

ORIGINAL ARTICLE BY

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ABSTRACT

OBJECTIVE

To assess the diagnostic properties of serum YKL-40 cervical squamous cell carcinomas (SCC) diagnosis.

METHODS

Blood samples were collected from 56 patients who attended the Obstetrics and Gynecology Clinic at Khon Kaen Hospital, Thailand. Serum YKL-40 levels were detected using enzyme-linked immunosorbent (ELISA) assay in 56 patients with SCC and in 40 healthy women donors as a control.

RESULTS

Serum YKL-40 was significant higher in those with SCC (median, 63.5 ng/ml; interquartile range (IQR), 23 to 309) compared to the controls (median, 20.8 ng/ml; IQR, 3 to 97). At the cut-off value of 30.5 ng/ml of serum YKL-40, area under the curve of the receiver operator characteristic curve was 0.958 (95% confidence interval (CI), 0.916 to 0.999) for SCC diagnosis with 90.6% accuracy, 94.6% sensitivity, 87.5% specificity. No association of high serum YKL-40 level and overall survival time was found.

CONCLUSION

The study suggest the diagnostic potential of serum YKL-40 for SCC diagnosis.

INTRODUCTION

Cervical cancer was accounted for one-third of women cancer worldwide between 2014 and 2018.¹⁻² Although most cervical cancer patients undergo standard treatment related to their tumor stages, some are resistant and develop recurrence and metastasis afterward.³ Some glycoproteins are found to be associated with treatment response.³⁻⁶ For instance, YKL-40, a type of glycoprotein, acts as an angiogenic factor is found to be associated with cancer metastasis and it is linked to clinical applications as a prognostic marker and targeted therapy for cervical cancer.⁷ It is a mammalian chitinase-like protein with no chitinase activity, and is generally known as chitinase 3 like 1 (CHI3L1) or human cartilage glycoprotein-39.⁸ It is found in various cell types such as synoviocytes, chondrocytes, macrophages, neutrophils, and vascular smooth muscle cells.⁸⁻¹⁰ The increase of serum YKL-40 is reported in many solid tumors such as breast cancer, colorectal cancer, lung cancer, ovarian cancer and is associated with short disease-free survival time.¹¹⁻¹⁷ However, the evidence for its linkage to cervical squamous cell carcinoma (SCC) is still scarce. Hence, the aim of this study is to identify the diagnostic properties of serum YKL-40 for cervical SCC diagnosis.

METHODS

STUDY DESIGN AND ETHICAL APPROVAL

This was a cross-sectional diagnostic study with follow up of a component of the cohort of patients with cervical SCC to identify their free survival time. Khon Kaen Hospital Institutional Review Board in Human Research

approved the present study protocol with KE 58029. Written informed consent was attained from all patients. Blood samples were derived from 56 cervical cancer patients who attended the Obstetrics and Gynecology Clinic at Khon Kaen Hospital, Thailand. All were new firstly diagnosed of cervical SCC at the hospital. They were all histopathologically confirmed as SCC and were staged according to The International Federation of Gynecology and Obstetrics (FIGO) staging. Sera were separated from collected blood by centrifugation for 10 min with speed of 3,000 rounds per minute (rpm) at room temperature, and then stored in aliquots at -20°C until the enzyme-linked immunosorbent (ELISA) assay. Sera of 40 healthy female donors with negative human immunodeficiency virus (HIV) and hepatitis B virus (HBV) were provided by Blood Bank, Khon Kaen Hospital as the control. Characteristics of SCC patients are shown in Table 1.

SERUM YKL-40 ASSAY

Serum YKL-40 was determined in duplicate by a commercial two-site, sandwich ELISA method (Boster's biological technology, Pleasanton, California, the United States of America) according to the manufacturer's recommendations. The limit of detection of this assay was 10 pg/ml.

DIAGNOSIS AND SURVIVAL DATA

The diagnosis of cervical SCC was confirmed histopathologically by the pathologist at Khon Kaen Hospital. Their survi

STATISTICAL ANALYSIS

To test the normality of data, Shapiro-Wilk was applied. The difference of each parameter between two groups was analyzed using Mann-Whitney U test. Scatter plot was produced for visualizing the differences between groups. The performance of a

Table 1. Characteristics of the patients with cervical squamous cell carcinoma

Characteristic	Values (n=56)
Age-years	
Median	54
Interquartile range	41-66
FIGO stage-no. (%)	
IIA	6 (11)
IIB	33 (59)
IIIA	2 (3)
IIIB	14 (25)
IVB	1 (2)
Histological differentiation-no. (%)	
Well	6 (11)
Moderately	28 (50)
Poorly	22 (39)
No differentiation report	1 (2)

FIGO, International Federation of Gynecology and Obstetrics.

diagnostic test was evaluated using receiver operator characteristic (ROC) curve and area under its curve together with sensitivities and specificities from various cut-off points. The selected cut-off value was determined using Youden's Index. To estimate the probability of survival time, Kaplan-Meier was performed. $P < 0.05$ was considered statistically significant. The SPSS Ver. 23 statistical programs and graph with Graph Pad Prism5 software were applied for all statistical analysis.

