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ORIGINAL ARTICLE

Diagnostic Value of Serum N1-Methylnicotinamide in Cervical Cancer Patients

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SUMMARY

Background: **The current study evaluated the level of serum *N*1-methylnicotinamide (me-NAM) in cervical cancer patients and further explored whether serum me-NAM was related to the prognosis of cervical cancer.**
Methods: **Fifty-eight cases of cervical intraepithelial neoplasia patients, 78 cases of cervical cancer patients, and 52 healthy women were included in the present study. Serum me-NAM concentrations were determined by liquid chromatography with tandem mass spectrometry. Receiver operating characteristic (ROC) curve was used to assess me-NAM as a biomarker and Kaplan-Meier analysis was carried out to evaluate the survival rate.**
Results: **Our data showed that the level of serum me-NAM in cervical cancer patients was significantly higher than that in the cervical intraepithelial neoplasia group and the healthy control group. Furthermore, the level of me-NAM in cervical cancer tissues of stage I, II, III, and IV was higher than that of those without lymph node metastasis. The area under the receiver operating characteristic curve (ROC) for me-NAM was higher than that of squamous cell carcinoma antigen (SCC Ag) and carbohydrate antigen 125 (CA125) when comparing cervical cancer from CIN or healthy control. The combination of me-NAM and SCC Ag or CA125 could improve the diagnostic efficiency better than SCC Ag or CA125 alone. Compared with me-NAM low expression group, the survival rate and time of me-NAM high expression group were lower and shorter.**
Conclusions: **Altogether, elevated serum me-NAM levels contribute to the progression of cervical cancer and may be used as a marker for the prognosis of patients with cervical cancer.**
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KEY WORDS

cervical cancer, *N*1-methylnicotinamide, nicotinamide N-methyltransferase, prognosis

INTRODUCTION

Cervical cancer is one of the most common gynecological malignancies, and its incidence rate is only inferior to that of breast cancer [1,2]. With the development of technology, the treatment of cervical cancer has gradually changed from surgery to a combination of traditional and new adjuvant therapy [3,4]. However, the recurrence rate of cervical cancer diagnosed at the late or metastatic stages is still high, and the therapeutic efficacy is not satisfactory [5,6]. Therefore, screening methods for early tumor detection are of great importance to

reduce mortality from cervical cancer.

Nicotinamide N-methyltransferase (NNMT), an S-adenosyl-L-methionine-dependent cytoplasmic enzyme, was first identified in the liver and is shown to play a key role by modulating nicotinamide and some pyridine derivative methylations [7,8]. Overexpression of NNMT is widely reported in different tumors, including liver cancer, ovarian cancer, and breast cancer [9-11]. A recent study has shown that NNMT is overexpressed in the tissues of cervical squamous cell carcinoma [12]. However, whether NNMT could be used as a prognostic indicator in the circulating system deserves further exploration. It is reported that NNMT mainly catalyzes methylation of nicotinamide to generate N1-methylnicotinamide (me-NAM) [13]. me-NAM is suggested to be an indicator of NNMT activity since it can only be derived from the methylation reaction catabolized by NNMT in the liver [8]. Hence, we evaluated the level of serum me-NAM and further explored whether serum me-NAM is related to the prognosis of cervical cancer.

MATERIALS AND METHODS

Patient samples

From January 2016 to January 2019, the clinical data of 136 patients with HPV infection who underwent cervical exfoliative cytology examination in the outpatient department of Qingdao Eighth People's Hospital were collected. Based on histopathological diagnosis, they were divided into 58 cases of cervical intraepithelial neoplasia (CIN) group and 78 cases of cervical cancer group. 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 approved by the ethic committee of Qingdao Eighth People's Hospital.

Detection of serum me-NAM

In the CIN group and cervical cancer group, 3 mL of elbow vein blood was extracted on an empty stomach in the early morning before the operation. Then, the blood samples were centrifuged at 2,500 g for 15 minutes, and the serum was stored at -70°C for future use. On the day of physical examination, 3 mL of elbow vein blood was taken from the control group, and the blood samples were processed as described as above. Serum me-NAM concentrations were determined by liquid chromatography with tandem mass spectrometry (Agilent 6430 Triple Quad liquid chromatograph/mass spectrometer) as previously described [14].

Statistical analysis

The data was expressed as the mean ± standard error (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 were used for comparisons of two or more groups. Receiver operating characteristic (ROC) curves were used to assess me-NAM 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). χ^2 test was used to analyze the relationship between serum me-NAM and clinical data among the three groups of subjects. $p < 0.05$ showed that the difference was statistically significant.

RESULTS

Comparison of serum me-NAM levels among three groups

The level of serum me-NAM in cervical cancer group was significantly higher than that in CIN group and healthy control group, while the difference between CIN group and healthy control group was not statistically significant (Figure 1).

The relationship between the level of serum me-NAM and the clinicopathological parameters of cervical cancer patients

The level of me-NAM in cervical cancer tissues of stage I, II, III, and IV was higher than that of those without lymph node metastasis (Table 1). Furthermore, the level of serum me-NAM was not related to age, tumor diameter, pathological type, and degree of tissue differentiation (Table 1).

