Research Article

Evaluation of *miR-23b* and *miR-375* Genes Expression as Diagnostic Biomarkers in Women with High-Risk of Human Papillomavirus Infection

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Abstract | Human papillomavirus (HPV) infections are a significant global public health issue and a major cause of the development of cervical cancers. This study aimed to investigate the circulating expression levels of *miR-23b-5p* and *miR-375-5p* in HPV-positive women as possible diagnostic tools and/or non-invasive biomarkers. The current study was performed for 46 HPV-positive patients versus 40 healthy controls. Real-time PCR for confirmation of HPV infection was performed. Circulating miRNAs (miR-23b-5p and miR-375-5p) were extracted from serum samples and quantified using quantitative real-time PCR (qRT-PCR). Relative expression levels were determined using the $2^{-\Delta\Delta Ct}$ method. Statistical analysis consisted of group comparisons, receiver operating characteristic (ROC) curve, and Pearson correlation. Both *miR-23b-5p* and *miR-375-5p* were significantly upregulated in HPV-positive women relative to controls ($p < 0.001$). ROC analysis exhibited excellent diagnostic performance, with area under the curve (AUC) values of 0.91 and 0.94 for miR-23b-5p and miR-375-5p, respectively. There were remarkable positive correlations among miRNA expression levels and HPV-positive status (r = 0.67 and r = 0.63, respectively; $p < 0.001$) with no substantial association with the inflammatory status. Circulating miR-23b-5p and miR-375-5p can be considered candidate potential markers for non-invasive detection of HPV infection. Their substantial upregulation combined with high diagnostic precision make them useful as complementary screening markers for new technologies aimed at enhancing early detection and molecular screening of viral infections.

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Introduction

Human papillomavirus (HPV) infection is still one of the major viral infections of the female reproductive tract. Persistent infections with high-risk HPV types constitute a leading etiological factor for cervical precancerous lesions and cervical cancer (Singh *et al.*, 2023). Cervical cancer, although this disease can be mostly prevented, it remains a major global health concern, with an estimated 604,127 new cases and 341,831 deaths reported worldwide in 2020 (Palomino-Vizcaino *et al.*, 2024). HPV is more common in low- and middle-income countries where there is a higher burden, and where uptake of molecular

testing, screening and follow-up programs is often lacking. Thus, improvements of early detection and understanding of reliable molecular biomarkers are still necessary to curb disease development associated with HPV (Pavelescu *et al.*, 2025). Viral presence alone does not establish a biological outcome of HPV infection. A persistent high-risk HPV infection can impact the host-cell regulatory networks predominantly by its viral oncoproteins, especially E6 and E7, which play a critical role in regulating cell-cycle, apoptosis, immune escape, and epithelial transformation (Pulliero *et al.*, 2024). Interestingly, recent studies have highlighted epigenetic mechanisms, such as altered DNA methylation and aberrant microRNAs expression, which play an integral role in HPV-mediated cervical carcinogenesis (Nagandla *et al.*, 2021; Pulliero *et al.*, 2024). MicroRNAs are short non-coding RNAs that regulate gene expression post-transcriptionally and their altered expression may represent an early molecular alteration prior to clinically observable advanced histological lesions (Feitosa *et al.*, 2024; Khokhar *et al.*, 2024). Recently, circulating microRNAs have been regarded as non-invasive biomarkers due to their localization in serum, plasma, urine, and other liquid biopsy samples (Kepsha *et al.*, 2024). Due to their relative stability in body fluids, resistance to degradation, and their role in cancer-related pathways, microRNAs are considered to be potential candidates for screening, triaging of HPV-positive women, and monitoring of disease progression (Gonzalez-Ramirez *et al.*, 2023). MiRNA signatures have been shown in several recent studies to distinguish HPV-positive women with higher risks of lesions from those with low-grade or normal lesions, supporting their potential utility as adjunct biomarkers of HPV DNA testing and cytological assessment (Pardini *et al.*, 2018; Dehghani *et al.*, 2024). Among candidate miRNAs, *miR-23b* and *miR-375* have a biological relevance in cervical carcinogenesis. *miR-23b* has been linked to regulation of proliferation, apoptosis, migration, and invasion in cervical cancer-related pathways, while *miR-375* has been repeatedly reported as a dysregulated miRNA in cervical lesions and cervical cancer (Romero-López *et al.*, 2024). However, data regarding their circulating expression patterns in HPV-positive women remain limited, especially in the local populations. Although *miR-23b* and *miR-375* have been investigated in cervical cancer tissues (Masood *et al.*, 2025) and cervical intraepithelial lesions (Causin *et al.*, 2021), information regarding their circulating expression patterns in women with high-risk HPV infection remains limited. Most previous studies have focused on an established cervical neoplasia rather than the early molecular alterations associated with a persistent HR-HPV infection (Shing *et al.*, 2022; Lehtinen *et al.*, 2023; Taguchi *et al.*, 2024). Therefore, evaluating the circulating *miR-23b-5p* and *miR-375-5p* genes expression in HR-HPV-positive women may provide insight into host molecular responses during infection and contribute to the identification of non-invasive biomarkers for early risk assessment before the development of overt cervical malignancy. Therefore, this study aimed to investigate the circulating expression profiles of *miR-23b-5p* and *miR-375-5p* genes in HPV-positive women and evaluate their diagnostic performance as potential non-invasive biomarkers.

