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ORIGINAL ARTICLE

Increased ADAM12 Expression Predicts Poor Prognosis in Cervical Cancer Patients before General Anesthesia

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SUMMARY

Background: **The current study aims to investigate the expression and diagnostic value of ADAM12 in patients with cervical cancer before general anesthesia.**
Methods: **Seventy-eight cases of cervical cancer patients were included in the present study. RT-PCR and western blot were used to detect the expression of ADAM12 in cervical cancer tissues and adjacent tissues. Meanwhile, the expression of secretory ADAM12 in serum of cervical cancer patients and healthy people was detected by ELISA. The relationship between ADAM12 expression and prognosis of cervical cancer patients was analyzed. ROC analysis was carried out to explore the diagnostic value of ADAM12.**
Results: **Our data showed that the expression of ADAM12 mRNA and protein in cervical cancer tissues was significantly up-regulated compared with the adjacent tissues. ELISA assay showed that the content of ADAM12 in serum of cervical cancer patients was significantly higher than that of healthy people. Furthermore, ADAM12 expression was closely related to tumor invasion, TNM stage, lymph node metastasis and tumor differentiation. Kaplan-Meier survival analysis showed that the overall survival rate of patients with high ADAM12 was significantly lower than that of patients with low ADAM12 expression. The AUC of ADAM12, CEA, CA125, and SCC for cervical cancer was 0.893, 0.510, 0.769 and 0.550, respectively, while the highest value of AUC was 0.946 by the combination of the four indexes.**
Conclusions: **In summary, increased expression of ADAM12 in cervical cancer patients can be used as an independent prognostic marker.**
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KEY WORDS

cervical cancer, ADAM12, tissues, serum, prognosis

INTRODUCTION

Cervical cancer is the third most common gynecological cancer in the world. Cervical cancer has a higher incidence in developing countries, and it is the main gynecological disease of female cancer deaths [1,2]. Studies have confirmed that 90% of cervical cancer is accompanied by persistent high-risk human papillomavirus (HPV) infection [3-5]. With the continuous development of cervical cytology, some cervical cancer and precancerous lesions were found and treated actively, so that the incidence rate and mortality of cervical cancer

were reduced to some extent [6]. However, due to the occult incidence of cervical cancer, there are still some patients who have entered the middle and late stage when they are diagnosed and missed the best time of treatment resulting in poor prognosis [7]. Therefore, the early diagnosis and monitoring of cervical cancer is of great significance for the development of a reasonable treatment plan.

ADAM12 belongs to the A Disintegrin And Metalloproteases (ADAM) protein family and is involved in cell adhesion via the proteolytic cleavage of substrates from producing cells [8]. Increasing evidence has indicated that ADAM12 widely interacts with cancer-related substrates, including epidermal growth factor (EGF) and Sonic Hedgehog (SHH) [9-11]. Enhanced expression of ADAM12 has been indicated in various tumors, including breast cancer, pancreatic cancer, pituitary adenomas [12-14]. A previous study suggests that ADAM9 expression is increased in Grade 3 cervical intraepithelial neoplasia (CIN3) lesions and squamous cell carcinomas (SCC) of the uterine cervix [15]. However, the expression of ADAM12 in cervical cancer patients before general anesthesia has not been reported. In this study, ADAM12 expression was detected in cervical cancer tissues and adjacent tissues. Furthermore, we also analyzed the level of serum ADAM12 and evaluated its diagnostic value in cervical cancer patients.

