Correlation between Serum SCCA and CYFRA 21-1, Tissue Ki-67, and Clinicopathological Factors in Patients with Esophageal Squamous Cell Carcinoma

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Background: *Squamous cell carcinoma antigen (SCCA) and CYFRA 21-1 have been reported as useful tumor markers for esophageal squamous cell carcinoma (ESCC), but no information has yet been reported about the relationship between these serum tumor markers and tissue proliferative activity (Ki-67) in ESCC patients.*
Objective: *To study the correlation between SCCA, CYFRA 21-1, Ki-67, and clinicopathological factors in ESCC patients.*
Material and Method: *Pretreatment SCCA and CYFRA 21-1 serum levels were measured, while the expression of Ki-67 was assessed on tumor tissue. The associations between these biomarkers, clinicopathological factors, and overall survival were analyzed.*
Results: *One hundred sixty six patients participated in this study. Elevated SCCA and CYFRA 21-1 were found in 78.9% and 50.0% of the patients, respectively, while 42.8% had both serum markers elevated. The SCCA and CYFRA 21-1 levels were not correlated ($p = 0.128$) to each other, nor to age, sex, T, N, M, location, grade, or Ki-67. High Ki-67 expression levels were significantly correlated with T4 ($p = 0.010$), M1 ($p = 0.010$), and poor grade ($p = 0.015$) but not to age, sex, N, or location. Levels of SCCA, CYFRA 21-1, and Ki-67, alone or in any combination, were not correlated to survival of patients.*
Conclusion: *The authors showed that Ki-67 in tumor tissue is probably a more reliable marker than serum SCCA and CYFRA 21-1 in predicting the clinical course of ESCC.*

Keywords: *SCCA, CYFRA 21-1, Ki-67, Esophagus cancer, Squamous cell*

J Med Assoc Thai 2016; 99 (3): 331-7
Full text. e-Journal: *http://www.jmatonline.com*

Esophageal squamous cell carcinoma (ESCC) is the most common type of esophageal cancer and has poor prognosis. Most of patients present with advanced, inoperable disease, and tumors usually have characters of rapid growth, lymph node metastases, and resistance to treatment. Even in potentially resection, the 5-year survival rate was fewer than 30%[1].

A number of clinicopathological factors such as depth of tumor invasion (T), lymph node metastasis (N), and distant organ metastasis (M) are commonly used to predict survival. However, several investigations of ESCC including the serum and tissue biological markers should be correlated with clinicopathological features to improve the evaluation of patient outcomes.

Some earlier reports have shown useful prognostic results through evaluating squamous cell carcinoma antigen (SCCA) and cytokeratin 19 fragments (CYFRA 21-1) as serum tumor markers for ESCC patients[2-7]. Other studies have examined the use of the Ki-67 nuclear antigen as a tissue marker of cell proliferation in several tumors including ESCC[8-10]. However, there is to date no report examining possible correlations between these serum markers and tissue markers. Therefore, the aim of the present study was to examine the clinical usefulness and any possible association of these biological markers to the clinicopathological factors of locally advanced and advanced ESCC patients.

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Material and Method

Patients

One hundred sixty six patients with primary locally advanced (T3-4, N0, M0 or any T, N1, M0) or advanced (any T, any N, M1) ESCC who underwent treatment at Prince of Songkla University Hospital in southern Thailand between January 2002 and December 2006 were investigated. Patient recruitment

and sample collection were performed with protocols approved by the Institution's Ethics Committee. All specimens were obtained from patients prior to treatment with informed consent. The diagnosis of ESCC was confirmed by endoscopic biopsy of the esophagus and staging was assessed by computed tomography of the thorax and abdomen, according to the American Joint Committee on Cancer TNM classification of the esophagus 6th edition[11]. Most of the patients were scheduled for posttreatment follow-up every one to three months for two years and then every six months until five years. After that, the patients were examined once yearly.

Analysis for SCCA, CYFRA 21-1, and Ki-67

Pretreatment serum specimens were stored at -25°C until assay. Serum SCCA levels were measured by enzyme immunoassay using Abbott reagent set. Serum CYFRA 21-1 levels were measured by electrochemiluminescent assay using Roche Diagnostic reagent set. The cut-off concentration of SCCA was 1.5 ng/mL and CYFRA 21-1 was 3.5 ng/mL, according to the manufacturer's instructions[12,13]. Tissue expression of Ki-67 was assessed on paraffin-embedded tissue blocks by immunohistochemistry and analyzed by pathologists. The percentages of Ki-67-reactive cancer cells were calculated with a light microscope that showed nuclear staining among 1,000 cancer cells in more than three fields of a specimen. It was considered high expression when the nuclear staining was above 10%[14].

