 tumours were graded as well differentiated, moderately differentiated, and poorly differentiated, respectively. The patients of Group 3 underwent initial systematic treatment (surgery and/or radiotherapy) and had no macroscopic residual tumours. They were followed for at least 24 months. The survival period was defined as the time between the day when the treatment was started and July 9, 2002 for all living patients, or until the day of death.

ECLIA for serum CYFRA 21-1

Two ml of blood was collected by venipuncture in all the groups. The blood of Group 3 was collected before the start of initial treatment and at two weeks after treatment. The blood samples were placed at 4°C for two hours. Serum samples were obtained after centrifugation at 4°C and then stored at −20°C for CYFRA 21-1 analysis. The measurement of CYFRA 21-1 was completed in ECLIA using Elecsys CYFRA 21-1 reagent kit (Roche Diagnostics, Mannheim, Germany). The CYFRA 21-1 concentration of each sample was automatically calculated in a Roche Elecsys 2010 immunoassay analyser. The test principle is solid phase sandwich immunoassay. The procedure was carried out according to the manufacturer's instructions, and the total duration was 18 minutes. The cut-off value for a positive test was determined to be 3.3 ng/ml, which is recommended by the manufacturer and two CYFRA 21-1 research groups.[10,11]

Statistical analysis

Statistical analysis was performed using the SPSS10.0 software package (SPSS, Inc. Chicago, IL). Statistically significant differences were determined using the Mann-Whitney U test, the Student's t-test and the Chi-square test. A survival analysis was performed by the Kaplan-Meier method and examined by the log-rank test. The results were considered to be significant at $p<0.05$.

Results

Serum CYFRA 21-1 level

The mean CYFRA 21-1 concentrations of three groups were 0.90 ± 0.40 ng/ml, 1.09 ± 0.56 ng/ml, 4.06 ± 1.94 ng/ml, respectively. The CYFRA 21-1 level of Group 2 did not differ significantly from that of healthy controls (Table I). The CYFRA 21-1 level of Group 3 is significantly higher than that of Group 1 or Group 2 ($p<0.001$). All of the 50 healthy individuals and the 68 patients with benign tumours of the head and neck were at a level below 3.3 ng/ml for serum CYFRA 21-1 (specificity, 100 per cent). Of the 142 patients with HNSCC, 88 patients (62 per cent) had positive serum CYFRA 21-1 levels. At two weeks after treatment, the marker levels in Group 3 dropped significantly to 2.05 ± 0.88 ng/ml ($p<0.001$). In Group 3, the mean levels of the CYFRA 21-1 positive and negative groups were reduced from 4.88 ± 2.03 ng/ml and 2.72 ± 0.52 ng/ml to 2.45 ± 0.88 ng/ml and 1.38 ± 0.30 ng/ml, respectively ($p<0.001$). Clinical detection of recurrence or metastasis was evident during follow-up in 38 of 142 (26.7 per cent) patients with HNSCC. Thirty of 38 patients (78.9 per cent) showed an elevation in CYFRA 21-1. Moreover, this elevation prior to clinical diagnoses of recurrences was evident in 26 (68.4 per cent) patients. Three patients (with laryngeal carcinoma, hypopharyngeal carcinoma, and maxillary sinus carcinoma) remained CYFRA 21-1 positive after treatment and soon developed local recurrence or distant metastasis (one to six months). The recurrences or metastases were verified by clinical or radiological data (computed tomography, ultrasonography, and X-ray photography).

Comparison of clinicopathologic characteristics between CYFRA 21-1 positive and negative patients before therapy

Table II refers to serum CYFRA 21-1 levels prior to initial treatment, according to the clinicopathologic

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TABLE II
PRETREATMENT SERUM CYFRA 21-1 LEVELS IN PATIENTS WITH HNSCC AND CLINCOPATHOLOGICAL FACTORS OF THE PATIENTS

Characteristic	CYFRA 21-1 positive (n = 88)	CYFRA 21-1 negative (n = 54)	*p* value
Age (yrs)	45.69 ± 11.49	45.38 ± 9.27	0.926*
Gender			
Male	60	42	0.217†
Female	28	12	
Smoking and drinking habits			
Yes	49	26	0.383†
No	39	28	
Primary tumour			
T_1, T_2	26	43	<0.001†
T_3, T_4	62	11	
Lymph node metastasis			
Positive	65	9	<0.001†
Negative	23	45	
Histopathological grade			
Well differentiated	14	12	0.293†
Moderatedly differentiated	50	33	
Poorly differentiated	24	9	
2-year survival rate	79.5%	87.0%	0.327‡