RESULTS

PATIENTS

In the present study, we collected sera from 56 histopathological confirm of cervical squamous cell carcinoma. Most of them were age more than 50 years old. More than half were in Stage IIB with moderately or poorly differentiation (Table 1).

SERUM YKL-40 AS A DIAGNOSTIC MARKER

The medians of YKL-40 in healthy controls and SCC patients were 20.8 ng/ml (IQR, 3 to 97) and 63.5 ng/ml (IQR, 23 to 309), respectively, and the latter was higher significantly ($P < 0.0001$; Mann-Whitney test) (Figure 1, Panel A).

From various cut-off values, the area under the ROC curve (AUC) was 0.958 (95% confidence interval (CI), 0.916 to 0.999) (Figure 1, Panel B). From Youden's Index, the selected cut-off value was 30.5 ng/ml. At this point, the sensitivity and specificity of YKL-40 for cervical SCC diagnosis were 94.6 and 87.5%, respectively. Moreover, the accuracy, negative predictive value (NPV) and positive predictive value (PPV) were 90.6%, 91.9% and 89.9%, respectively, and they are all shown in Table 2.

SERUM YKL-40 AND SURVIVAL TIME

From Figure 2, more than half of cases died at the end of the follow-up period. Fifty-four out of 56 cases of cervical SCC, one was lost to follow-up and another died within the first four weeks, were divided into low and high YKL-40 level regarding the median of its median, 63.5 ng/ml. No association of serum YKL-40 levels with overall survival time were observed ($P = 0.986$).

DISCUSSION

YKL-40 at the cut-off value of 30.5 ng/ml could be used as a diagnostic marker with high sensitivity and specificity, 94.6% and 87.5%, respectively, with the AUC of 0.958. While the previous study used the cut-off value of 92.2 ng/ml and the result showed that the AUC, specificity, and sensitivity were 0.898, 89% and 75%, respectively.¹¹ However, these were merely the findings to assess the YKL-40 for diagnosis of the cervical adenocarcinoma.

The increase of serum YKL-40 level was reported in many solid tumors such as breast cancer, colorectal cancer, lung cancer, ovarian cancer, glioblastoma and cervical cancer which was associated with short disease free survival time.¹¹⁻¹⁷ Furthermore, the up-regulated of YKL-40 was reported in advanced cervical cancers.⁶ Single nucleotide polymorphisms of YKL-40 are associated with overall survival and recurrence of cervical cancer patients.¹⁸ The study demonstrated that serum YKL-40 between healthy controls and SCC patients was significantly different, which agreed with the previous study.¹¹ It also showed that serum YKL-40 was correlated with disease free survival indicating the usefulness as an unfavorable prognostic marker¹¹ while Kaplan-Meier analysis in the present study didn't show any correlation between serum YKL-40 level and OS. Hence, this may be due to the small number of cases included in our study.

In conclusion, the present study found that serum YKL-40 was relatively higher in those with cervical SCC than that of healthy controls. It also found to have high sensitivity and specificity

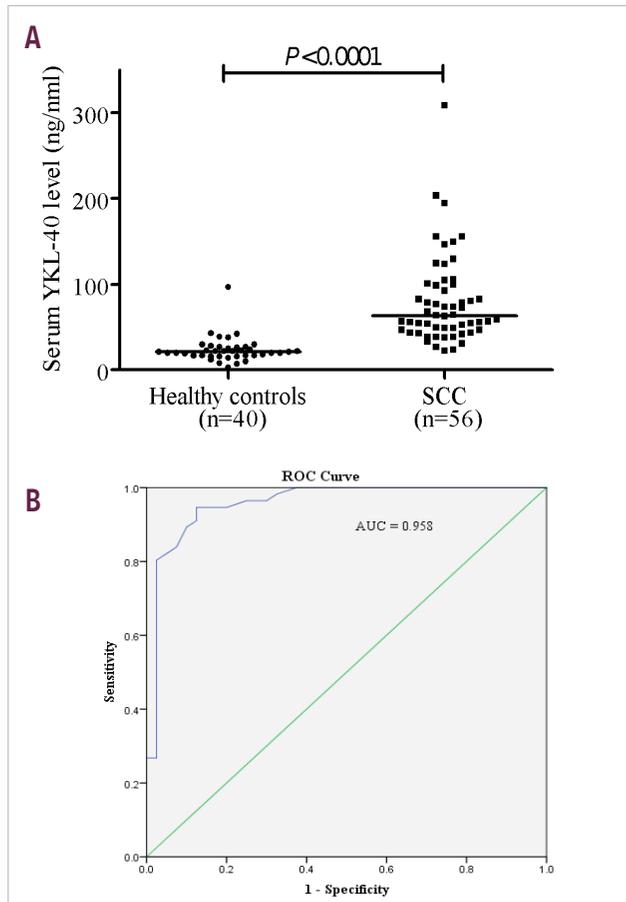


Figure 1. Scatter plot and receiver operator characteristic curve.