Statistical analysis

Overall survival was calculated from the date of histological diagnosis to the date of death or last follow-up. Patients who were lost to follow-up were considered to be censored.

Data were analyzed to verify the associations between biological markers (SCCA, CYFRA 21-1, and Ki-67) and clinicopathological characters. Chi-square test was applied to detect the differences between groups. Differences were considered statistically significant if the *p*-value fell below 0.05. The survival probabilities were calculated using the method of Kaplan and Meier. All statistical analyses were carried out with SPSS software (SPSS, Chicago, IL).

Results

Clinicopathological characteristics

One hundred and sixty six patients (144 males and 22 females) were included in the study, with median age 62 years (39-90), location of tumor upper:middle:lower 31:82:53, and histological grade well:moderate:poor 50:73:43. Seventy-one (43%) patients received surgery, 37 (22%) received chemoradiotherapy, and 58 (35%) received palliative treatment. The median follow-up period was 159 days (range 2-38 months). The median overall survival was 11.0 months. Overall survival curves according to treatments were shown in Fig. 1.

SCCA concentrations

The mean SCCA concentration in the 166 patients was 5.48±2.94 ng/mL (median 2.90 ng/mL, range 0.50-59.80). At the cut-off point of 1.5 ng/mL, 131 of the 166 patients (78.9%) had positive results for SCCA. No significant differences were observed between SCCA concentrations categorized by sex, age, T, N, M staging, location of tumor, or pathological grading (Table 1), or between positive and negative SCCAs and overall survival ($p = 0.76$) shown in Fig. 2.

CYFRA 21-1 concentrations

The mean CYFRA 21-1 concentration in the 166 patients was 8.43±0.43 ng/mL (median 3.53 ng/mL, range 0.52-173.20). At the cut-off point of 3.5 ng/mL, 83 patients (50.0%) had positive results for CYFRA 21-1. There were no significant associations between CYFRA 21-1 levels and patient clinicopathological factors (Table 1). No significant differences were observed between positive and negative CYFRA 21-1 levels and overall survival rates ($p = 0.23$) shown in Fig. 3.

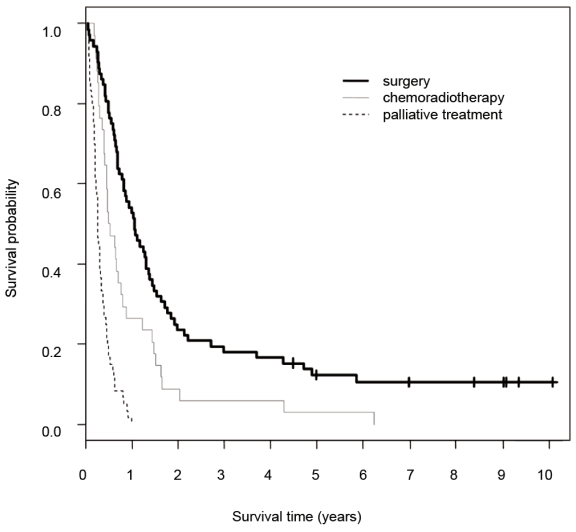

Fig. 1 Overall survival compared on the types of treatment.

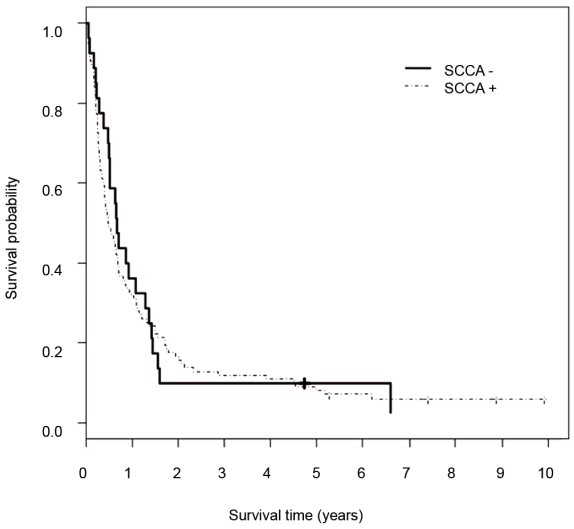

Fig. 2 Overall survival compared on SCCA.