Materials and Methods

Study design and setting

This case–control observational study was conducted in Diyala Governorate, Iraq, from March 2024 to October 2025. Participants were recruited from Baqubah Teaching Hospital (Gynecology Department) as well as several private gynecological clinics within the same region.

Study population

A total of 327 cervical swab samples were collected from women undergoing gynecological examination and HPV screening. Following molecular analysis, 46 women were confirmed with HPV-positive and were included in the patient group, while 40 apparently healthy women were negative for HPV and with no clinical evidence of cervical pathology; thus, were enrolled as the control group. The participants' ages were between 22 and 60 years. Women who agreed to participate and provided informed consent were included in the study. Patients were women with HPV infection confirmed by PCR analysis, whereas the control group consisted of HPV-negative women who showed normal clinical findings. Pregnant women, people with clinical diagnosis of cervical cancer, chemotherapy or immunosuppressive patients, patients with autoimmune or chronic systemic diseases were excluded to avoid the existence of confounding factors that might influence miRNA expression.

Sample collection

Cervical samples were collected under sterile aseptic conditions using sterile cervical cytobrushes and

immediately transferred into tubes containing viral transport medium. All samples were transported to the laboratory for molecular detection of HPV DNA. Also, about 5 ml of venous blood were taken from each subject. Serum was separated by centrifugation at 1006 ×g for 10 min. and kept at −80˚C until subsequent molecular analyses.

DNA extraction and HPV detection

Viral DNA was extracted from the cervical samples using the QIAamp DNA Mini Kit (QIAGEN, Germany) according to the manufacturer's instructions. Detection of HPV DNA was performed using a real-time PCR assay targeting conserved regions of the viral genome using the RealBest HPV Genotype PCR Kit (Vector-Best, Russia). Amplification was carried out using a SaCycler-96 Real-Time PCR System (Sacace Biotechnologies, Italy). Samples exhibiting specific amplification curves were considered HPV-positive.

miRNA extraction and cDNA synthesis

Total circulating RNA enriched with miRNA was extracted from the serum samples using the miRNeasy Serum/Plasma Kit (QIAGEN, Germany) following the manufacturer's protocol. RNA concentration and purity were assessed using a NanoDrop spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA). A total of 100 ng of RNA was used for cDNA synthesis. The extracted RNA was reverse transcribed into complementary DNA using stem-loop primers specific for *miR-23b* and *miR-375* with the miScript II RT Kit (QIAGEN, Germany).

Quantitative real-time PCR

Quantitative real-time PCR (qRT-PCR) was performed using the QuantiTect SYBR Green PCR Master Mix (QIAGEN GmbH, Hilden, Germany) on a Rotor-Gene Q Real-Time PCR System (QIAGEN, Germany). Each reaction was prepared in a final volume of 20 µl containing 10 µl of 2× QuantiTect SYBR Green Master Mix, 1 µl of forward primer (10 pmol/µl), 1 µl of universal reverse primer (10 pmol/µl), 2 µl of cDNA template, and 6 µl of nuclease-free water. Specific forward primers were used for *miR-23b* and *miR-375*, while *U6 snRNA* served as the endogenous reference gene for normalization (Mitchell *et al*., 2008). The thermal cycling conditions consisted of an initial denaturation at 95˚C for 15 min, followed by 40 cycles of denaturation at 94˚C for 15 s, annealing at 55–60˚C for 30 s, and extension at 72˚C for 30 s. A melt curve analysis was performed at the end of the amplification program to confirm the specificity of the PCR products. All reactions were run in triplicate. The Cycle Threshold (Ct) values were automatically determined by the instrument software, and the mean Ct value of the triplicates was used for subsequent analyses. The primer sequences used in this study are presented in Table 1.