Panel A, median line graphics of serum YKL-40 levels in healthy controls and those with cervical squamous cell carcinoma. Horizontal bars indicate the medians of YKL-40 level; Panel B, receiver operator characteristic curve of YKL-40 levels for cervical squamous cell carcinoma diagnosis.

at the cut-off value of 30.5 ng/ml. However, its association with overall survival was not observed. Further studies with a larger sample size is also required before the wild application of serum YKL-40 in terms of a screening and diagnostic marker for cervical SCC.

Table 2. Sensitivities and specificities at different cut-off values of the serum YKL-40 for cervical SCC diagnosis

Cut-off value	Sensitivity	Specificity
23.50	98.2	67.5
24.50	96.4	70
25.50	96.4	72.5
26.50	96.4	75
27.50	94.6	80
29.00	94.6	82.5
30.50	94.6	87.5
32.00	92.9	87.5
35.50	91.1	87.5
38.50	89.3	90
40.50	83.9	92.5
42.50	82.1	95
43.50	80.4	97.5
45.50	76.8	97.5
48.00	73.2	97.5

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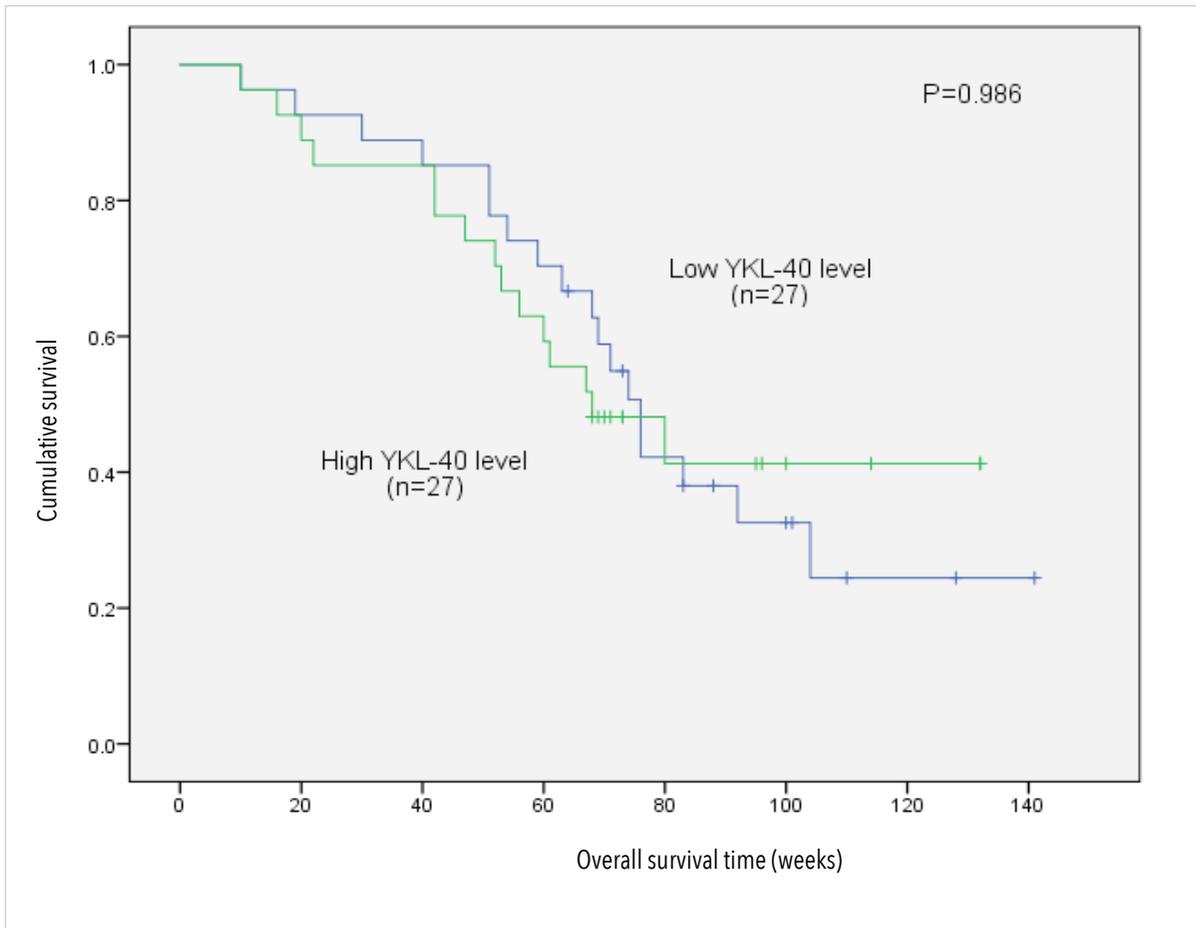


Figure 2. Kaplan-Meier survival curve

Survival curve of low vs. high serum YKL-40 levels at 63.5 ng/ml as a cut-off value.

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