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ORIGINAL RESEARCH

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Three serum miRNAs as potential biomarkers for early diagnosis of cervical cancer

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Abstract

This study aimed to analyze the changes associated with serum miRNAs in patients with cervical intraepithelial neoplasia (CIN) and cervical cancer in predicting early-stage cervical cancer. A total of 229 serum samples were collected to extract miRNAs. The expression level of miR-146b-5p, miR-192-5p and miR-122-5p were determined *via* qRT-PCR (Quantitative Real-time PCR), and their relative expressions were calculated using the equation: $2^{-\Delta\Delta Ct}$. We observed significantly higher expression levels of serum miR-146b-5p, miR-192-5p and miR-122-5p in the cervical diseases (CIN and cervical cancer) compared with controls. The expression levels of serum miR-146b-5p, miR-192-5p and miR-122-5p in cervical cancer were significantly higher than in controls. The relative expressions of serum miR-146b-5p and miR-192-5p in CIN were significantly higher than controls. However, there was no difference in three miRNAs between CIN and cervical cancer. The relative expressions of serum miR-146b-5p in patients classified as FIGO (International Federation of Gynaecology and Obstetrics) stage I were significantly higher than in controls and CIN. The AUC (area under the ROC curve) of the three miRNAs combined with SCC (squamous cell carcinoma antigen) in diagnosing cervical cancer was 0.840, and the sensitivity and specificity were 0.632 and 0.957, respectively. Serum miR-146b-5p, miR-192-5p and miR-122-5p showed promising efficacy as noninvasive biomarkers to accurately diagnose cervical cancer and demonstrated certain early diagnostic values, especially miR-146b-5p and miR-192-5p. The combined use of the three serum miRNAs with SCC could further improve the diagnostic efficiency for cervical cancer.

Keywords

Cervical cancer; Cervical intraepithelial neoplasia; miR-146b-5p; miR-192-5p; miR-122-5p

1. Introduction

Cervical cancer is the most common gynecological malignant tumor threatening women's health and life. According to the World Health Organization (WHO) estimation, in 2018, there were about 570,000 new cervical cancer cases and 311,000 deaths worldwide [1], and in 2020, just two years later, the estimated new cervical cancer cases and deaths increased to 604,000 and 342,000 worldwide [2].

Human papillomavirus (HPV) infection, a special type of sexually transmitted disease, is an important cause of cervical intraepithelial neoplasia (CIN) and cervical cancer and was reported to be the main etiology in nearly 80% of all cervical cancer cases [3]. In recent decades, the widespread application of cervical cytology screening has enabled the early detection and treatment of cervical cancer and its precancerous lesions. In developed countries, the expanded production and vaccination campaigns of the HPV vaccine have significantly reduced cervical cancer incidence rate and mortality.

MicroRNA (miRNA) is an endogenous single-stranded non-coding RNA with 21∼25 nucleotides in length and multiple regulatory functions in eukaryotes and viruses. It is highly conserved in evolution [4]. Studies have shown that miRNA plays an important regulatory role in cell proliferation, apoptosis, differentiation, metabolism and tumorigenesis [5–7]. In recent years, the relationship between miRNA and cervical cancer has also been widely investigated [8–11].

Pathological diagnosis remains the gold standard for the diagnosis of cervical cancer. However, the high incidence rate and mortality of cervical cancer make it urgent to use newer experimental methods as attempts to improve the early detection and diagnosis of the disease. Thus, it could be of great significance to analyze the mechanisms of cell carcinogenesis at the molecular level and find novel and more effective biomarkers of cervical cancer for its early clinical diagnosis and prevention.

In an earlier study, we reported our preliminary sequencing results and serum miRNAs expression profile in cervical can-

cer, cervical lesions and healthy controls *via* Solexa sequencing. Further analysis of the screening results identified three differentially expressed and potentially significant miRNAs: hsa-miR-146b-5p (miR-146b-5p), hsa-miR-192-5p (miR-192-5p) and hsa-miR-122-5p (miR-122-5p). Thus, in this study, we evaluated the significance of these miRNAs in early cervical cancer diagnosis by detecting their expression levels in the serum samples of CIN and cervical cancer patients.

2. Materials and methods

2.1 Patients and controls

For this study, the data of 229 cases, comprising 61 CIN cases, 69 cervical cancer cases and 99 controls, were retrieved and assessed. All serum samples were collected at the Chinese PLA (People's Liberation Army) General Hospital. All patients were diagnosed with CIN or cervical cancer and were hospitalized and treated at the Department of Gynecology of our hospital from March 2017 to May 2019. The control group comprised individuals who received routine physical examinations in our hospital during the same period.

The pathological results were confirmed by histopathology after tumor resection, and the tumor was staged according to the standards of the International Federation of Obstetrics and Gynecology [12]. For patients unsuitable for surgical treatment, the histopathological characteristics and tumor stages were confirmed by histobiopsy and imaging.

2.2 Serum sample collection

The serum samples of all patients in this study were collected before any treatment procedure (*i.e.*, surgery, chemotherapy or radiotherapy). Venous blood from all participants on an empty stomach was drawn in the morning into vacuum blood collection vessels (Greiner bio-one VACUETTE® Blood Collection Tubes), centrifuged at 3500 rpm (2390 × g) for 7 minutes to separate serum, and followed by a 15 minutes high-speed centrifugation at 12,000 × g. Lastly, the supernatant sera were stored at −80 ℃ ultra-low temperature refrigerator for further analysis.