MATERIALS AND METHODS

Patient samples

The study was a retrospective study. Here, we screened 150 patients for enrollment in the study and 136 patients were included since 14 patients withdrew or were lost to follow-up. The clinical data of 136 patients with HPV infection who underwent cervical exfoliative cytology were collected from the The Fourth Hospital of Shijiazhuang City. The patients were divided into cervical intraepithelial neoplasia (CIN) group (58 cases) and cervical cancer group (78 cases). The cervical cancer tissues and adjacent tissues were taken from cervical cancer patients after giving 1 mg/kg propofol injection for general anesthesia. Inclusion criteria: (1) all patients were diagnosed as cervical lesions for the first time; (2) no treatment related to cervical diseases was carried out in the past 3 months; (3) no B vitamins were taken in the past 1 month; (4) no anti-cancer treatment such as endocrine therapy, radiotherapy, and chemotherapy was carried out before operation; (5) all patients signed the informed consent before operation. Exclusion criteria: (1) pregnant or lactating women; (2) history of cervical conization; (3) patients with severe liver and kidney dysfunction; (4) history of other tumors; (5) reproductive tract inflammation or other gynecological complications. In addition, 52 healthy women in the same period were selected as the control group, aged 20 - 60 years, with an average age of (41.8 ± 7.7) years. This study was conducted in compliance with the Helsinki Declaration. The protocol and laboratory manuals for this study were reviewed and approved by the ethical committee of The Fourth Hospital of Shijiazhuang City. All patients' participation was voluntary, and all enrolled participants were given the right to refuse or exit the study at any time. Written informed consent was obtained from each participant at the time of enrollment for the present study.

Enzyme-linked immunosorbent assay (ELISA)

In the CIN group and cervical cancer group, 3 mL of elbow vein blood was collected on an empty stomach in the early morning before general anesthesia. The blood was centrifuged at 3,000 g for 15 minutes. The serum was collected and then stored in a refrigerator at -70°C. On the day of physical examination, 3 mL of elbow vein blood was taken from the control group, and the sample processing method was the same as above. Then, the level of serum ADAM12 was detected by a Human ADAM12 ELISA Kit (ab171346, Abcam).

RT-PCR

Total RNA was isolated from the serum samples using RNAVzol LS (Vigorous Biotechnology Beijing Co., Ltd., Beijing, China) according to the manufacturer's protocol. The concentration and the purity of RNA samples were determined by measuring the optical density (OD) 260/OD280. RNA was reverse transcribed into cDNA using One Step PrimeScriptTM RT-PCR Kit (Tarkara, Japan). qPCR was performed using SYBR Green Supermix (Bio-Rad Laboratories, Inc., Hercules, CA, USA) according to the instruction. Relative mRNA expression was normalized to β-actin using the $2^{-\Delta\Delta Cq}$ method [16]. The primers used in the present study were listed as follows:

ADAM12-F:
5'-CGAGGGGTGAGCTTATGGAAC-3';
ADAM12-R:
5'-GCTTTCCCGTTGTAGTCGAATA-3';
β-actin-F:
5'-TGACGTGGACAGCCGCAAAG-3';
β-actin-R:
5'-CTGGAAGGTGGACATCCGCAAAG-3'.

Western blot

A total protein extraction kit (Beijing Solarbio Science & Technology Co., Ltd.) was used to isolate the protein and the protein was separated using 12% SDS-PAGE, transferred onto polyvinylidene difluoride (PVDF) membranes and blocked with 5% fat-free milk at room temperature for 2 hours. Membranes were incubated with primary antibodies against ADAM12 (Abcam, Cambridge, UK), and GAPDH (Cell Signaling Technology, Inc.) at 4°C overnight. Membranes were subsequently incubated with horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG (both 1:5,000; cat. no. ZB-2301; Beijing Zhongshan Golden Bridge Biotechnology Co., Beijing, China) for 2 hours at room temperature, followed by three washes with TBST. Enhanced

chemiluminescence (EMD Millipore, Billerica, MA, USA) was used to determine the protein concentrations according to the manufacturer's protocol.

Statistical analysis

The data were represented as the mean ± standard deviation (SD). The two-tailed unpaired Student's *t*-tests were used for comparisons of two groups. The one-way ANOVA multiple comparison test (SPSS 20.0) followed by Tukey's post hoc test was used for comparisons of two more groups. Kaplan-Meier analysis followed by log rank test was used to test the total survival of patients with cervical cancer. Receiver operating characteristic (ROC) curves were used to assess serum ADAM12 as a biomarker, and the area under the curve (AUC) was reported (version 20.0, IBM SPSS Statistics for Windows; IBM Corp, Armonk, NY, USA). $p < 0.05$ was considered significant.