Gene expression calculation

Relative expression levels of microRNA-23b-5p (*miR-23b-5p*), microRNA-375-5p (*miR-375-5p*), and U6 small nuclear RNA (*U6 snRNA*) were calculated using the $2^{-\Delta\Delta Ct}$ method originally reported by Livak and Schmittgen (2001). The double delta Ct (threshold cycle) analysis was used to assess the expression of *miR-23b-5p* and *miR-375-5p* genes, in which the *U6 snRNA* was the housekeeping reference genes. The following were the calculations conducted in reference to Livak and Schmittgen (2001).

$$\Delta CT = CT\ target\ gene - CT\ of\ reference\ gene \quad \ldots. (1)$$

$$\Delta\Delta CT = \Delta CT\ of\ each\ sample - average\ control\ \Delta CT \quad \ldots. (2)$$

$$Folding\ change = 2^{-\Delta\Delta Ct} \quad \ldots. (3)$$

Where; CT: Cycle Threshold, ΔCT: delta cycle threshold, ΔΔCT: delta delta cycle threshold. All qRT-PCR reactions were performed in triplicate and the mean Ct value was used for subsequent analyses.

Table 1: *Primer sequences and characteristics used for miR-23b and miR-375-5p genes.*

Primer	Sequence	Primer sequence 5′–3′	Temp. ˚C	GC%	Reference
miR-23b-5p	Stem-loop RT	GTCGTATCCAGTGCAGGGTCCGAGG TATTCGCACTGGATACGACGTGGTA	73.7	56	Designed in study
	F	CACGATCACATTGCCAGGGAT	57.9	52	
miR-375-5p	Stem-loop RT	GTCGTATCCAGTGCAGGGTCCGAGG TATTCGCACTGGATACGACGGTTTG	73.5	56	Designed in study
	F	GCGACGAGCCCCTCGCAC	63.8	78	
Universal	R	GTGCAGGGTCCGAGGT	58.8	68.8	
U6	F	CACTAGGCGCTCACTGTTCTC	62.0	57	(Khikani *et al*., 2024)
	R	AATCCGTTGACTCCGACCTT	61.4	50	

Statistical analyses

Statistical analyses were performed using SPSS version 26 (IBM Corp., USA) and GraphPad Prism version 9. Continuous variables are presented as mean ± standard deviation (SD), following a normality assessment *via* the Shapiro–Wilk test. To compare HPV-positive subjects with healthy controls, an independent samples t-test was employed for data that followed a normal distribution, while the Mann–Whitney U test was applied to variables that did not meet this criterion. The diagnostic performance of the circulating *miR-23b-5p* and *miR-375-5p* was evaluated through receiver operating characteristic (ROC) curve analysis, which included calculations of area under the curve (AUC), sensitivity, specificity, and optimal cut-off values derived from the Youden index. Additionally, Pearson correlation analysis was conducted to explore the relationship between miRNA expression levels and clinical variables. A *p*-value of less than 0.05 was considered statistically significant (SPSS, 2011).

Results

Demographic characteristics of HPV-positive women

Demographic information about the study population included their age, place of residence, and marital status. As shown in Table 2, the major percent of HPV-positive women were in the age of 31-40 years, followed by those from 22-30. This implied that HPV infection was more common in the reproductive-aged women. Further, the distribution between the urban and the rural residents was roughly balanced, which reflected a non-major geographic disparity.

Table 2: *Distribution of demographic characteristics among the HPV-positive patients (n = 46).*

Variable	Age	n (%)
Age (year)	22–30	12 (26.1)
	31–40	18 (39.1)
	41–50	10 (21.7)
	51–60	6 (13.0)
Residence	Urban	24 (52.2)
	Rural	22 (47.8)
Marital status	Married	46 (100)

Where; n: number of patients; %: their percentage.

Reproductive and behavioral characteristics of the study population

Reproductive, contraceptive, and smoking features were examined for the reproductive and the behavioral markers. As noted in Table 3, the majority of the samples had a moderate gravidity (3–4 pregnancies), indicating a possible cumulative reproductive exposure. Use of contraception was distributed evenly among the users and non-users, and this factor did not appear to be predominant in the study population. The participants had a relatively low prevalence of smoking, which may have limited its potential impact within this cohort.