2.3 miRNA extraction

Total RNA isolation from the serum was performed using the Ambion® mirVana™ miRNA Isolation Kit (Cat# AM1560, Ambion, Austin, TX, USA) following the manufacturer's instructions. RNA concentration was measured using the Thermo Scientific NanoDrop 2000C Spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA) at the 260 nm (A260) absorbance.

2.4 Quantitative Real-Time PCR (RT-qPCR)

First, 5 μL of total RNA was reversed transcribed into cDNA using the TIANGEN miRNA First Strand cDNA Synthesis Kit (KR211, TIANGEN Biotech Co., Ltd., Beijing, China). Quantitative real-time PCR (RT-qPCR) was performed on the 7500 Fast Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). Then, 2 μL of reversed transcribed cDNA was used for the amplification reaction. The TIANGEN miRcute Plus miRNA qPCR Detection Kit (KR211, TIANGEN Biotech Co., Ltd., Beijing, China) was used for the RT-qPCR amplification of the miRNA (SYBR Green, FP411). U6 snRNA was used as the endogenous control. The primers of the investigated three miRNAs in RT-qPCR and U6 endogenous control were purchased from TIANGEN Biotech (Beijing) Co., Ltd. The final reaction volume was 20 μL. All the reactions were conducted in triplicate.

2.5 miRNA relative expression analysis (fold change)

The relative expression levels of the markers were analyzed using the equation: $2^{-\Delta\Delta Ct}$ [13], whereby $\Delta\Delta\mathrm{Ct} = (\mathrm{Ct}_{target\ miRNA} - \mathrm{Ct}_{U6})_{Patient} - (\mathrm{Ct}_{target\ miRNA} - \mathrm{Ct}_{U6})_{Control}$.

2.6 Detection of tumor markers

The tumor markers of alpha-fetoprotein (AFP), carcinoembryonic antigen (CEA), carbohydrate antigen 125 (CA125), carbohydrate antigen 199 (CA199) and carbohydrate antigen 724 (CA724) were detected by Roche Cobas8000-E602 automatic electrochemiluminescence analyzer (Cobas8000, Roche Diagnostics, Basel, Switzerland). The reagents were provided by Roche Diagnostics (Shanghai) Co., Ltd. Additionally, the serum levels of squamous cell carcinoma (SCC) antigen were detected by the Abbott Architect i2000SR automatic immune analyzer (Abbott Diagnostics, Lake Forest, IL, USA).

2.7 Statistical analysis

Statistical analyses were performed using the SPSS statistical software version 17.0 (SPSS Inc., Chicago, IL, USA). Quantitative data were uniformly expressed as median (Q25–Q75) and assessed using the Mann-Whitney U test. For the three miRNAs, we constructed a subject operating characteristic (ROC) curve and calculated the corresponding area under the ROC curve (AUC) to evaluate the specificity and sensitivity of cervical intraepithelial neoplasia and cervical cancer prediction. Spearman correlation analysis was used for correlation analysis. p value < 0.05 was used to denote statistical significance.

3. Results

3.1 Basic clinical data of controls and patients

Clinical data were queried from the medical record query system of the Chinese PLA General Hospital. The patients in the disease group had confirmed diagnoses of CIN and/or cervical cancer after clinicopathological assessment. All study participants had no other major diseases, including serious cardiovascular diseases, autoimmune diseases, nervous system diseases and other concurrent cancers. There were 130 cases in the disease group, comprising 61 CIN cases and 69 cervical cancer cases. The controls comprised 99 healthy women. Of the investigated cases, 52.3% of patients with cervical diseases come to our hospital because of vaginal bleeding. Clinical data of patients based on gynecological examinations were

also retrieved and assessed. Tumor marker assessment showed that the levels of CEA and SCC in the disease group were significantly higher than controls (Table 1).

3.2 Comparison of clinical data between CIN and cervical cancer

The age, BMI (body mass index), CEA, SCC, number of births and age at menarche of patients from the cervical cancer group were significantly higher than the CIN group ($p < 0.05$). The percentages of vaginal bleeding and contact bleeding in patients with cervical cancer were higher than CIN, 72.5% and 73.9%, respectively, and the lesions of 75.4% of the cervical cancer patients were in the anterior part of the uterus. Other differences between CIN and cervical cancer, such as gynecological examination, are shown in Table 2.

3.3 Serum miRNA expression in cervical diseases and controls

Compared with the controls, the relative expressions of serum miR-146b-5p ($p < 0.001$), miR-192-5p ($p < 0.001$) and miR-122-5p ($p = 0.037$) were significantly increased in the cervical diseases group. Additionally, the relative expression levels of serum miR-146b-5p, miR-192-5p and miR-122-5p in the cervical disease group were 1.80, 2.52 and 2.85 times, respectively (Fig. 1).

3.4 Comparison of serum miRNA relative expression between CIN and cervical cancer

The relative expression of serum miR-146b-5p, miR-192-5p and miR-122-5p in patients with cervical cancer was significantly higher than in controls, with the relative expression level being 2.04, 2.67 and 4.33 times higher than the control groups, respectively. For patients with CIN, the relative expression of serum miR-146b-5p and miR-192-5p was significantly higher by 1.57 and 2.36 times than controls, respectively. However, we observed no significant difference in the relative expression of serum miRNA between CIN and cervical cancer patients (Fig. 2).