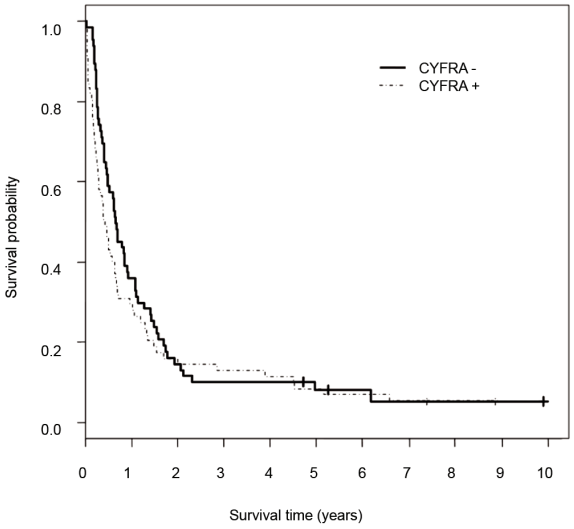

Fig. 3 Overall survival compared on CYFRA 21-1.

Table 1. The relationship between SCCA, CYFRA, Ki-67, and clinicopathological characteristics in the study patients (mean ± SD)

Characteristic (n)	SCCA	*p*-value	CYFRA	*p*-value	Ki-67	*p*-value
Sex		0.19		0.55		0.95
Male (144)	5.24±7.90		7.57±15.11		22.89±20.97	
Female (22)	7.31±8.36		14.97±43.84		19.19±21.96	
Age (years)		0.77		0.40		0.47
<60 (70)	6.74±10.46		11.00±27.97		19.54±19.14	
≥60 (96)	4.39±4.63		6.19±9.81		24.65±22.31	
Tumor invasion		0.93		0.67		0.01
T2 (3)	3.10±2.15		5.52±3.56		22.90±21.24	
T3 (109)	5.38±8.54		9.19±24.07		21.94±20.80	
T4 (54)	5.91±7.11		7.33±12.27		28.67±32.87	
Nodal metastases		0.75		0.48		0.98
N negative (15)	3.92±2.62		4.87±2.95		24.50±17.55	
N positive (151)	5.64±8.26		8.79±21.32		22.22±21.39	
Metastases		0.46		0.51		0.01
M negative (94)	5.33±7.39		6.50±20.11		20.20±20.26	
M positive (72)	5.74±8.76		11.11±20.91		26.51±22.09	
Stage		0.76		0.58		0.12
2 (13)	3.83±2.74		4.53±2.45		27.82±21.36	
3 (80)	5.59±7.93		6.77±21.81		19.84±20.45	
4 (73)	5.48±8.61		10.91±20.57		25.14±21.80	
Location		0.72		0.35		0.64
Upper (31)	5.13±8.70		5.22±5.19		15.33±17.77	
Mid (82)	6.14±8.90		9.22±24.55		24.34±23.05	
Lower (53)	4.64±5.88		8.71±17.92		23.03±18.58	
Grading		0.60		0.42		0.01
Well (50)	7.30±9.37		6.48±12.19		11.63±20.77	
Moderate (73)	4.98±6.03		8.73±25.93		19.27±16.40	
Poor (43)	4.73±4.06		13.74±26.35		27.23±26.10	

SCCA = squamous cell carcinoma antigen

Seventy-one (42.8%) patients had both positive serum SCCA and CYFRA 21-1 and 22 patients (13.2%) had both negative serum SCCA and CYFRA 21-1. However, there were no significant correlations between either of these groups and overall survival ($p = 0.67$) shown in Fig. 4.

Ki-67 expressions

Mean Ki-67 expression in the 166 patients was 22.41±21.04% (median 15%, range 0-80). At the cut-off point of 10%, 96 patients (57.8%) had high expressions for Ki-67. There were significant relationships noted between Ki-67 and T ($p = 0.010$), M ($p = 0.010$), and grade ($p = 0.015$) (Table 1). No significant associations were noted between low or high Ki-67 and overall survival ($p = 0.30$) shown in Fig. 5.

Association of SCCA, CYFRA 21-1, and Ki-67

There were no significant associations found between SCCA and CYFRA 21-1 ($p = 0.128$), SCCA and Ki-67 ($p = 0.163$), or CYFRA 21-1 and Ki-67 ($p = 0.131$).

