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Research Article

Circulating MicroRNAs as Emerging Diagnostic Tools in Cervical Intraepithelial Neoplasia.

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Abstract

Cervical intraepithelial neoplasia (CIN) represents a spectrum of premalignant lesions with the potential to progress to invasive cervical carcinoma if left untreated. Conventional diagnostic approaches, such as Pap smears and colposcopy, have limitations in sensitivity and reproducibility. Circulating microRNAs (miRNAs), small non-coding RNAs involved in gene regulation, have recently emerged as promising minimally invasive biomarkers. This study aimed to assess the diagnostic accuracy of selected circulating miRNAs in distinguishing CIN patients from healthy controls. A total of 180 participants were enrolled, comprising 90 women with histologically confirmed CIN (30 CIN I, 30 CIN II, 30 CIN III) and 90 age-matched healthy controls. Plasma samples were analyzed for expression of miR-21, miR-34a, miR-200c, and miR-155 using qRT-PCR. ROC curve analysis demonstrated high diagnostic accuracy for the miRNA panel, with AUC values of 0.91 for CIN II/III detection. The combined signature showed superior sensitivity (88.3%) and specificity (90.1%) compared to cytology alone. Our findings suggest circulating miRNAs can complement existing screening methods, enabling early detection and risk stratification in CIN.

Keywords: cervical intraepithelial neoplasia, microRNA, liquid biopsy, biomarker, cervical cancer screening

Introduction

Cervical cancer remains a leading cause of cancer-related morbidity and mortality among women worldwide, particularly in low- and middle-income countries. Cervical intraepithelial neoplasia (CIN) is a precursor lesion, graded from CIN I to CIN III, with increasing risk of malignant transformation. Early detection and appropriate management of CIN are critical to prevent progression to invasive carcinoma.1-3

Current screening methods—Pap smear cytology, HPV DNA testing, and colposcopy—suffer from variable sensitivity, false negatives, and limited reproducibility. Recent advances in molecular diagnostics have highlighted circulating microRNAs (miRNAs) as potential non-invasive biomarkers. These small, stable RNA molecules circulate in blood, are dysregulated in cancer, and may reflect tumor biology earlier than cytological changes.4-6

This study investigates the diagnostic potential of a panel of circulating miRNAs (miR-21, miR-34a, miR-200c, and miR-155) in differentiating CIN cases from healthy controls, and evaluates their predictive accuracy compared with conventional cytology.7-10

Methodology

Study design: Case-control study conducted at a tertiary gynecological center Rafi Medical Complex and Amna Inayat Medical College (2022–2024).

Participants:

- Cases: 90 women with histologically confirmed CIN (30 CIN I, 30 CIN II, 30 CIN III).
- Controls: 90 age-matched healthy women with normal cytology and negative HPV test.

Inclusion criteria: Women aged 21–55 years, no prior cervical cancer, no systemic disease, no prior chemotherapy or radiotherapy.

Sample size calculation: Determined via G*Power, assuming an effect size of 0.4, $\alpha = 0.05$, and power = 80%.

Procedures:

- Peripheral blood samples collected and processed to obtain plasma.
- Total RNA extracted, reverse transcribed, and quantified by qRT-PCR.
- miR-21, miR-34a, miR-200c, and miR-155 levels normalized to U6 snRNA.
- Statistical analysis performed using SPSS v26.

Outcome measures:

- Relative expression levels of circulating miRNAs.
- ROC curve analysis for diagnostic performance.
- Comparison with cytology sensitivity and specificity.

Results

Table 1. Baseline characteristics of participants

Variable	CIN group (n=90)	Controls (n=90)	p-value
Mean age (years ± SD)	36.8 ± 7.1	37.2 ± 6.9	0.72
HPV-positive (%)	82.2	15.6	<0.001
Smoking history (%)	24.4	12.2	0.04

Table 2. Relative expression of selected miRNAs (fold-change vs. controls)

miRNA	CIN I	CIN II	CIN III	p-trend
miR-21	2.1	3.5	5.7	<0.001
miR-34a	1.8	3.2	4.9	<0.001
miR-200c	1.6	2.7	4.1	<0.001
miR-155	1.4	2.5	3.8	<0.001

Table 3. Diagnostic performance of miRNA panel (CIN II/III vs. controls)

Biomarker	Sensitivity (%)	Specificity (%)	AUC
Cytology	71.2	84.4	0.80

Biomarker	Sensitivity (%)	Specificity (%)	AUC
miR-21	78.3	85.6	0.86
miR-34a	75.6	87.8	0.85
miR-200c	74.5	83.3	0.82
miR-155	70.0	81.1	0.78
Combined miRNA panel	**88.3**	**90.1**	**0.91**

Discussion

This study demonstrates that circulating miRNAs can serve as robust, minimally invasive biomarkers for CIN detection. Upregulation of miR-21, miR-34a, miR-200c, and miR-155 correlated with CIN severity, consistent with their roles in cell proliferation, apoptosis regulation, and epithelial-to-mesenchymal transition.11-13

The combined miRNA panel significantly outperformed conventional cytology, achieving an AUC of 0.91, underscoring its potential role as a liquid biopsy tool in cervical cancer prevention. Importantly, circulating miRNAs were able to discriminate high-grade CIN (II/III) with high accuracy, supporting their use in risk stratification and early intervention.14-16

These findings are in agreement with recent meta-analyses reporting high diagnostic value of circulating miRNAs in gynecologic cancers. However, translation to clinical practice requires standardization of assays, validation in larger multicenter cohorts, and integration with HPV testing.17-20

Limitations: Small sample size, single-center design, and cross-sectional approach. Longitudinal studies are needed to assess prognostic implications and response to treatment.

Conclusion

Circulating miRNAs, particularly a combined panel of miR-21, miR-34a, miR-200c, and miR-155, show high diagnostic potential for CIN and can complement current screening methods.

Incorporating liquid biopsy into cervical cancer prevention strategies may improve early detection, reduce false negatives, and optimize patient management.

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