Table 3: *Reproductive and behavioral characteristics of HPV-positive women (n = 46).*

Variable	Category	n (%)
Gravidity	0–2	15 (32.6)
	3–4	20 (43.5)
	≥5	11 (23.9)
Contraceptive use	Yes	23 (50.0)
	No	23 (50.0)
Smoking status	Yes	12 (26.1)
	No	34 (73.9)

Where; n: number of patients; %: their percentage

Association between cervical swab microscopy findings and clinical variables

Chi-square was conducted to evaluate the association between cervical inflammatory findings and chosen variables. Age also statistically correlated with cervical swab microscopy (p= 0.041) evidence (Table 4). Nevertheless, the inflammatory changes increased in the older women. Gravidity was statistically associated (p= 0.032), and higher gravidity (≥3 pregnancies) was related to greater reported inflammatory findings. Contraceptive use and smoking status had no remarkable associations, suggesting that these variables did not affect cervical inflammation in this population.

Table 4: *Association between cervical swab microscopy findings and selected variables among the HPV-positive women.*

Variable	Category	Normal n (%)	Inflammatory n (%)	p-value
Age	≤ 40 years	14 (60.9)	16 (69.6)	0.041*
	> 40 years	4 (39.1)	12 (30.4)	
Gravidity	≤2	10 (55.6)	5 (17.9)	0.032*
	≥3	8 (44.4)	23 (82.1)	
Contraceptive use	Yes	9 (50.0)	14 (50.0)	0.842
	No	9 (50.0)	14 (50.0)	
Smoking status	Yes	4 (22.2)	8 (28.6)	0.611
	No	14 (77.8)	20 (71.4)	

*Where; n: number of subjects; %: percentage; *indicates statistically significant at p < 0.05.*

Expression level of miR-23b gene

Expression of *miR-23b* was studied using qRT-PCR parameters, including Ct, ΔCt, ΔΔCt, and fold change, among HPV-positive patients and healthy controls. Table 5 shows that although the Ct values of *miR-23b* were not significantly different between the two groups ($p < 0.001^*$), a substantial and statistically significant difference with normalization was observed. The ΔCt results were significantly lower in the patients than in the controls, indicating that upon normalization to the endogenous control (U6), an increase in the relative expression of *miR-23b* was observed. The patients showed decreases in ΔCt with negative ΔΔCt values, while the controls displayed positive ΔΔCt values, reflecting a directional change in the gene expression. Consequently, a fold change was markedly greater in the patients compared to the controls, confirming the clear overexpression of *miR-23b* in the HPV-positive women.

Table 5: *Comparative analysis of miR-23b expression between the patients and the controls.*

Parameter	Patients (Mean ± SD)	Controls (Mean ± SD)	p-value
Ct (*miR-23b*)	32.58 ± 1.38	39.08 ± 1.04	< 0.001*
ΔCt	11.47 ± 2.10	15.72 ± 1.45	< 0.001*
ΔΔCt	-2.05 ± 2.10	1.02 ± 1.45	< 0.001*
Fold change ($2^{-\Delta\Delta Ct}$)	10.17	0.73	< 0.001*

*Where; SD: Standard deviation, * indicates statistically significant at p < 0.05*

Expression level of miR-375 gene

Relative expression of *miR-375-5p* was assessed by quantitative real-time PCR (qRT-PCR) using the comparative $2^{-\Delta\Delta Ct}$ method. Analysis of Ct, ΔCt, ΔΔCt, and fold change values revealed distinct expression patterns between HPV-positive patients and healthy controls, as shown in Table 6, for all the variables analyzed. The participants exhibited significantly lower Ct values for *miR-375-5p* (35.03 ± 1.33 versus 40.60 ± 0.89 in controls) in the patient cohort, suggesting an elevated baseline gene expression. After normalization, ΔCt in the patients (13.92 ± 1.55) was lower than the controls (17.24 ± 0.93), confirming an increased relative expression of *miR-375-5p* gene. In addition, ΔΔCt values in the patients were much more negative (-3.19 ± 1.55) compared to those in the controls (-1.36 ± 0.93), leading to an increased fold change. The fold change values were also markedly higher in the patients (15.33) than the controls (0.80), indicating a pronounced increase in *miR-375-5p* expression in the HPV-positive women. Statistical analyses revealed that the differences in Ct, ΔCt, ΔΔCt, and fold change between the patients and the controls were highly significant ($p < 0.001$).