RESULTS

Increased expression of ADAM12 in cervical cancer tissues

RT-PCR was used to detect the expression of ADAM12 mRNA in 78 cases of cervical cancer patients. The results showed that the relative mRNA level of ADAM12 in 78 cases of cervical cancer tissues was 11.11 ± 0.54, which was significantly higher than that in adjacent tissues (1.29 ± 0.49) (Figure 1A). Furthermore, western blot showed that the expression of ADAM12 in cervical cancer tissues was significantly increased compared with that in adjacent tissues (Figure 1B).

Expression of ADAM12 and clinical characteristics in cervical cancer patients

Then, we analyzed the relationship between the expression of ADAM12 and the clinicopathological characteristics of cervical cancer patients. The results indicated that the increased expression of ADAM12 was closely related to tumor invasion, TNM stage, lymph node metastasis, and tumor differentiation (Table 1).

Serum ADAM12 was increased in cervical cancer patients

Furthermore, the serum ADAM12 content in the cervical cancer group was significantly higher than that in the CIN group and healthy control group, while the difference between the CIN group and healthy control group was not statistically significant ($p > 0.05$), as shown in Figure 2A. Additionally, our data showed that serum ADAM12 was significantly enhanced in cervical cancer patients with TNM stage of III + IV compared with TNM stage of I + II (Figure 2B). Meanwhile, higher serum ADAM12 level was also identified in patients with lymph node metastasis of N2 + N3 compared with that of N0 + N1 (Figure 2C).

Diagnostic value of serum ADAM12 in cervical cancer patients

The common cervical cancer markers include carcinoembryonic antigen (CEA), carbohydrate antigen 125 (CA125), squamous cell carcinoma antigen (SCC), but their sensitivity and specificity are not high [17]. Here, we compared the diagnostic value of serum ADAM12 with CEA, CA125, SCC. The results showed that the sensitivity and specificity of serum ADAM12 in the cervical cancer group were 75.1% and 79.8%, respectively, which was higher than those in CEA, CA125 and SCC. The AUC of ADAM12, CEA, CA125, and SCC for cervical cancer was 0.893, 0.510, 0.769, and 0.550 respectively, while the highest value of AUC was 0.946 by the combination of the four indexes (Figure 3). Hence, the combined detection efficiency of serum ADAM12, CEA, CA125, and SCC was the highest.

High serum ADAM12 predicted poor prognosis of cervical cancer patients

Based on the median level of serum ADAM12, cervical cancer patients were further divided into two groups, high ADAM12 group (> 11.11 μg/mL) and low ADAM12 group (≤ 11.11 μg/mL). Kaplan-Meier analysis showed that the patients with high expression of ADAM12 had poor prognosis. The clinical median survival time of patients with high expression of serum ADAM12 was 29.5 months, and that of low ADAM12 expression was 49.4 months, with a significant difference ($p = 0.004$, Figure 4).

DISCUSSION

Cervical cancer is currently a major cause of cancer deaths among women around the world [18]. However, if cervical cancer is detected during its earlier stages, the 5-year survival rate may be improved [5]. Therefore, early detection of cervical cancer is essential for favorable prognosis [19]. Currently, tumor markers, such as CEA, CA125, and SCC, are used in the evaluation of cervical cancer [20]. However, these markers are questioned due to their low potential for early detection. ADAM12, has been linked to the development and progression of multiple diseases, including liver fibrogenesis, and various cancers [21,22]. Mohammed S. et al. showed that the expression of ADAM12 was upregulated as cervical cells progressed from dysplastic to malignant lesions compared to normal cervical cells [23]. However, whether ADAM12 was dysregulated in cervical cancer patients has not been explored.