Discussion

ESCC is still one of the most lethal cancers with a very poor outcome. ESCC is characterized by late stage diagnosis and aggressive behavior[1]. In recent years, biochemical markers in combination with TNM stage have helped clinicians to predict the clinical outcome of this disease. The most commonly used serum tumor markers for ESCC are SCCA and CYFRA 21-1, while the tissue proliferative activity of cancer cells as measured by Ki-67 levels has been demonstrated to have some use as a prognostic factor for patients with different types of cancers, including ESCC[2-10].

SCCA

Elevated SCCA levels were previously reported in many squamous cell carcinomas such as of the cervix, head and neck, lung, and esophagus[15,16]. Increased SCCA concentrations (>1.5 ng/mL) in ESCC ranging from 17 to 57% have been reported[2,4,7,17]. In the present study, the high pretreatment SCCA levels were founded as high as 78.9% of patients.

Using SCCA as a prognostic factor in ESCC is still somewhat controversial. Two studies found that high concentrations of pretreatment SCCA in ESCC were associated with deep tumor invasion and poor prognosis[3,4], but a number of other studies have found no correlation between SCCA and tumor invasion and survival[7,17,18].

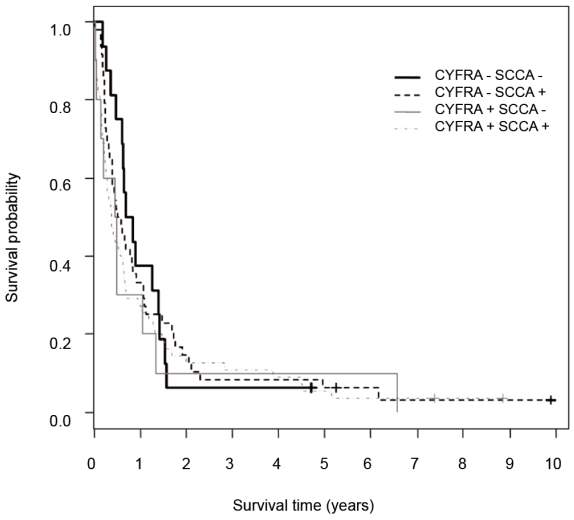

Fig. 4 Overall survival compared on SCCA and CYFRA 21-1.

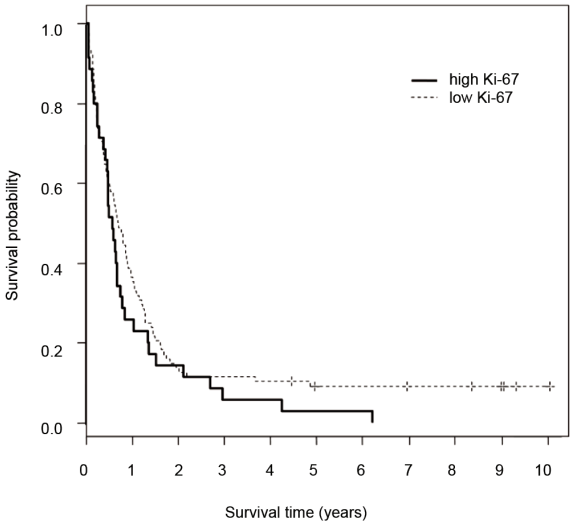

Fig. 5 Overall survival compared on Ki-67.

In our study, a high pretreatment SCCA concentration was not found to be correlated with clinicopathological characters such as sex, age, T, N, M, location of tumor, pathological grading, or overall survival.

CYFRA 21-1

CYFRA 21-1 is an epitope of a polypeptide that is released following cell death. Cytokeratins are intermediate filaments of the cytoskeletons of both normal and malignant epithelia[19]. CYFRA 21-1, which recognizes a soluble cytokeratin-19 fragment,

is elevated in squamous cell carcinoma of the lung, cervix, head and neck, and esophagus[2,5,6,20], with increase of CYFRA 21-1 concentrations (>3.5 ng/mL) ranging from 20 to 63%[2,5-7,20]. In the present study, the high pretreatment CYFRA 21-1 levels were found in 50% of patients.

Previous studies have found correlations between high pre-treatment CYFRA levels in ESCC, deep tumor invasion, and short survival rates[2,5,6,18]. In comparing CYFRA and SCCA in ESCC, some studies have found CYFRA to be superior to SCCA in tumor invasion and survival correlation[2,18,21,22].

In our study, a high pretreatment CYFRA concentration was not found to be correlated with clinicopathological characters such as sex, age, T, N, M, or location of tumor, pathological grading, or overall survival.

Ki-67

Ki-67 is a marker of cell proliferation, expressed in all cell-cycle phases except in G0, the resting phase. Ki-67 levels are low in the G1 and S phases, and rise to their peak levels in mitosis. High levels of Ki-67 have been associated with poor survival in several tumors, including ESCC[8-10].