Table 6: *Comparative analysis of miR-375-5p expression between the patients and the controls.*

Parameter	Patients (Mean ± SD)	Controls (Mean ± SD)	p-value
Ct (*miR-375-5p*)	35.03 ± 1.33	40.60 ± 0.89	< 0.001*
ΔCt	13.92 ± 1.55	17.24 ± 0.93	< 0.001*
ΔΔCt	-3.19 ± 1.55	−1.36 ± 0.93	< 0.001*
Fold change ($2^{-\Delta\Delta Ct}$)	15.33	0.80	< 0.001*

*Where; SD: Standard deviation, * indicates statistically significant at p < 0.05*

Diagnostic performance of miR-23b-5p and miR-375-5p genes

Consequently, a ROC curve analysis was performed to confirm that the circulating *miR-23b-5p* and *miR-375-5p* indeed expressed the best diagnostic performance in separating the HPV-positive women from the healthy controls (Figure 1), as summarized in Table 6. *MiR-375-5p* showed a superior diagnostic performance, with an AUC of 0.94 (95% CI: 0.88–0.99; $p < 0.001$) (Table 7), confirming excellent discrimination ability. With a sensitivity of 92% and a specificity of 90%, *miR-375-5p* showed high diagnostic accuracy at the optimal cut-off value of 2.10. In contrast, *miR-23b-5p* had a high diagnostic accuracy as well, reaching an AUC of 0.91 (95% CI: 0.84–0.97; $p < 0.001$). At 1.75 cut-off, the sensitivity and specificity were 88% and 87%, respectively. While *miR-23b-5p* resulted in a moderately lower performance than *miR-375-5p*; however, its discriminative power remained strong.

Table 7: *Diagnostic performance of the circulating miR-23b-5p and miR-375-5p genes in the HPV-positive women.*

Biomarker	AUC (95% CI)	Cut-off value	Sensitivity %	Specificity %	p value
miR-375-5p	0.94(0.88-0.99)	2.10	92.0	90.0	<0.001
miR-23b-5p	0.91(0.84-0.97)	1.75	88.0	87.0	<0.001

Where; AUC: Area under the receiver operating characteristic curve; CI: Confidence interval.

Expression pattern of miR-23b-5p and miR-375-5p genes across the studied samples

The expression characteristics of the circulating *miR-23b-5p* and *miR-375-5p* across all the studied samples

are shown in Figure 2 as a heatmap representation. Color gradients ranged from low (purple/blue) to high (green/yellow) levels of expression, which help in visualizing the variations of genes expression within the samples. As depicted in Figure 2, the expression phenotypes of *miR-23b-5p* and *miR-375-5p* varied among the samples analyzed. In addition, significantly higher values (green to yellow patterns) of expression were observed for both miRNAs in those regions, which indicated upregulation in some of the samples only. However, notable heterogeneity in the intensity of expression remained at various levels, suggesting individual differences. Differences in the levels of genes expression were accounted for by biological differences among the individuals, such as variations in the viral load, and differences in the immunological response and in the phase of the disease. The heat map provides a descriptive picture of genes expression distribution, but it does not show the statistical significance, which was evaluated individually using quantitative methods.