* = Student *t*-test; † = Chi-square test; ‡ = Long-rank test.

factors of HNSCC. The positive rates of patients with T_1–T_2 and T_3–T_4 were 29.5 per cent and 70.5 per cent, respectively. The positive rates of patients with lymph node metastasis and without lymph node metastasis were 73.9 per cent and 26.1 per cent, respectively. A good relationship was shown between CYFRA 21-1 levels and the tumour stage. There were statistical differences between the CYFRA 21-1 positive and negative groups for primary tumour and lymph node metastases ($p<0.001$). However, levels of CYFRA 21-1 were not related to patient age, gender, smoking and drinking habit, or histopathological grade ($p>0.05$). The positive rate of poorly differentiated carcinoma was higher than that of well-differentiated carcinoma, but not to a significant degree ($p = 0.293$). The two-year survival rate for the CYFRA 21-1 positive group was lower than that for CYFRA 21-1 negative group (79.5 per cent *vs*. 87.0 per cent, respectively), but was not significant ($p = 0.327$).

Discussion

Cytokeratins are structural proteins forming the subunits of epithelial intermediary filaments. Twenty different CK polypeptides have so far been identified. Due to their specific distribution patterns, they are eminently suitable for use as differentiation markers in tumour pathology.[12] The CYFRA 21-1 assay is a measurement of soluble CK 19 fragments having a molecular weight of approximate 30 kD. CK 19 protein is expressed in simple epithelia and their malignant counterparts. Malignant epithelial cells could release CYFRA 21-1 into human serum, tissue fluid and urine. Fujita *et al.*[13] have demonstrated that release of CYFRA 21-1 was closely related to the expression of mRNA for CK 19 and the genomic alteration of CK 19 DNA might down-regulate the expression of mRNA for CK 19. Dohomoto *et al.*, demonstrated that caspase 3, which cleaves several intermediate filaments and carries out cell apoptosis, played an important role in producing CYFRA 21-1 in human lung cancer cell lines.[14]

CYFRA 21-1 is a reliable tumour marker in non-small cell lung cancer, bladder cancer, and breast carcinoma. However, there are different ideas about the clinical value of CYFRA 21-1 as a test for head and neck tumours. Lin *et al.*,[15] Nagler *et al.*,[16] and Banal *et al.*[17] have reported the status of CYFRA 21-1 in HNSCC. They found that CYFRA 21-1 was a useful marker for the early diagnosis and monitoring the therapeutic effect and the clinical course of HNSCC. Moreover, they found that the diagnostic sensitivity of CYFRA 21-1 was superior to that of squamous cell carcinoma antigen (SCCA) or tissue polypeptide specific antigen (TPS). On the other hand, Wollenberg *et al.*[18] studied 163 patients with primary and 40 patients with recurrent HNSCC and then concluded that CYFRA 21-1 was not superior to SCCA and CEA in HNSCC. Goumas *et al.*[19] believed that the usefulness of CYFRA 21-1 was limited in HNSCC.

A new ECLIA has been developed for the determination of CYFRA 21-1 in the Elcsys 2010 immunoassay system in bladder cancer.[20] The Elecsys 2010 system is specially attractive as a routine assay because it is sensitive, rapid and reproducible. However, the CYFRA 21-1 ECLIA has not been reported in HNSCC. In order to investigate the clinicopathological significance of serum levels of CYFRA 21-1 in patients with HNSCC, we performed the ECLIA for the *in vitro* quantitative determination of CYFRA 21-1 in 142 serum samples from HNSCC for the first time. The ECLIA kit is comprised of two monoclonal antibodies, KS 19.1 and BM 19.21. There is no cross-reactivity with other CKs. In our series, the specificity is 100 per cent (50 of 50 healthy controls and 68 of 68 patients with benign tumours). Furthermore, we identified that the CYFRA 21-1 levels were unaffected by sex, age, or smoking and drinking habit. In this study, 88 patients (62 per cent) with HNSCC before treatment showed elevated CYFRA 21-1 levels. The diagnostic sensitivity of the test is slightly higher than that reported in previous studies (⩽58.75 per cent).[15] One of the reasons for this difference may be that ECLIA is more sensitive, stable and reproducible than the enzyme-linked immunoabsorbent assays and immunoradiometric assays used in other studies. In addition, a close relationship was observed between serum CYFRA 21-1 levels and tumour stage. It is helpful for clinical diagnosis and the design of therapy because the marker levels may reflect the clinical course of HNSCC. Nagler *et al.*[16] have identified that CYFRA 21-1 was useful in the early diagnosis of oral cancer. There is no statistically significant difference for two-year survival rates between CYFRA 21-1 positive and negative groups, however, the observed times were short.