In the current study, we showed novel data that the expression of ADAM12 in cervical cancer tissues was significantly higher than that in adjacent tissues. Meanwhile, the expression level of ADAM12 in serum of patients with cervical cancer was significantly higher than that of CIN patients and healthy people. The oncogenic role of ADAM12 has been shown in other tumors [22, 24]. Consistent with these observations, we found that

Table 1. The expression of ADAM12 and clinicopathological characteristics of cervical cancer patients.

	n	ADAM12 level	p
Age (years)			> 0.05
≤ 60	34	11.12 ± 4.34	
> 60	44	10.56 ± 5.23	
Tumor diameter (cm)			> 0.05
≤ 3	28	11.25 ± 4.36	
> 3	50	10.78 ± 3.87	
HPV infection			> 0.05
Yes	61	10.23 ± 3.65	
No	17	11.89 ± 4.32	
Histological type			> 0.05
AD	37	10.67 ± 3.58	
SCC	41	11.78 ± 5.42	
Tumor invasion (T)			< 0.01
T1 + T2	48	5.67 ± 3.21	
T3 + T4	30	16.58 ± 5.96	
Tumor differentiation			< 0.01
Good + medium	46	4.35 ± 3.11	
Poor	32	13.12 ± 5.23	
TNM stage			< 0.01
I + II	43	3.24 ± 14.56	
III + IV	35	15.34 ± 3.98	
Lymph node metastasis			< 0.05
N0 + N1	37	4.78 ± 2.67	
N2 + N3	41	12.89 ± 3.85	

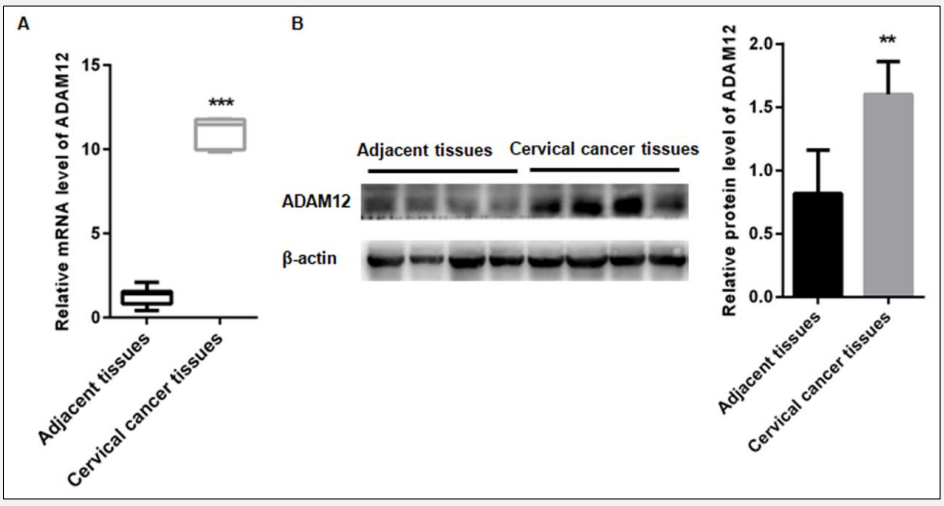

Figure 1. RT-PCR was used to detect the expression of ADAM12 mRNA in 78 cases of cervical cancer patients.

(A) The relative mRNA level of ADAM12 in cervical cancer tissues was significantly higher than that in adjacent tissues. (B) Western blot showed that the expression of ADAM12 in cervical cancer tissues was significantly increased compared with that in adjacent tissues. ** p < 0.01, *** p < 0.001 vs. control.