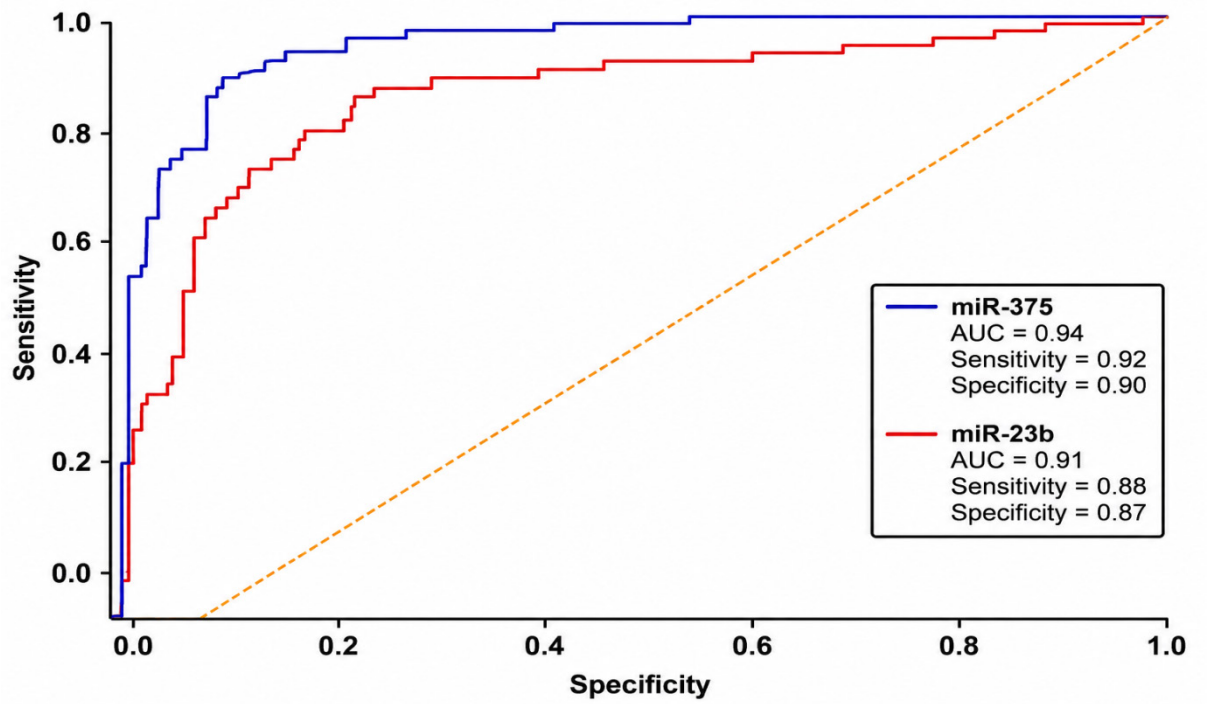

Figure 1: *Receiver operating characteristic curve (ROC) analysis of the circulating miR-23b-5p and miR-375-5p genes in the HPV-positive women.*

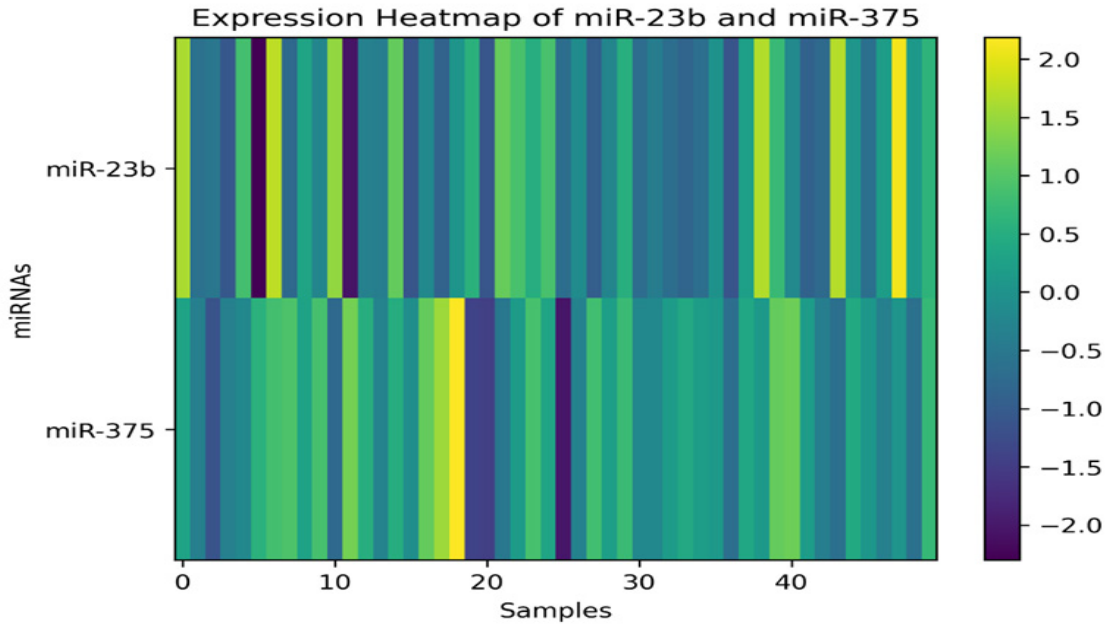

Figure 2: *Heatmap representation of miR-23b-5p and miR-375-5p genes expression across the studied samples.*

