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Clinical Research Paper

Clinical significance of combined examination of pretreatment serum CYFRA21-1 and SCCAg in cervical cancer patients

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Background and Objective: Cytokeratin 19 fragment antigen 21-1 (CYFRA21-1) can be used for the quantitative examination of fragment of cytokeratin 19, and is a valuable tumor marker in various malignancies. This study was to investigate the significance of pretreatment serum CYFRA21-1 and squamous cell carcinoma antigen (SCCAg) in diagnosis and their correlations to the clinicopathologic features of cervical carcinoma. Methods: One hundred cervical carcinoma patients underwent pretreatment serum CYFRA21-1 and SCCAg evaluation; 20 healthy women were subjected as control. The specificity and sensitivity of CYFRA21-1 and SCCAg as diagnostic indexes were analyzed; their correlations to clinicopathologic features were investigated through univariate and multivariate analyses. Results: The specificity of CYFRA21-1 and SCCAg in diagnosing cervical cancer were both 100%. The sensitivity of CYFRA21-1 and SCCAg in diagnosing cervical cancer were 36.0% and 47.0% respectively, without significant difference. The combined examination of CYFRA21-1 and SCCAg elevated the sensitivity to 60.0%, which was significantly higher than that of examining CYFRA21-1 alone. Univariate analysis showed elevation of CYFRA21-1 was related with FIGO stage and tumor size; elevation of SCCAg was related with pathologic type, tumor size, deep stromal invasion and pelvic node metastasis. Multivariate analysis showed that elevation of CYFRA21-1 had no relationship with any factors, while elevation of SCCAg was related with deep stromal invasion and pelvic node metastasis. The sensitivity of SCCAg in predicting pelvic node metastasis and deep stromal invasion were significantly higher than those of CYFRA21-1 (75.0% vs. 29.2%, p = 0.001; 55.8% vs. 26.9%, p = 0.024), and the addition of CYFRA21-1 to SCCAg could not significantly improve the sensitivity compared with SCCAg alone (79.2% vs. 75.0%, p > 0.05; 63.5% vs. 55.8%, p > 0.05). Conclusion: The value of pretreatment serum CYFRA21-1 as predictor of pelvic node metastasis and deep stromal invasion is less significant compared with that of SCCAg. For cervical squamous cell cancer, SCCAg is the preferred tumor marker.

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Tumor progression is the key factor for determining treatment of cervical cancer and predicting the prognosis. Although the close relation of tumor size, depth of stromal invasion and lymph node metastasis to the prognosis of cervical cancer has been extensively reported, the aforementioned data mainly depend on postoperative pathologic diagnosis, and it is difficulty to accurately evaluate before operation. Investigation of valuable tumor markers would facilitate the estimation of tumor progression before treatment and therefore could be referenced for therapeutic regimens.

Cytokeratin 19 fragment antigen 21-1 (CYFRA21-1), which can be used for quantitative detection of cytokeratin 19 fragment, is a promising tumor marker for squamous lung cancer.[1] Its significance in diagnosis of cervical cancer has also been paid more and more attention in recent years,[2,3] but relevant studies have seldom been reported in China. In this study, 100 patients with initial diagnosis of cervical cancer were tested for pretreatment serum level of CYFRA21-1, which was further compared with pretreatment serum level of squamous cell carcinoma antigen (SCCAg) to investigate the clinical significance of CYFRA21-1 in predicting the progression of cervical cancer.

Materials and Methods

Patients. A total of 100 patients with cervical cancer, who received treatment in the Department of Gynecology, Cancer Center, Sun Yat-sen University between October 2006 and October 2007, were selected according to the following criteria: (1) naïve patients with pathologically confirmed cervical cancer; (2) patients with complete clinical data, and without present or previous concomitant primary malignancies. Twenty healthy women who received simultaneous screening for gynecologic malignancies at our gynecological outpatient clinic were regarded as controls.

Clinical data. The median age of the 100 patients was 45 years (range, 22–66 years). According to the staging criteria of FIGO issued in 1995, 38 patients had stage IB1 tumor, 16 had stage IB2 tumor, 21 had stage IIA tumor, 13 had stage IIB tumor, 10 had stage IIIB tumor, one had stage IVA tumor, and one had stage IVB tumor.