Furthermore, we compared the relative expression of the three serum miRNA levels of CIN and early cervical cancer (classified as FIGO stage I). The results showed that the degree of miR-146b-5p in FIGO stage I patients was significantly higher than that in CIN patients, while the level of miR-192-5p and miR-122-5p was not significantly different between the two groups. The miR-146b-5p, miR-192-5p and miR-122-5p levels in patients classified as FIGO stage I were significantly higher than in controls (Fig. 3).

3.5 ROC curve analysis of serum miR-146b-5p, miR-192-5p and miR-122-5p and tumor markers for diagnosing CIN

The diagnostic efficacy of CIN was evaluated by ROC curve analysis, which showed that the AUC of 0.646 (95% CI: 0.511–0.781, $p = 0.034$), 0.839 (95% CI: 0.751–0.927, $p < 0.001$) and 0.568 (95% CI: 0.422–0.714, $p = 0.325$) for miR-146b-5p, miR-192-5p and miR-122-5p, respectively. The sensitivity and specificity of miR-192-5p were 0.720 and 0.836, respectively, which were superior compared to the other two miRNAs (Fig. 4 and Table 3). Comparatively, the AUC for CEA was 0.542 (95% CI: 0.449–0.636, $p = 0.369$) and that of SCC was 0.603 (95% CI: 0.449–0.758, $p = 0.177$).

3.6 ROC curve analysis of serum miR-146b-5p, miR-192-5p and miR-122-5p and tumor markers for diagnosing cervical cancer

The diagnostic efficacy of cervical cancer was evaluated by ROC curve analysis, which showed that the AUC of miR-146b-5p was 0.720 (95% CI: 0.607–0.833, $p < 0.001$), while that of miR-192-5p was 0.775 (95% CI: 0.669–0.880, $p < 0.001$) and that of miR-122-5p was 0.644 (95% CI: 0.523–0.765, $p = 0.017$), respectively. The specificity of miR-146b-5p and miR-192-5p was 0.918 and 0.902, respectively, and the sensitivity of miR-122-5p was the best, at 0.697. Next, we included the three serum miRNAs, CEA and SCC into the binary logistic regression analysis using the Conditional forward method. After screening, the three serum miRNAs and SCC were entered into the model, which generated prediction probability using ROC curve analysis. The AUC under the ROC curve of the three miRNAs combined with SCC for diagnosing cervical cancer was 0.840 and demonstrated a sensitivity and specificity of 0.632 and 0.957, respectively (Fig. 5 and Table 4).

3.7 Correlation analysis of three serum miRNAs

Correlation analysis showed that serum miR-146b-5p was positively correlated with miR-192-5p ($r = 0.629$, $p < 0.001$) and miR-122-5p ($r = 0.711$, $p < 0.001$). Additionally, we also observed a positive correlation between miR-192-5p and miR-

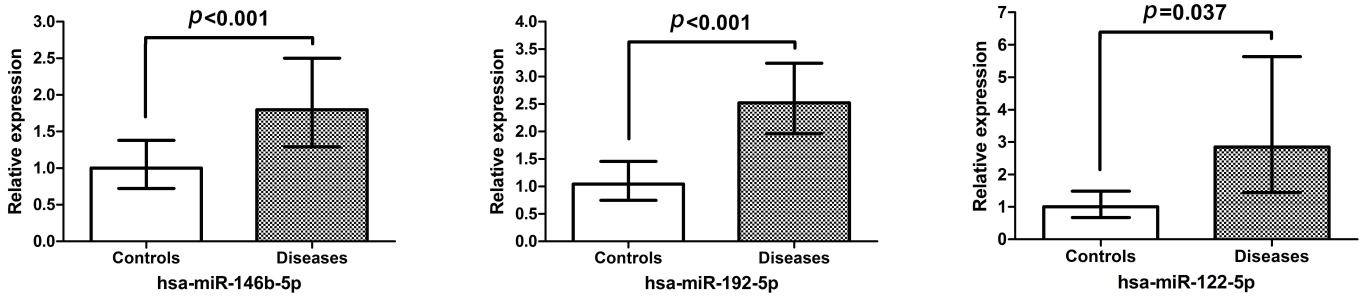

FIGURE 1. Serum miRNA expression in cervical diseases (CIN and cervical cancer) and controls.

TABLE 1. Demographic and clinical characteristics of the patients and controls.