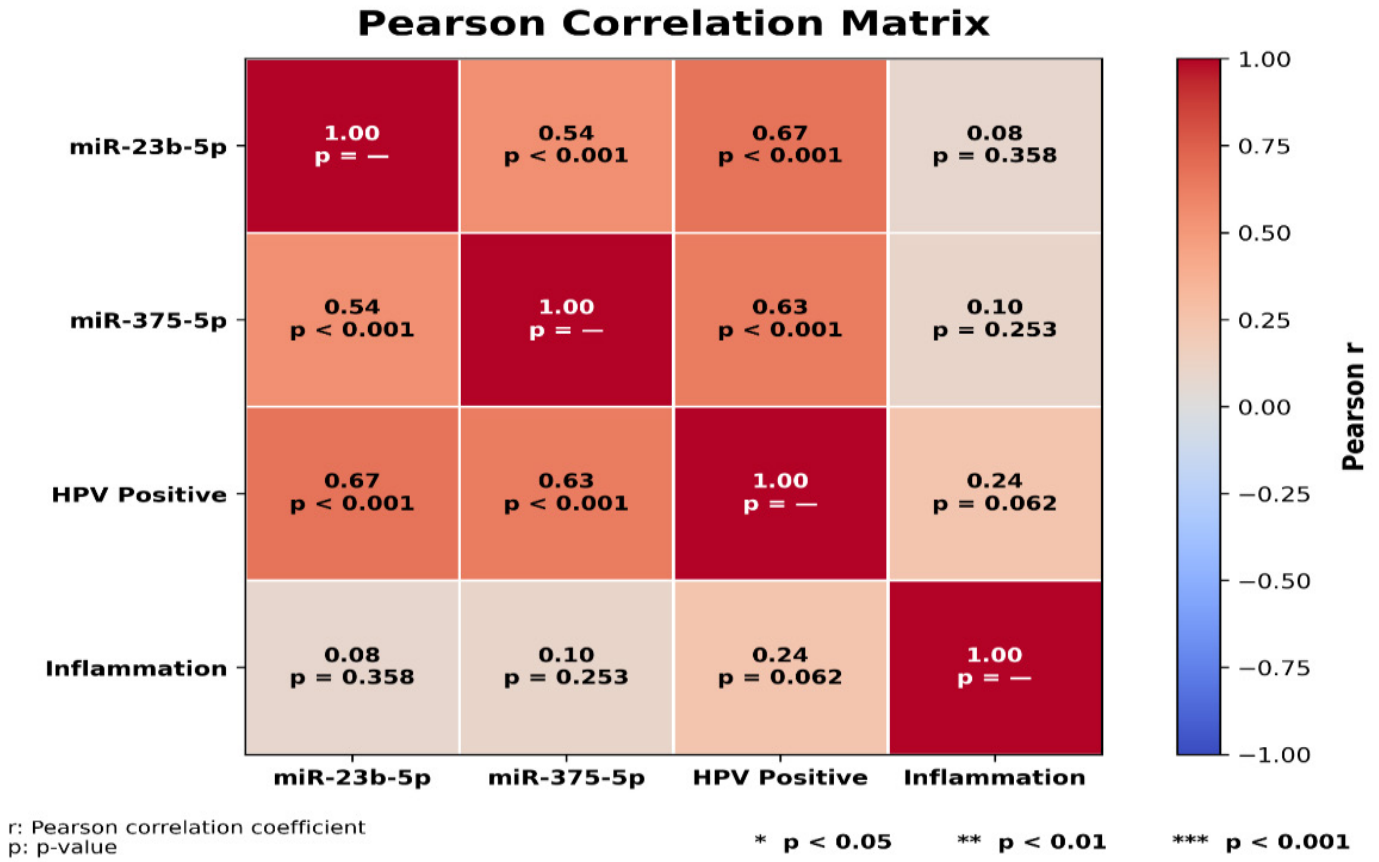

Figure 3: *Pearson correlation matrix illustrating the relationships among the circulating miR-23b-5p, miR-375-5p genes, HPV-positive status, and inflammatory status. The color scale ranges from blue (negative correlation) to red (positive correlation), representing the strength and the direction of the Pearson correlation coefficients (r). Corresponding p-values are displayed within each cell to indicate the statistical significance.*

Correlation analysis between miR-23b-5p and miR-375-5p genes expression

Pearson correlation analysis was conducted to evaluate the relationships among the circulating *miR-23b-5p*, *miR-375-5p* genes, HPV-positive status, and inflammatory status, as illustrated in Figure 3. The analysis revealed that both the *miR-23b-5p* and *miR-375-5p* expression levels were substantially and positively correlated with the HPV-positive status. Specifically, *miR-23b-5p* demonstrated a correlation coefficient of r= 0.67 ($p < 0.001$), while the *miR-375-5p* had a correlation coefficient of r= 0.63 ($p < 0.001$), implying that higher levels of these miRNAs were closely correlated with HPV infection. There was also a moderate positive correlation between *miR-23b-5p* and the *miR-375-5p* (r= 0.54, $p < 0.001$), which implied a coordinated expression of the two biomarkers. Conversely, no statistically significant association was detected between either the miRNAs or the inflammatory status (*miR-23b-5p*: r = 0.08, $p = 0.358$; *miR-375-5p*: r = 0.10, $p = 0.253$). Likewise, the association between the HPV-positive status and the inflammation was not statistically significant (r= 0.24, $p = 0.062$).