Diagnostic efficacy of serum me-NAM in cervical cancer patients

ROC analysis was performed to evaluate the diagnostic value of me-NAM compared with serum biomarkers, SCC Ag or CA125. When comparing cervical cancer with healthy control, the AUC for serum me-NAM was 0.71, which was higher than that of SCC Ag (0.68) and CA15-3 (0.51) (Figure 2A). Moreover, the AUC for combined use of me-NAM, SCC Ag and CA125 was 0.78, which was higher than SCC Ag or CA125 alone (Figure 2A).

When comparing cervical cancer with CIN, we found the AUC of serum me-NAM was 0.71, which was also higher than that of SCC Ag (0.67) or CA125 (0.52) (Figure 2B). After combining serum me-NAM with SCC Ag or CA125, the AUC was 0.77, which was also higher than that of SCC Ag or CA125 alone (Figure 2B). These data suggested that combining serum me-

Table 1. The relationship between serum me-NAM and the clinicopathological parameters of cervical cancer patients.

	n (78)	Serum me-NAM level (ng/L)
Age (years)		
≤ 55	**37**	**16.78 ± 2.13**
> 55	**41**	**16.68 ± 3.24**
Tumor diameter (cm)		
≤ 3	**38**	**17.12 ± 2.54**
> 3	**40**	**16.48 ± 2.87**
Histological type		
Squamous cell carcinoma	**66**	**16.45 ± 2.87**
Adenocarcinoma	**12**	**17.23 ± 2.52**
Differentiation		
High	**16**	**16.34 ± 2.56**
Medium	**37**	**15.67 ± 1.98**
Poor	**25**	**18.12 ± 5.12**
FIGO stage		
I	**27**	**5.98 ± 0.95**
II	**38**	**11.67 ± 1.23** *
III/IV	**13**	**19.67 ± 2.36** **, #
Lymph node metastasis		
No	**61**	**16.56 ± 2.87**
Yes	**17**	**16.98 ± 2.13**

*** p < 0.05, ** p < 0.01 vs. stage I, # p < 0.05 vs. stage II.**

NAM with SCC Ag or CA125 could improve the diagnostic performance of cervical cancer.

The relationship between serum me-NAM and the prognosis of cervical cancer

According to the mean value of relative expression of me-NAM in cervical cancer, patients with cervical cancer were divided into low expression group (50 cases) and high expression group (28 cases). The median follow-up time was 32 months. For me-NAM low expression group, the survival rate was 88.00% (44/50) and the survival time was 56.9 ± 2.3 months. For me-NAM high expression group, the survival rate and the survival time were 67.86% (19/28) and 46.5 ± 3.8 months, respectively. Compared with me-NAM low expression group, the survival rate and time of me-NAM high expression group were lower and shorter (Figure 3).

DISCUSSION

Among females, cervical cancer (CC) was the fourth leading cause of cancer death around the world in 2018 [15]. Serum tumor biomarkers, including squamous cell carcinoma antigen (SCC Ag) and carbohydrate antigen 125 (CA125), are shown to be effective for CC diagnosis [16]. However, due to the poor sensitivity and specificity, their clinical applications are substantially restricted [17]. Hence, to develop reliable and noninvasive biomarkers is especially important for early detection of cervical cancer.

Recently, overexpression of NNMT has been identified in cervical cancer tissues [14]. Me-NAM is an endogenous metabolite of nicotinamide, which is catalyzed by NNMT in the liver and easily detected in the circulating system [18]. In the present study, we showed novel data that serum me-NAM, an indicator of NNMT activity, is strongly associated with the prognosis of cervical cancer.

In this study, we confirmed that the level of serum me-NAM in cervical cancer patients was significantly higher than that in CIN group and healthy control group. It is shown that FIGO staging is an important factor in predicting the prognosis [19,20]. Hence, we analyzed the level of serum me-NAM according to FIGO staging. Our data showed that the level of serum me-NAM increased with FIGO tumor stage, indicating that the level of me-NAM might occur at the early stage of cervical cancer.

SCC Ag and CA125 have been used as cervical cancer biomarkers, but the sensitivity and specificity to the diagnosis of cervical cancer is still limited [21,22]. Here, the AUC of me-NAM was higher than that of SCC Ag and CA125. SCC Ag and CA125 showed low sensitivity in detecting cervical cancer, which is in line with previous research. More importantly, combining me-NAM with SCC Ag and CA125 led to a higher AUC than single use of SCC Ag or CA125 alone, indicating that serum me-NAM could improve diagnostic values.

In addition, the follow-up results of this study showed that compared with the low expression group of me-NAM, the high expression group of me-NAM had lower survival rate and shorter survival time, which confirmed that NNMT could promote the proliferation and invasion of cervical cancer cells [14]. Therefore, for patients with high expression of me-NAM, it is necessary to strengthen postoperative monitoring and reasonably apply auxiliary anti-tumor therapy to improve the postoperative survival rate and prolong the postoperative survival time.