Characteristics	Classification	Patients (130 cases)	Controls (99 cases)	*p* value
Age (yr, M (Q25–Q75))		45 (38–53)	29 (24–42)	<0.001
Tumor markers				
	AFP (μg/L, M (Q25–Q75))	3.03 (1.47–4.04)	2.59 (1.93–3.42)	0.864
	CEA (μg/L, M (Q25–Q75))	1.48 (1.09–3.16)	1.08 (0.77–1.62)	0.002
	CA125 (u/mL, M (Q25–Q75))	14.94 (9.85–21.63)	14.99 (11.65–21.08)	0.953
	CA199 (u/mL, M (Q25–Q75))	9.65 (7.19–13.96)	11.33 (7.13–16.10)	0.698
	CA724 (u/mL, M (Q25–Q75))	2.26 (1.31–6.31)	1.87 (1.08–4.73)	0.324
	SCC (ng/mL, M (Q25–Q75))	1.2 (0.7–3.7)	0.7 (0.5–1.0)	<0.001
BMI (kg/m^2, M (Q25–Q75))		23.0 (21.5–25.0)		
Body Surface Area (m^2, M (Q25–Q75))		1.64 (1.57–1.72)		
Reason for Seeking Medical Care				
	Vaginal bleeding	52.3% (68/130)		
	Physical examination	33.8% (44/130)		
	Cervical lesions	6.9% (9/130)		
	Abnormal vaginal discharge	3.8% (5/130)		
	Others	3.1% (4/130)		
Number of pregnancies (n, M (Q25–Q75))		3 (2–4)		
Number of births (n, M (Q25–Q75))		1 (1–2)		
Age at menarche (yr, M (Q25–Q75))		14 (14–16)		
Menopausal status				
	Postmenopausal	30.8% (40/130)		
	Premenopausal	69.2% (90/130)		
CIN classification (%)		46.9% (61/130)		
	CIN I	3.3% (2/61)		
	CIN I–II	3.3% (2/61)		
	CIN II	13.1% (8/61)		
	CIN II–III	26.2% (16/61)		
	CIN III	54.1% (33/61)		
Cervical Cancer (FIGO Stage)		53.1% (69/130)		
	Stage I	58.0% (40/69)		
	Stage II	40.6% (28/69)		
	Stage III	1.4% (1/69)		
Gynecological examination				
Examination of the vulva				
	Atrophy	3.1% (4/130)		
	Varicose Veins of the Vulva	0.7% (1/130)		
Vaginal examination				
	Disappearance of fornix	1.5% (2/130)		
	Poor ductility	1.5% (2/130)		
	Abnormal vaginal mucosa	2.3% (3/130)		
	Vaginal odor	12.3% (16/130)		
	Bloody secretion	12.3% (16/130)		

TABLE 1. Continued.

Characteristics	Classification	Patients (130 cases)	Controls (99 cases)	*p* value
Cervical examination				
	Cervical erosion	46.9% (61/130)		
	Cauliflower-like tumor	23.1% (30/130)		
	Hypertrophy	30% (39/130)		
	Atrophy	2.3% (3/130)		
	Cervical cyst	9.2% (12/130)		
	Contact bleeding	50.8% (66/130)		
	Cervical bleeding	20.8% (27/130)		
Uterine examination				
	Anterior uterus	66.9% (87/130)		
	Median uterus	12.3% (16/130)		
	Posterior uterus	20.8% (27/130)		
	Atrophy	8.5% (11/130)		
	Enlarge	11.5% (15/130)		
	Hypertrophy	1.5% (2/130)		
	Surface irregularity	11.5% (15/130)		
	Poor mobility	6.2% (8/130)		
	Tenderness	11.5% (15/130)		
Uterine adnexa examination				
	Adnexal mass	2.3% (3/130)		
	Tenderness	0.8% (1/130)		
Involving glands		40.0% (52/130)		
HPV test	Positive	21.5% (28/130)		

AFP: alpha-fetoprotein; CEA: carcinoembryonic antigen; CA: carbohydrate antigen; SCC: squamous cell carcinoma antigen; CIN: cervical intraepithelial neoplasia; HPV: human papillomavirus; BMI: body mass index; FIGO: International Federation of Gynaecology and Obstetrics.

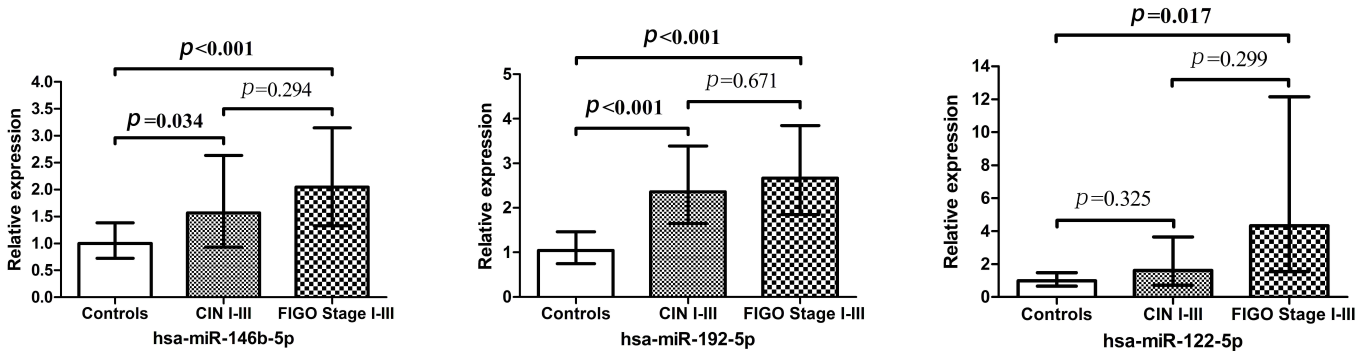

FIGURE 2. Comparison of serum miRNA relative expression between CIN and cervical cancer. CIN: cervical intraepithelial neoplasia; FIGO: International Federation of Gynaecology and Obstetrics.

TABLE 2. Comparison of clinical data between cervical intraepithelial neoplasia (CIN) and cervical cancer (CC).