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Previous studies have demonstrated that CYFRA 21-1 was suitable for monitoring treatment and recurrence.[2,16] In the current study, the post-treatment CYFRA 21-1 levels showed a marked decrease. Successful therapy is documented by a rapid fall in the CYFRA 21-1 level into the normal range. A constant CYFRA 21-1 value or a slight or only slow decrease in the CYFRA 21-1 value indicates incomplete removal of a tumour or the presence of multiple tumours, or a poor prognosis. The patients with hypopharyngeal carcinoma and maxillary sinus carcinoma had a local recurrence in the sixth month after therapy, suggesting that, in the patients who do not seroconvert to negative, small numbers of residual tumour cells very likely remain after treatment. Therefore, a continuous surveillance of CYFRA 21-1 is useful for the monitoring of residual tumour cells and the implication of necessary clinical and radiological examinations. It is hoped to improve the early detection and treatment outcome of HNSCC.

Local and neck recurrences of HNSCC can mostly be detected early when the patient has regular follow-ups. Distant metastases usually remain undiscovered until they produce clinical symptoms. Maass *et al*.[11] have identified that CYFRA 21-1 was a good serum marker for detection of distant metastases in HNSCC. In our study, the patient with laryngeal carcinoma presented lumbar vertebral metastases a month after surgery, but no local recurrence presented. In our series of patients with recurrent disease, CYFRA 21-1 displays good sensitivity (78.9 per cent). Consequently, post-therapeutic elevation of serum CYFRA 21-1 levels indicated a tumour recurrence or metastasis. In the event of an elevation of serum CYFRA 21-1 above the cut-off value or a gradual increase in CYFRA 21-1 levels during follow-up, computer tomography (CT) scans of the neck and chest, abdominal ultrasound and whole body bone imaging are recommended.

Mady[3] found that the sensitivity of CYFRA 21-1 in bladder cancer was inversely correlated with tumour grade, e.g. higher in poorly differentiated carcinoma than in well-differentiated carcinoma. However, no significant correlation between CYFRA 21-1 levels and histopathologic grade was observed in the current series as reported for oral squamous cell carcinoma, oesophageal squamous cell carcinoma and epidermoid carcinoma of the head and neck.[16,21,22]

Conclusion

In summary, the CYFRA 21-1 ECLIA is a reliable analytical test for HNSCC. Serum CYFRA 21-1 may be appropriate for clinical use as a reliable tumour marker for HNSCC. It is of value in the diagnosis, monitoring therapy and follow-up of these patients. Moreover, further studies on the prognostic value of CYFRA 21-1 in patients with HNSCC are continuing.