Of the 100 patients, 77 underwent radical hysterectomy and bilateral pelvic lymph node dissection, without any pathologic residual tumor on surgical margin; 23 underwent radiotherapy due to advanced tumor or surgical contraindication. The median age of women in control group was 39 years (range, 33–47 years).

Testing of serum CYFRA21-1 and SCCAg. Informed consent was obtained from each subject. Venous blood sample (3 mL) was obtained from each subject (before treatment), and serum was separated. Serum CYFRA21-1 titer was detected with electrochemiluminescence (ECL) using CYFRA21-1 quantitative detection kit (Roche Diagnostic, Germany). The reference value was 0.1–3.3 mg/L; a serum titer of > 3.3 mg/L was considered as elevation. Serum SCCAg titer in cervical cancer patients was simultaneously detected with microparticle enzyme immunoassay (MEIA) using IMX Automated Immunoassay System (Abbott, US). The normal reference value was ≤ 1.5 mg/L; a serum titer of > 1.5 mg/L was considered as elevation.

Statistical analysis. All statistical analyses were done using the SPSS 10.0 statistics software. Quantitative data were analyzed with Chi-square test. Logistic regression was used for multivariate analysis on factors influencing serum CYFRA21-1 and SCCAg evaluation. $p < 0.05$ was considered significant.

Results

Correlation of serum CYFRA21-1 and SCCAg level to cervical cancer. The serum levels of CYFRA21-1 and SCCAg in control group were within normal range, among which serum CYFRA21-1 level was (1.32 ± 0.7) mg/L (range, 0.55–3.12 mg/L), and serum SCCAg level was (0.48 ± 0.3) mg/L (range, 0.2–0.9 mg/L). The specificity of both markers in diagnosing cervical cancer was 100%.

Before treatment, serum CYFRA21-1 level was elevated in 36 cervical cancer patients, and serum SCCAg level was elevated in 47 patients. The specificity of CYFRA21-1 for the diagnosis of cervical cancer before treatment was 36.0%, and the specificity of SCCAg was 47.0%. As shown in Table 1, 13 patients with normal level of SCCAg had elevated level of CYFRA21-1, while 24 patients with normal level of CYFRA21-1 had elevated level of SCCAg. There was no significant difference between the elevation of these two serum markers ($p > 0.05$). The sensitivity of combined detection of CYFRA21-1 and SCCAg for diagnosing cervical cancer reached up to 60.0%, significantly higher than that of detecting CYFRA21-1 alone ($p = 0.001$), but not significantly higher than that of detecting SCCAg alone ($p = 0.065$).

As shown in Table 2, CYFRA21-1 elevation was significantly more common in patients with disease of stage IIB and above, with a ratio of 64.0% ($p = 0.001$); while SCCAg elevation was not significantly different among patients with diseases of different stages ($p > 0.05$). Comparing patients of the same stage, SCCAg elevation was significantly more common than CYFRA21-1 elevation in IB1 patients ($p = 0.037$).

Correlation of serum CYFRA21-1 and SCCAg levels to clinicopathologic factors of cervical cancer. In the univariate analysis, CYFRA21-1 elevation was significantly related with tumor size ($p = 0.020$); SCCAg elevation was significantly related with pathologic type ($p = 0.008$), tumor size ($p = 0.033$), deep stromal invasion ($p = 0.001$), and pelvic lymph node metastasis ($p < 0.001$) (Table 3).

Logistic regression analysis showed that SCCAg elevation was significantly related with were deep stromal invasion ($p = 0.028$) and pelvic lymph node metastasis ($p = 0.011$); no clinical or pathological factor was related with CYFRA21-1 elevation.