Characteristics	Classification	CIN (n = 61)	Cervical cancer (n = 69)	*p* value
Age (yr, M (Q25–Q75))		42 (33.0–49.5)	47 (39.5–56.5)	0.006
BMI (kg/m^2, M (Q25–Q75))		22.1 (21.1–24.2)	24.0 (22.2–26.3)	0.003
Body Surface Area (m^2) (m^2, M (Q25–Q75))		1.62 (1.56–1.67)	1.66 (1.57–1.73)	0.118
Number of pregnancies (n, M (Q25–Q75))		3 (2–4)	3 (2–4)	0.445
Number of births (n, M (Q25–Q75))		1 (1–1.5)	1 (1–2)	0.028
Age at menarche (yr, M (Q25–Q75))		14 (13–15)	15 (14–17)	0.011
Menopausal status				
	Postmenopausal	19.7% (12/61)	40.6% (28/69)	0.013
	Premenopausal	80.3% (49/61)	59.4% (41/69)	
Tumor markers				
	AFP (μg/L)	2.31 (1.36–4.11)	3.03 (1.70–4.04)	0.770
	CEA (μg/L)	1.16 (0.95–1.44)	1.87 (1.26–3.59)	0.046
	CA125 (u/mL)	16.84 (10.83–20.54)	14.75 (9.83–28.91)	0.887
	CA199 (u/mL)	9.83 (7.73–11.96)	9.46 (7.06–15.50)	0.797
	CA724 (u/mL)	3.07 (1.38–5.79)	2.07 (1.31–6.70)	0.730
	SCC (ng/mL)	0.9 (0.5–1.2)	1.4 (0.8–4.6)	0.006
Reason for Seeking Medical Care				
	Vaginal bleeding	29.5% (18/61)	72.5% (50/69)	<0.001
	Physical examination	52.4% (32/61)	17.4 (12/69)	<0.001
	Cervical lesions	11.5% (7/61)	2.9% (2/69)	0.082
	Abnormal vaginal discharge	3.3% (2/61)	4.3% (3/69)	1.000
	Others	3.3% (2/61)	2.9% (2/69)	1.000
Gynecological examination				
Examination of the vulva				
	Atrophy	0% (0/61)	5.8% (4/69)	0.122
	Varicose Veins of the Vulva	1.6% (1/61)	0% (0/69)	0.469
Vaginal examination				
	Disappearance of fornix	0% (0/61)	2.9% (2/69)	0.498
	Poor ductility	1.6% (1/61)	1.4% (1/69)	1.000
	Abnormal vaginal mucosa	0% (0/61)	4.3% (3/69)	0.247
	Vaginal odor	6.6% (4/61)	17.4% (12/69)	0.058
	Bloody secretion	1.6% (1/61)	21.7% (15/69)	<0.001
Cervical examination				
	Cervical erosion	62.3% (38/61)	33.3% (23/69)	0.001
	Cauliflower-like tumor	1.6% (1/61)	42.0% (29/69)	<0.001
	Hypertrophy	37.7% (23/61)	23.2% (16/69)	0.086
	Atrophy	1.6% (1/61)	2.9% (2/69)	1.000
	Cervical cyst	14.8% (9/61)	4.3% (3/69)	0.066
	Contact bleeding	24.6% (15/61)	73.9% (51/69)	<0.001
	Cervical bleeding	14.8% (9/61)	26.1% (18/69)	0.133

TABLE 2. Continued.

Characteristics	Classification	CIN (n = 61)	Cervical cancer (n = 69)	*p* value
Uterine examination				
	Anterior uterus	57.4% (35/61)	75.4% (52/69)	0.042
	Median uterus	16.4% (10/61)	8.7% (6/69)	0.195
	Posterior uterus	26.2% (16/61)	15.9% (11/69)	0.194
	Atrophy	4.9% (3/61)	11.6% (8/69)	0.216
	Enlarge	9.8% (6/61)	13.0% (9/69)	0.596
	Hypertrophy	1.6% (1/61)	1.4% (1/69)	1.000
	Surface irregularity	9.8% (6/61)	13.0% (9/69)	0.596
	Poor mobility	0% (0/61)	11.6% (8/69)	0.007
	Tenderness	1.6% (1/61)	20.3% (14/69)	0.001
Uterine adnexa examination				
	Adnexal mass	1.6% (1/61)	2.9% (2/69)	1.000
	Tenderness	1.6% (1/61)	0% (0/69)	0.469
Involving glands		60.7% (37/61)	21.7% (15/69)	<0.001
HPV test	Positive	29.5% (18/61)	14.5% (10/69)	0.054

AFP: alpha-fetoprotein; CEA: carcinoembryonic antigen; CA: carbohydrate antigen; SCC: squamous cell carcinoma antigen; HPV: human papillomavirus.

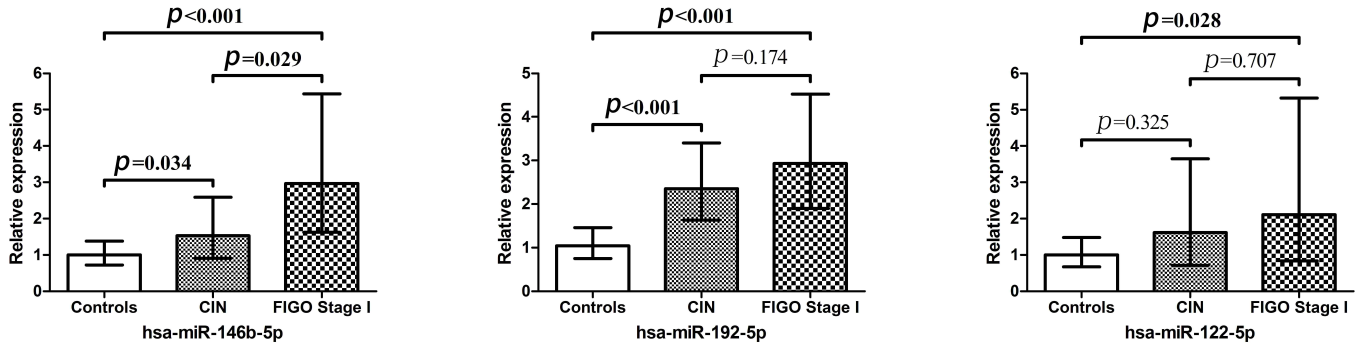

FIGURE 3. Comparison of serum miRNA relative expression between CIN and FIGO Stage I. CIN: cervical intraepithelial neoplasia; FIGO: International Federation of Gynaecology and Obstetrics.