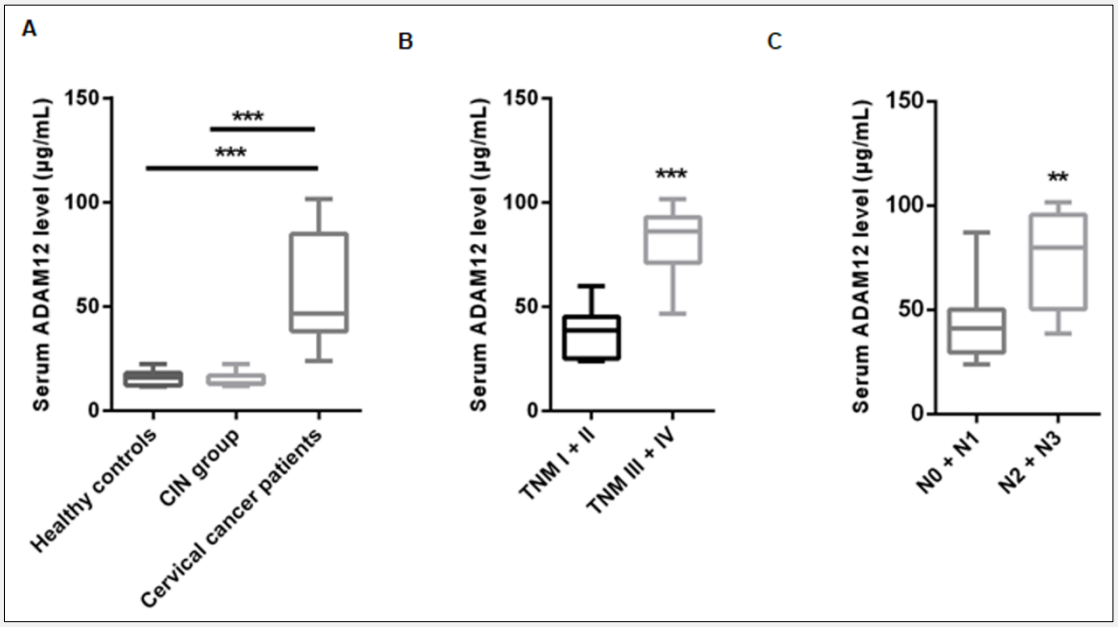

Figure 2. ELISA assay was performed to analyze the expression of serum ADAM12 in cervical cancer patients, CIN group, and healthy controls.

(A) The serum ADAM12 content in the cervical cancer group was significantly higher than that in the CIN group and healthy control group. (B) Serum ADAM12 was significantly enhanced in cervical cancer patients with TNM stage of III + IV compared with TNM stage of I + II. (C) Higher serum ADAM12 level was also identified in patients with lymph node metastasis of N2 + N3 compared with that of N0 + N1. ** p < 0.01, *** p < 0.001 vs. control.

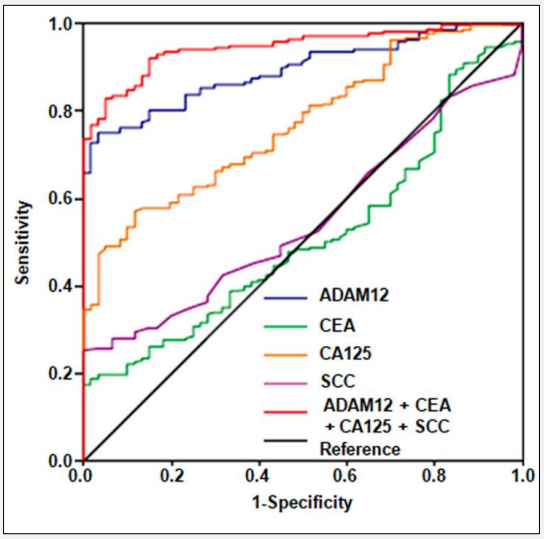

Figure 3. ROC analysis was performed to analyze the diagnostic value of serum ADAM12, CEA, CA125, and SCC in cervical cancer patients.