In conclusion, me-NAM is highly expressed in cervical cancer patients and is related to the occurrence and development of cervical cancer. The prognosis of patients with low expression of me-NAM is better than that of patients with high expression of me-NAM. Therefore, me-NAM is expected to be a new index to evaluate the prognosis of patients with cervical cancer.

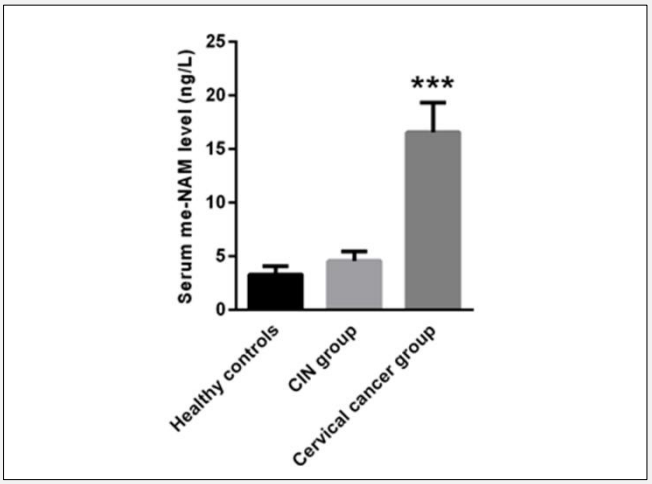

Figure 1. The level of serum me-NAM in the cervical cancer group was significantly higher than that in the CIN group and healthy control group.

***** p < 0.001 vs. healthy control group.**

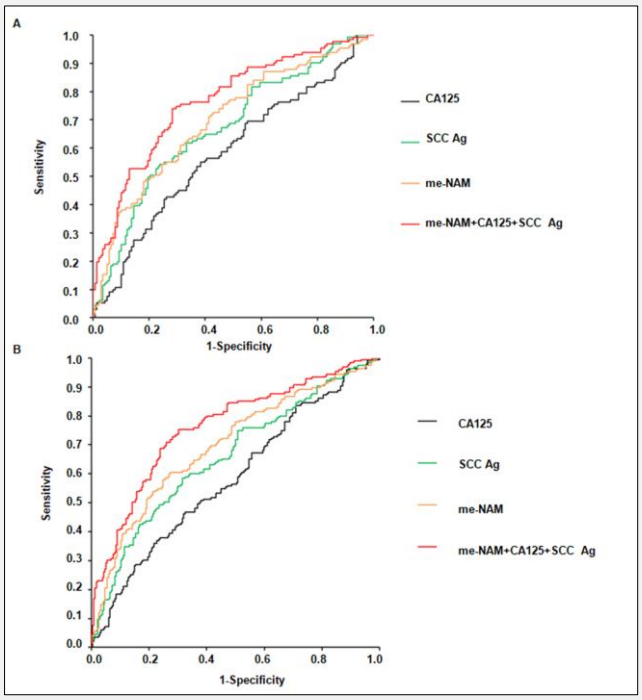

Figure 2. ROC analysis showed that serum me-NAM could differentiate cervical cancer patients from CIN and healthy control group.

(A) The AUC for combined use of me-NAM, SCC Ag, and CA125 was higher than SCC Ag or CA125 alone when comparing cervical cancer with healthy control. (B) After combining serum me-NAM with SCC Ag or CA125, the AUC was also higher than that of SCC Ag or CA125 alone when comparing cervical cancer with CIN.

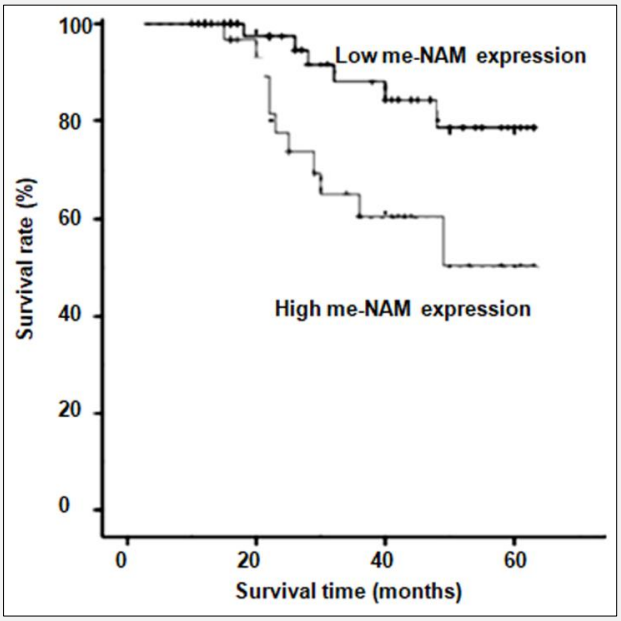

Figure 3. Kaplan-Meier analysis showed that the survival rate and time of the me-NAM high expression group were lower and shorter than that of the me-NAM low expression group.

Declaration of Interest:

We declare no conflicts of interest.

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