TABLE 3. Diagnostic efficacy of three serum miRNAs and tumor markers in diagnosing CIN.

Characteristics	AUC	SE	AUC 95% CI		Sensitivity	Specificity	*p* value
			Lower	Upper			
hsa-miR-146b-5p	0.646	0.069	0.511	0.781	0.400	0.869	0.034
hsa-miR-192-5p	0.839	0.045	0.751	0.927	0.720	0.836	<0.001
hsa-miR-122-5p	0.568	0.074	0.422	0.714	0.240	0.967	0.325
CEA (μg/L)	0.542	0.048	0.449	0.636	0.344	0.788	0.369
SCC (ng/mL)	0.603	0.079	0.449	0.758	0.500	0.731	0.177

SE: Standard Error; CI: Confidence Interval; AUC: area under the ROC curve. p value < 0.05 was considered significant.

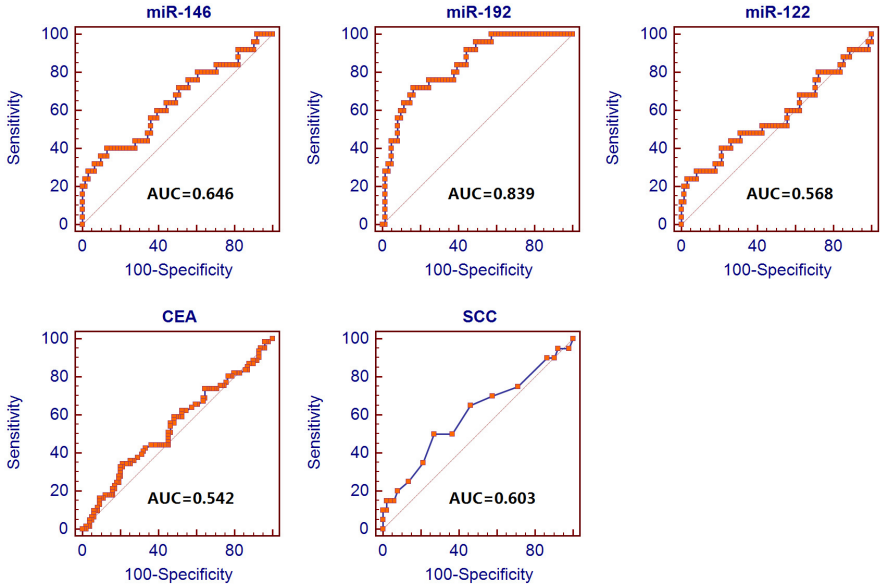

FIGURE 4. Receiver operating characteristic (ROC) curve analysis of three serum miRNAs and tumor markers in diagnosing CIN. AUC: area under the ROC curve; CEA: carcinoembryonic antigen; SCC: squamous cell carcinoma antigen.

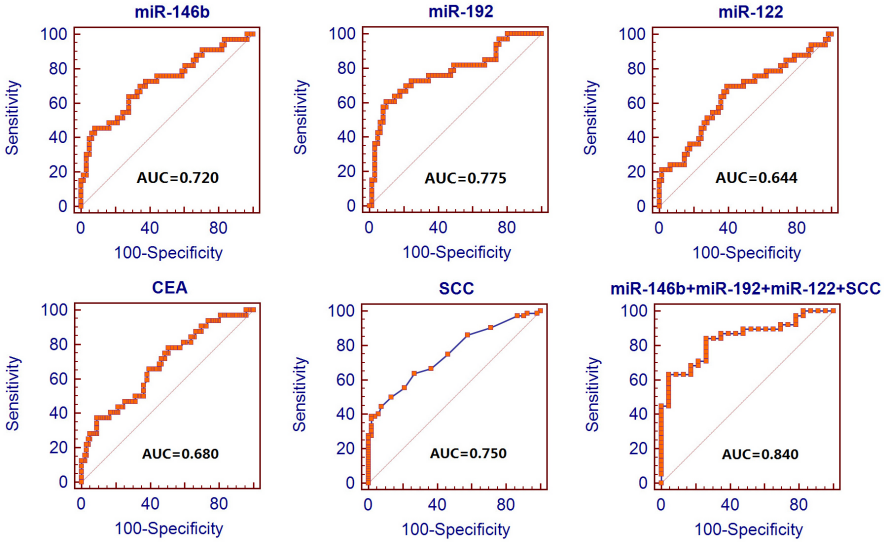

FIGURE 5. Receiver operating characteristic (ROC) curve analysis of the proposed three serum miRNAs and tumor markers in diagnosing cervical cancer. AUC: area under the ROC curve; CEA: carcinoembryonic antigen; SCC: squamous cell carcinoma antigen.

TABLE 4. Diagnostic efficacy of three serum miRNAs and tumor markers in diagnosing cervical cancer.