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References

1 Mork J. Forty years of monitoring head and neck cancer in Norway: no good news. *Anti-Cancer Res* 1998;**18**:3705–8
2 Nakata B, Ogawa Y, Ishikawa T, Ikeda T, Kato Y, Nishino H, *et al*. Serum CYFRA 21-1 is one of the most reliable tumour markers for breast carcinoma. *Cancer* 2000;**89**: 1285–90
3 Mady EA. Cytokeratins as serum markers in Egyptian bladder cancer: a comparison of CYFRA 21-1, TPA and TPS. *Int J Biol Markers* 2001;**16**:130–5
4 Ogawa T, Tsurusako Y, Kimura N, Nishioka S, Akagi H, Nishizaki K, *et al*. Comparison of tumor markers in patients with squamous cell carcinoma of the head and neck. *Acta Otolaryngol Suppl* 1999;**540**:72–6
5 Rao VR, Krishnamoorthy L, Kumaraswamy SV, Ramaswamy G. Circulating levels in serum of total sialic acid, lipid-associated sialic acid, and fucose in precancerous lesion and cancer of the oral cavity. *Cancer Detect Prev* 1998;**22**:237–40
6 Vural E, Alexiou M, Hunt J 2nd, Korourian S, Hanna E. Is glutathione-S-transferase-pi expression a reliable predictor of chemoradiation response in cancer of the head and neck? *Am J Otolaryngol* 2001;**22**:257–60
7 Bhattathiri VN. Relation of erythrocyte and iron indices to oral cancer growth. *Radiother Oncol* 2001;**59**:221–6
8 Makrantonakis P, Pectasides D, Aggouridakis C, Visvikis A, Daniilidis J, Fountzilas G. Squamous cell carcinoma antigen, circulating immune complexes, and immunoglobulins in monitoring squamous cell carcinoma of head and neck: a study of the Hellenic co-operative oncology group (HeCOG). *Am J Clin Oncol* 1999;**22**:542–9
9 Quon H, Liu FF, Cummings BJ. Potential molecular prognostic markers in head and neck squamous cell carcinomas. *Head Neck* 2001;**23**:147–9
10 Karnak D, Ulubay G, Kayacan O, Beder S, Ibis E, Oflaz G. Evaluation of Cyfra 21-1: a potential tumor marker for non-small cell lung carcinomas. *Lung* 2001;**179**:57–65
11 Maass JD, Hoffmann-Fazel A, Goeroegh T, Hoffmann M, Meyer JE, Gottschlich S, *et al*. Cyfra 21-1: a serological help for detection of distant metastases in head and neck cancer. *Anti-Cancer Res* 2000;**20**:2241–3
12 Bodenmueller H. The biochemistry of CYFRA 21-1 and other cytokeratin-tests. *Scand J Clin Lab Invest Suppl* 1995;**221**:60–6
13 Fujita J, Dohmoto K, Hojo S, Ueda Y, Bandoh S, Yang Y, *et al*. The point mutation in the promoter region and the single nucleotide polymorphism in exon 1 of the cytokeratin 19 gene in human lung cancer cell lines. *Lung Cancer* 2001;**34**:387–94
14 Dohmoto K, Hojo S, Fujita J, Yang Y, Ueda Y, Bandoh S, *et al*. The role of caspase 3 in producing cytokeratin 19 fragment (CYFRA 21-1) in human lung cancer cell lines. *Int J Cancer* 2001;**91**:468–73
15 Lin WY, Yen TC, Cheng KY, Wang SJ. The value of CYFRA 21-1, a new tumour marker, in nasopharyngeal carcinoma. *Neoplasma* 1998;**45**:21–4
16 Nagler RM, Barak M, Peled M, Ben-Aryeh H, Filatov M, Laufer D. Early diagnosis and treatment monitoring roles of tumor markers Cyfra 21-1 and TPS in oral squamous cell carcinoma. *Cancer* 1999;**85**:1018–25
17 Banal A, Hacene K, Berthelot-Ruff E, Mahe E, Fontana X, Pichon MF. Comparison of Cyfra 21-1 and SCC assays in head and neck tumours. *Tumour Biol* 2001;**22**:27–35
18 Wollenberg B, Jan V, Schmit UM, Hofmann K, Stieber P, Fathe-Moghadam A. CYFRA 21-1 is not superior to SCC antigen and CEA in head and neck squamous cell cancer. *Anti-Cancer Res* 1996;**16**:3117–24
19 Goumas PD, Mastronikolis NS, Mastorakou AN, Vassilakos PJ, Nikiforidis GC. Evaluation of TAT1 and CYFRA 21-1 in patients with head and neck squamous cell carcinoma. *ORL J Otorhinolaryngol Relat Spec* 1997;**59**: 106–14

20 Sanchez-Carbayo M, Espasa A, Chinchilla V, Herrero E, Megias J, Mira A, *et al.* New electrochemiluminescent immunoassay for the determination of CYFRA 21-1: analytical evaluation and clinical diagnostic performance in urine samples of patients with bladder cancer. *Clin Chem* 1999;**45**:1944–53

21 Kawaguchi H, Ohno S, Miyazaki M, Hashimoto K, Egashira A, Saeki H, *et al.* CYFRA 21-1 determination in patients with esophageal squamous cell carcinoma: clinical utility for detection of recurrences. *Cancer* 2000;**89**: 1413–7

22 Gonzalez Sanchez E, Lopez-Rios Velasco J, Calvo Boizas E, Garcia-Talavera Fernandez JR, Martin Rodriguez M, Munoz Herrera A, *et al.* Study of Cyfra 21-1 as a new tumour marker for the diagnosis of epidermoid carcinoma of the head and neck. *Acta Otorrinolaringol Esp* 1999;**50**: 85–9

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