Table 1 **Examination results of serum CYFRA21-1 and SCCAg in 100 cervical cancer patients**

SCCAg	CYFRA21-1		Total
	Negative	Positive	
Negative	40	13	53
Positive	24	23	47
Total	64	36	100

Table 2 **Correlation of serum CYFRA21-1 and SCCAg to FIGO stage of cervical cancer**

FIGO stage	Cases	CYFRA21-1		SCCAg	
		Positive [cases (%)]	Serum level (mg/L)	Positive [cases (%)]	Serum level (mg/L)
ⅠB1	38	6(15.8)	2.4±1.0	14(36.8)	2.1± 3.6
ⅠB2	16	7(43.8)	3.1±1.6	8(50.0)	4.3± 5.5
ⅡA	21	7(33.3)	4.3±6.2	8(38.1)	3.0± 5.7
ⅡB	13	7(53.8)	5.0±4.5	9(69.2)	12.4±17.8
Ⅲ–Ⅳ	12	9(75.0)	10.5±9.2	8(66.7)	12.5±18.5
Total	100	36(36.0)	4.2±5.2	47(47.0)	5.1±10.3

All values of serum level are presented as mean ± SD of relevant groups.

Significance of combined detection of CYFRA21-1 and SCCAg in predicting pelvic lymph node metastasis and deep stromal invasion

As shown in Table 3, among the 24 patients with pelvic lymph node metastasis, 18 had pretreatment SCCAg elevation, and seven had CYFRA21-1 elevation. The sensitivity of SCCAg in predicting pelvic lymph node metastasis was significantly higher than that of CYFRA21-1 (75.0% vs. 29.2%, $p = 0.001$). Among the 52 patients with deep stromal invasion, 29 had pretreatment SCCAg elevation, and 14 had CYFRA21-1 elevation. The sensitivity of SCCAg in predicting deep stromal invasion was also significantly higher than that of CYFRA21-1 (55.8% vs. 26.9%, $p = 0.024$).

As shown in Table 4, one patient with pelvic lymph node metastasis and four patients with deep stromal invasion had normal level of SCCAg but elevated level of CYFRA21-1. The sensitivity of combined detection of CYFRA21-1 and SCCAg was 79.2% in predicting pelvic lymph node metastasis and 63.5% in predicting deep stromal invasion, which were not significantly higher than that of detecting SCCAg alone ($p > 0.05$).

Discussion

Cytokeratin 19, the smallest member of cytokeratin family, belongs to a subfamily of intermediate filament proteins, which is extensively represented on the surface of normal tissues, such as squamous epithelia, acini, sudoriferous glands, trachea, endometria, colon and liver cells, as part of epithelial cytoskeleton. Normally, cytokeratin 19 is never or lowly expressed in peripheral blood, bone marrow or lymph nodes. In epithelial malignancies, activated protein enzymes accelerate the cellular degradation and release excessive soluble cytokeratin 19 fragments, resulting in elevated concentration

Table 3 **Correlations of pretreatment CYFRA21-1 and SCCAg to clinicopathologic factors of cervical cancer**

Factor		Cases	CYFRA21-1 > 3.3 mg/L (cases)	*P*	SCCAg > 1.5 mg/L (cases)	*P*
Age	≤35 years	15	5	0.815	8	0.594
	>35 years	85	31		39	
Pathologic type	Squamous	89	33	0.523	46	0.008
	Non-squamous	11	3		1	
Gross type	Exophytic	58	17	0.077	24	0.186
	Endophytic	42	19		23	
Tumor size	≤ 4 cm	58	15	0.020	22	0.033
	> 4 cm	42	21		25	
Grade	Ⅰ–Ⅱ	67	25	0.697	30	0.525
	Ⅲ	33	11		17	
Deep stromal invaison[a]	No	25	7	0.921	4	0.001
	Yes	52	14		29	
Pelvic node metastasis[a]	No	53	14	0.815	15	<0.001
	Yes	24	7		18	

Table 4 **Correlations of CYFRA21-1 and SCCAg to pelvic node metastasis and deep stromal invasion**

Factor	Pelvic node metastasis	Deep stromal invasion
SCCAg(−)/CYFRA21-1(−)	5	19
SCCAg(−)/CYFRA21-1(+)	1	4
SCCAg(+)/CYFRA21-1(−)	12	19
SCCAg(+)/CYFRA21-1(+)	6	10
Total	24	52

of soluble cytokeratin 19 fragments in tissue and humoral fluid.[3, 4] This is the mechanism that it is considered as a tumor marker.