Characteristics	AUC	SE	AUC 95% CI		Sensitivity	Specificity	*p* value
			Lower	Upper			
miR-146b-5p	0.720	0.058	0.607	0.833	0.455	0.918	<0.001
miR-192-5p	0.775	0.054	0.669	0.880	0.606	0.902	<0.001
miR-122-5p	0.644	0.062	0.523	0.765	0.697	0.607	0.017
CEA (μg/L)	0.680	0.055	0.573	0.787	0.375	0.909	0.002
SCC (ng/mL)	0.750	0.043	0.666	0.834	0.389	0.981	<0.001
miR-146b + miR-192 + miR-122 + SCC	0.840	0.050	0.742	0.938	0.632	0.957	<0.001

SE: Standard Error; CI: Confidence Interval; AUC: area under the ROC curve; CEA: carcinoembryonic antigen; SCC: squamous cell carcinoma antigen. p value < 0.05 was considered significant.

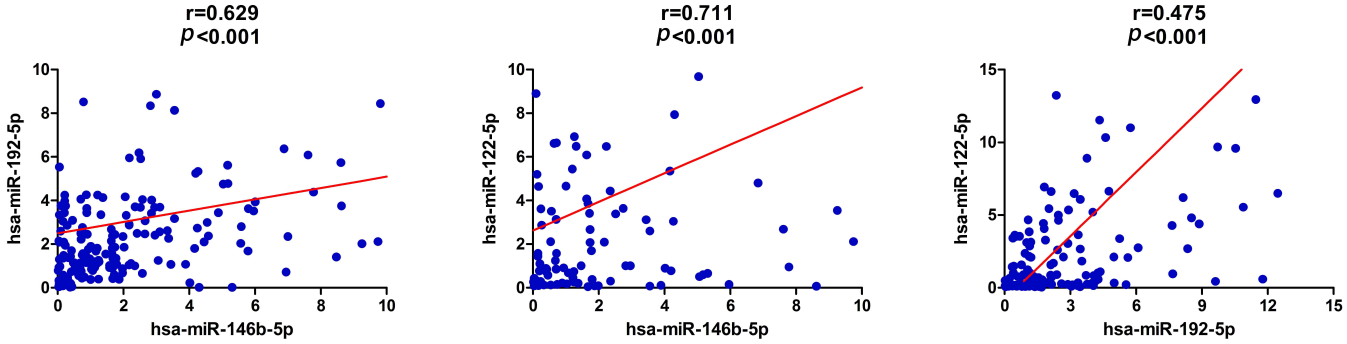

FIGURE 6. Correlation analysis of miR-146b-5p, miR-192-5p and miR-122-5p.

122-5p ($r = 0.475$, $p < 0.001$) (Fig. 6).

4. Discussion

The etiologies of cervical cancer have been shown to be mainly related to persistent HPV infection, sexual behavior and the number of deliveries. It is considered to be the most common gynecological malignant tumor in women [2, 14]. In recent years, the age for cervical cancer diagnosis has tended to be younger, and the universal application of cervical cytology screening has enabled the early detection and treatment of cervical cancer and precancerous lesions, which have led to a significant decrease in its incidence and mortality rates [15, 16]. However, the sensitivity of standard cervical cytology has remained low, with the detection of CIN being quite challenging, which has led to an urge to research more sensitive molecular diagnostic methods to improve their detection efficiency and accuracy. In this study, we assessed the differential expression of serum miR-146b-5p, miR-192-5p and miR-122-5p between CIN and cervical cancer patients and constructed the ROC curve for each miRNA. Additionally, we calculated the AUC under the ROC and evaluated the specificity and sensitivity of three miRNAs of interest to determine their significance for early cervical cancer diagnosis.

miRNAs are evolutionarily conserved non-coding small endogenous RNA molecules [17]. They can inhibit translation or induce mRNA degradation by pairing with 30 untranslated regions (30UTR) of specific target messenger RNAs (mRNAs) and play an important role as post-transcriptional gene regulators [18–20]. Studies have shown that miRNAs are also key in many cancer-related biological processes [21–23] and demonstrated important associations with cancer progression and metastasis. They were also reported to play a part in tumor inhibition or carcinogenesis [24, 25]. These indicate the potential promise of using miRNAs for diagnosing and predicting various diseases. Quantitative PCR is the most commonly used method to measure miRNA expression [26]. A large number of studies have shown that the abnormal expression of miRNA was closely related to the proliferation, apoptosis and invasion of cervical cancer cells, making miRNAs a new target for the diagnosis, treatment and prognosis evaluation of cervical cancer [27–32].