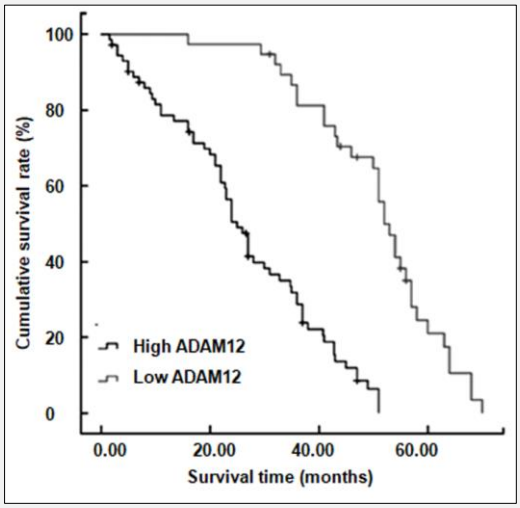

Figure 4. Kaplan Meier analysis showed that the patients with high expression of ADAM12 had poor prognosis.

serum ADAM12 was significantly upregulated in cervical cancer patients with higher TNM stage and lymph node metastasis. Therefore, it is feasible that ADAM12 results in increased malignant phenotype of cervical cancer.

CA-125, CEA, and SCC have long been used as tumor biomarkers in patients with cervical cancer [25,26]. However, these markers lack specificity. For instance, serum CEA, CA125, and SCC antigens were also shown to be important for the diagnosis in patients with resectable non-small cell lung cancer [27]. Here, we compared the diagnostic efficiency of serum ADAM12 with CA-125, CEA, and SCC. A previous finding has shown that CA-125 demonstrates a better diagnostic sensitivity than CEA and SCC [25]. Contrary to that study, we found that the sensitivity and accuracy of CA125 were significantly higher than CEA and SCC in cervical cancer patients. More importantly, serum ADAM12 was found to present the highest sensitivity, with an AUC of 0.893. As the result of combined analysis, the diagnostic value of ADAM12 + CEA + CA125 + SCC for cervical cancer was 0.946, which significantly enhanced the diagnostic efficiency. Therefore, serum ADAM12 was useful when combined with CEA, CA125, and SCC in the diagnosis of cervical cancer patients.

Here, we took the 5-year survival rate as the primary outcome. During the 5-year follow-up, 24 patients relapsed and 54 patients were disease-free. Kaplan-Meier survival analysis showed that the clinical prognosis of patients with high expression of ADAM12 was poor, and the clinical median survival time of patients with high expression of ADAM12 was significantly lower than that of patients with low expression. Hence, preoperative level of serum ADAM12 is useful for predicting the status of postsurgical high-risk factors in women with cervical cancer. In the future, it will be interesting to explore the secondary outcome, such as progression-free survival, partial response rate, and complete response rate.

Xiong et al. demonstrates that ADAM12 is highly expressed in cervical cancer tissues [28]. Shaker M. et al. reports that the mRNA levels of ADAM12 were enhanced in cervical cells progressed from dysplastic to malignant lesions compared to normal cervical cells [23]. These previous findings indicate the oncogenic role of ADAM12 in cervical cancer patients. In comparison with these observations, we showed novel data that ADAM12 was significantly increased in the serum of cervical cancer patients and elevated serum ADAM12 correlated with TNM stage and lymph node metastasis. More importantly, we investigated the diagnostic value of serum ADAM12 and demonstrated that serum ADAM12 was an effective biomarker in differentiating cervical cancer patients from controls. The strength of the present study includes: 1. Elevated serum ADAM12 was closely related to tumor invasion, TNM stage, lymph node metastasis, and tumor differentiation. 2. We evaluated the diagnostic value of serum ADAM12 in cervical cancer patients and compared it with the current existing serum markers. 3. Our data showed that higher serum ADAM12 predicted poor survival rate among cervical cancer patients.

However, there are limitations in the present study. First, the sample size is relatively small; therefore, a larger sample size is necessary in a future study. Secondly, the underlying mechanism by which ADAM12 regulates the progression of cervical cancer has not been

explored.

In conclusion, upregulated expression of ADAM12 is involved in the occurrence and development of cervical cancer and may be an important factor of poor prognosis in cervical cancer patients.

Declaration of Interest:

We declare no conflicts of interest.

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