As previously reported, CYFRA21-1 elevation was seen in 42–63% of cervical squamous cancer patients [3,6-9] and 25–40% of cervical adenocarcinoma or adenosquamous cell cancer patients,[3,7] which varied due to the differences in sample size, proportion of diseases of various stages and pathologic types, detection method and established cut-off value. When setting 1.2 mg/L as the cut-off value, CYFRA21-1 elevation was seen in 59% of stage I-II patients and in all stage III-IV patients.[10] Because serum CYFRA21-1 titer of above 1.2 mg/L is also frequent in healthy population or patients with benign disorders,[11] 3.3 mg/L is set as the upper limit currently. In our study, 3.3 mg/L was also set as cut-off value and our results revealed that the specificity of both CYFRA21-1 and SCCAg in cervical cancer reached up to 100%, while the sensitivity of CYFRA21-1 and SCCAg in diagnosing cervical cancer were 36% and 47%, respectively. When detecting CYFRA21-1 and SCCAg alone for diagnosis, there is no significant difference between their sensitivity. However, our results showed that the sensitivity of combined examination was significantly higher than that of detecting CYFRA21-1 alone, but not higher than that of detecting SCCAg alone; in FIGO stage IB1 patients, SCCAg elevation was significantly more common than CYFRA21-1 elevation. Our results indicate that the addition of SCCAg detection to CYFRA21-1 detection can help to improve the sensitivity of cervical cancer diagnosis, especially for the patients at early stage (IB1).

According to previous reports, serum CYFRA21-1 elevation is related with multiple clinicopathologic factors, such as stage, tumor size, stromal invasion depth, lymphovascular space involvement and lymph node metastasis.[3,6-10] However, these results are mainly from univariate analyses, and there are significant discrepancies among various reports due to the heterogeneity in the samples investigated. Our univariate analysis revealed that CYFRA21-1 was related with stage and tumor size, indicating that it can reflect the tumor burden in patients. However, multivariate analysis revealed that no factor was significantly related with CYFRA21-1 elevation, which may due to inadequate sample size. Our study showed that SCCAg elevation was significantly related with pelvic lymph node metastasis and deep stromal invasion, in consistence with our previous report.[12] In our study, SCCAg elevation was more specifically seen in cervical squamous cell cancer and showed no relationship with FIGO stage, in contrast, CYFRA21-1 elevation didn't vary significantly between cervical squamous cell cancer and non-squamous cell cancer, and was more frequently seen in patients of stage IIB and above, indicating that CYFRA21-1 detection can be applied to provide auxiliary information on monitoring the progression and clinical efficacy, and predicting prognosis of cervical cancer in patients with advanced stage tumor, non-squamous tumor or normal level of SCCAg. In our previous reports, pelvic lymph node metastasis, positive parametrial margin and deep stromal invasion were key prognostic factors for cervical cancer, and patients with these factors varied significantly in 5-year disease-free survival rate and relapse pattern.[13] Therefore, the correlations of pretreatment serum CYFRA21-1 and SCCAg levels to the above factors (except for positive parametrial margin because all surgical margins were negative) were also analyzed in this study. We found that the specificity of SCCAg elevation in predicting pelvic lymph node metastasis and deep stromal invasion were 75.0% and 55.8%, significantly higher than those of CYFRA21-1 elevation (29.2% and 26.9%); the addition of CYFRA21-1 detection to

SCCAg detection wouldn't improve the sensitivity, indicating the superiority of SCCAg over CYFRA21-1 in predicting key prognostic factors, hereby, SCCAg should be used as the preferred tumor marker for cervical cancer. However, this result may be related with the dominated proportion of squamous cell cancer.

Due to insufficient follow-up period, the association of CYFRA21-1 with the prognosis of cervical cancer couldn't be concluded in this study, which will be further studied. In previous reports, univariate analyses showed that CYFRA21-1 was related with tumor-free survival rate and overall survival rate of cervical cancer patients.[6, 7] However, as CYFRA21-1 had no obvious relationship with pelvic lymph node metastasis and deep stromal invasion in our study, we presume CYFRA21-1 elevation is unlikely to be associated with the prognosis.

In summary, CYFRA21-1 elevation is seen in about 40% of cervical cancer patients, and is related with stage and tumor size, but not related with pelvic lymph node metastasis and deep stromal invasion. For the patients with squamous-dominated cervical cancer, SCCAg is a preferred tumor marker. The clinical significance of CYFRA21-1 in non-squamous cancer is yet to be investigated.

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