Although the pathogenesis of tumors is different, tumors are usually characterized by unlimited proliferation and rapid metastasis. In this study, we observed that the expression levels of three serum miRNAs, miR-146b-5p, miR-192-5p and miR-122-5p, were upregulated in cervical cancer patients compared with controls. *In vitro* investigations showed that miR-146b-5p inhibited the G0/G1 proliferation cycle and downregulated the expression of HPV16 E7 in CaSki cervical cancer cells [33]. miR-146b-5p inhibited the growth and metastasis of various types of cancers [34]. Additionally, it was also reported that miR-146b-5p was associated with breast cancer, pancreatic cancer and thyroid cancer. miR-146b-5p was shown to downregulate BRCA1 (breast cancer susceptibility gene 1) expression in triple negative sporadic breast cancer [35], and miR-146b-5p targeted MMP16 to inhibit the migration and invasion of pancreatic cancer cells [36] and promoted metastasis and induced the epithelial-mesenchymal transformation of thyroid carcinoma by targeting ZNRF3 (zinc ring finger 3) [37]. miR-192-5p inhibited the proliferation and invasion of cervical cancer by targeting TRPM7 [38]. Previous studies confirmed that miR-192-5p inhibited tumorigenesis in many different cancers. The upregulation of miR-192-5p was shown to downregulate the activity of BMPR2 (bone morphogenetic protein type II) and inhibit the RhoA-ROCK-LIMK2 pathway to prevent the growth of colon cancer cells [39]. Additionally, the downregulation of mir-192-5p played a tumor-suppressive role in pancreatic ductal adenocarcinoma and demonstrated promising prospects as a biomarker for its diagnosis and prognosis evaluation [40]. Moreover, miR-192-5p was shown to inhibit the proliferation of lung cancer cells and led to apoptosis by binding to retinoblastoma [41]. The ROC analysis results of our present study showed that miR-146b-5p (AUC = 0.646) and miR-192-5p (AUC = 0.839) had certain diagnostic values in diagnosing CIN. In particular, serum miR-192-5p had the best diagnostic efficiency, with a sensitivity and specificity of 0.720 and 0.836, respectively. Recent studies showed that miR-192 could be a potential biomarker for the early detection of cervical cancer [42], which was consistent with the results of our study. Also, previous studies found that miR-122-5p inhibited the development of cervical cancer by targeting the oncogene RAD21 (human homolog of Schizosaccharomyces pombe radiation sensitive mutant 21) [43] and blocked the *HPV E6* gene and increased interferon signaling by interfering with cytokine signaling inhibitors in SiHa cells [44]. miR-122-5p was proven to play an anti-tumor role in many cancers, such as gastric cancer, liver cancer and melanoma [45–47].

Studies have also shown that the overexpression of miR-122-5p could trigger the cell cycle arrest and apoptosis of cancer cells by reducing the expression of Bcl-W (B-cell lymphoma w) and/or CCNG1 (cyclin G1) [48]. All these findings suggest that miR-146b-5p, miR-192-5p and miR-122-5p play a key role as signaling molecules in physiological and pathological events.

Surgery is usually recommended for patients with severe CIN. Patients who do not have fertility requirements can choose from cervical conization or total hysterectomy. We also focused on whether the investigated three miRNAs could be used as diagnostic markers for early cervical cancer. Our results showed that compared with controls, the relative expressions of serum miR-146b-5p and miR-192-5p in CIN patients were significantly higher by 1.57 and 2.36 times, respectively. Although there was no difference in the relative expression of miR-146b-5p, miR-192-5p and miR-122-5p between patients with CIN and cervical cancer, the relative expression demonstrated a gradual upward trend from CIN to cervical cancer (Fig. 2). Another study showed no difference in the relative expression of four serum miRNAs (miR-21, miR-200a, miR-25, miR-486-5p) between CIN and cervical cancer, which was concordant with our results to a certain extent [49]. Additionally, we further analyzed the distinction of three miRNAs in the serum of CIN and cervical cancer patients classified as FIGO stage I, and the results showed that the expression of miR-146b-5p in the serum of the cervical cancer patients was significantly higher than that of CIN patients and controls (Fig. 3). By analyzing the expression of serum miRNAs in patients with CIN and cervical cancer, we found that the three miRNAs had certain significance for early cervical cancer diagnosis. The results of our study also showed that when the three miRNAs were combined with CEA and SCC to diagnose cervical cancer, the best AUC of miR-146b-5p, miR-192-5p, miR-122-5p and SCC was 0.840, and the corresponding sensitivity and specificity were 0.632 and 0.957 respectively. Our result proved that the combined diagnosis of the three miRNAs with SCC had certain synergizing effects. ROC curve analysis showed that compared with any single miRNA-based detection, *i.e.*, SCC and CEA, the combination of our proposed three miRNAs and SCC had great clinical value for the diagnosis of cervical cancer.

5. Conclusions

In summary, the relative expression of serum miR-146b-5p, miR-192-5p and miR-122-5p in cervical cancer was significantly increased, and in CIN, the serum level miR-146b-5p and miR-192-5p were highly expressed. Serum miR-146b-5p, miR-192-5p and miR-122-5p demonstrated promising efficacies as noninvasive and potentially accurate biomarkers for the diagnosis of cervical cancer and showed certain value for the early diagnosis of cervical cancer, in particular, miR-146b-5p and miR-192-5p. Additionally, the combined detection of miR-146b-5p, miR-192-5p and miR-122-5p with SCC could improve the diagnostic efficiency of cervical cancer. Therefore, the identification and classification of cervical cancer-specific miRNAs could play an important role in improving the detection, treatment and outcomes of patients with CIN and cervical cancer.

AVAILABILITY OF DATA AND MATERIALS

The datasets generated and/or analyzed during the current study are not publicly available due to (reason why data are not public) but are available from the corresponding author on reasonable request.

AUTHOR CONTRIBUTIONS

JZN and XYW—Conception; XZ, MJZ and XJL—Extraction and amplification of RNA; DGL and MLJ—Interpretation or analysis of data; XJL—Preparation of the manuscript; XZ—Revision for important intellectual content; XYW—Supervision.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

All participants involved in this study provided written informed consent, and this research was approved by the Ethics Committee of Chinese PLA General Hospital (no. S2015-127-01).

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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