Over-expression of Ki-67 has been found to be associated with poorly-differentiated ESCC[8,23], and with deep tumor invasion and short survival[8-10]. Ki-67 has also been found to be a predictive factor for response to chemoradiation treatment of ESCC, and low levels of Ki-67 were associated with better response and longer survival[24,25].

Our results suggested that high levels of Ki-67 were correlated with deep tumor invasion (T), high metastasis (M), and poor differentiation of tumor grading, but not survival. However, it should be noted that this study was a retrospective study that did not control the types of treatment, and the treatment effected on survival.

Conclusion

The role of marker in predicting the clinical course of locally advanced and advanced ESCC, immunohistochemical Ki-67 expression in the tumor tissue is probably a more reliable marker than pretreatment levels of serum markers SCCA and CYFRA 21-1.

What is already known on this topic?

Previous studies reported using these markers, SCCA, CYFRA 21-1, and Ki-67 in ESCC patients. However, no study reported the association of these common markers.

What this study adds?

The authors showed that Ki-67 in tumor tissue is probably a more useful marker than serum marker SCCA and CYFRA 21-1.

Acknowledgements

The present study was supported by the grant from the Faculty of Medicine, Prince of Songkla University, Thailand. The authors are grateful to David Patterson for his assistance with the manuscript preparation.

Potential conflicts of interest

None.

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ความสัมพันธ์ระหว่าง SCCA, CYFRA 21-1 และ Ki-67 ในผู้ป่วยมะเร็งหลอดอาหารชนิดเซลล์สความัส

สมเกียรติ สรรพวีรวงศ์, พุฒิศักดิ์ พุทธวิบูลย์, ภัทรพิมพ์ สรรพวีรวงศ์, อนุพงศ์ นิติเรืองจรัส, ปลื้มจิต บุณยพิพัฒน์, มาริษา เขมะพันธุ์มนัส

ภูมิหลัง: *ได้มีการศึกษานำสารส่อมะเร็งในซีรัม squamous cell carcinoma antigen (SCCA) และ CYFRA 21-1 มาใช้กับผู้ป่วยมะเร็งหลอดอาหารชนิดเซลล์สความัส แต่ยังไม่เคยมีการศึกษาร่วมกันระหว่างสารส่อมะเร็งในซีรัมทั้ง 2 ชนิด กับตัวบ่งชี้การเพิ่มจำนวนในเนื้อเยื่อ Ki-67 มาก่อน*

วัตถุประสงค์: *เพื่อศึกษาความสัมพันธ์ระหว่าง SCCA, CYFRA 21-1 และ Ki-67 ในผู้ป่วยมะเร็งหลอดอาหารชนิดเซลล์สความัส*

วัสดุและวิธีการ: *ทำการศึกษาระดับ SCCA และ CYFRA 21-1 ในซีรัม และระดับ Ki-67 ในเนื้อเยื่อมะเร็งของผู้ป่วยมะเร็งหลอดอาหารชนิดเซลล์สความัส โดยศึกษาร่วมกับลักษณะทางคลินิก และพยาธิวิทยา*

ผลการศึกษา: *ในจำนวนผู้ป่วย 166 ราย พบว่ามีระดับ SCCA สูงร้อยละ 78.9 และระดับ CYFRA 21-1 สูงร้อยละ 50 โดยที่ร้อยละ 42.8 พบมีระดับสูงทั้ง SCCA และ CYFRA 21-1 ในทางสถิติไม่พบความสัมพันธ์ร่วมกันของสารส่อมะเร็งนี้กับ อายุ เพศ T, N, M, ตำแหน่งมะเร็ง เกรดทางพยาธิวิทยา และระดับ Ki-67 ในขณะที่ระดับ Ki-67 มีความสัมพันธ์อย่างมีนัยสำคัญทางสถิติกับ T4, M1 และลักษณะเกรดทางพยาธิวิทยาชนิดเลว*

สรุป: *การศึกษานี้แสดงความเกี่ยวข้องของสารส่อมะเร็งในซีรัมและตัวบ่งชี้การเพิ่มจำนวนในเนื้อเยื่อมะเร็งในผู้ป่วยมะเร็งหลอดอาหารชนิดเซลล์สความัส โดย Ki-67 จะสัมพันธ์กับลักษณะทางคลินิกและพยาธิวิทยามากกว่า SCCA และ CYFRA 21-1*