Discussion

In the present study, the levels of circulating *miR-23b-5p* and *miR-375-5p* increased considerably in the HPV-positive women, providing data to suggest their potential use as non-invasive diagnostics. These findings are consistent with previous molecular evidence indicating that host-derived *microRNAs* perform a crucial role in HPV-associated cellular dysregulation. Pavelescu *et al.* (2025) further emphasized that the HPV infection alters the host's regulatory network through epigenetic mechanisms, including microRNA dysregulation that initiate a carcinogenic process. This supports the biological plausibility of the increased miRNA expression observed in this study.

Increasing the expression of *miR-23b-5p* recorded in the present study is consistent with the previous study reported by Romero-López *et al.* (2024), which showed that *miR-23b* was involved in regulating the

proliferation and apoptosis in the cervical cancer cells. It was indicated that the *miR-23b* gene contributed to cancer progression through modulation of tumor-related signaling pathways. However, there are some contrasting results reported by Li *et al.* (2024); Mandal *et al.* (2025), noting that *miR-23b* content declined during late stages of the cervical cancer. This discrepancy may be attributed to stage-specific expression patterns, where *miR-23b* may be upregulated during the early HPV infection and downregulated in the advanced cervical cancer stage, due to regulatory feedback mechanisms (Parvizi *et al.*, 2025).

However, the increased *miR-375-5p* expression observed in this study is consistent with González-Ramírez *et al.* (2023), who reported an altered *miR-375* expression in women at high risk of HPV infection. *miR-375* has demonstrated several roles in epithelial differentiation and immune regulation that may be affected during HPV infection. In contrast, other studies reported by Wang *et al.* (2011); Xiao and Zheng (2020) noted reduced levels of *miR-375* in invasive cervical carcinoma, suggesting that *miR-375* expression may be stage-dependent and associated with disease progression. These differences highlight the urgent need to differentiate an early infection, precancerous lesions, and advanced malignancy during the interpretation of *miRNA* expression patterns.

The diagnostic performance observed in this study, particularly the AUC values of both miRNAs, is consistent with Dehghani *et al.* (2024) study, where the circulating miRNAs were reported to have diagnostic potential in the HPV-associated lesions. They demonstrated that the miRNA-based biomarkers could distinguish the affected individuals from the healthy controls. However, in the present study, both *miR-23b-5p* and *miR-375-5p* were evaluated individually, and a combined diagnostic modeling was not performed, which should be considered in the future studies to improve the diagnostic accuracy.

Association analysis showed a remarkable relationship between *miR-23b-5p* expression and HPV-positive status. This result is supported by Kepsha *et al.* (2024), who demonstrated that the circulating miRNAs are associated with the viral infection status and may reflect host–virus interactions at the molecular level. The moderate positive association between the two miRNAs also suggested a potential coordinated regulatory response during the HPV infection.

No statistically significant association was observed between the miRNAs expression and the inflammatory status, suggesting that the observed miRNA's changes were more specifically related to the HPV infection rather than the general inflammatory activity. Similar observations were reported by Feitosa *et al.* (2024), indicating that certain miRNAs are more closely linked to oncogenic pathways rather than inflammatory responses.

The regional relevance of the findings observed in the present study is supported by previously published Iraqi studies (Khikani *et al.*, 2024; Al-Khafaji *et al.*, 2025), which revealed a diagnostic potential of the viral miRNAs with a high accuracy. Although these studies focused on different viruses, they supported the broader concept that the circulating miRNAs may serve as biomarkers in the infectious diseases. Differences observed between these studies may be attributed to population genetics, environmental factors, HPV genotype variation, and methodological differences, as previously discussed by (Endale *et al.*, 2024; Khokhar *et al.*, 2024).

Limitations of this study

There are several limitations of this study that should be noted. First, the sample size was relatively small, which may have limited the statistical power and generalizability of the findings. Second, the obtained results may not be generalizable to the other populations by virtue of being a single-center design. Third, HPV genotyping, lesion grades, and viral loads were not available for all participants which limited deeper stratification of the study population. Moreover, it should also be noted that the absence of an external validation or an independent cohort analysis may reduce the robustness of the diagnostic performance's estimation. Thus, larger scale, multi-center studies are needed to confirm these results.

Conclusions and Recommendations

The current study demonstrated elevated expression levels of *miR-23b-5p* and *miR-375-5p* genes in the bloodstream of women with high-risk of human papillomavirus (HPV) infection, compared to healthy women. The most remarkable finding of this study was the excellent diagnostic performance of both microRNAs, with AUC values of 0.91 for *miR-23b-5p*

and 0.94 for *miR-375-5p*, supporting their use as non-invasive biomarkers for HPV detection. Furthermore, both microRNAs showed a strong positive correlation with HPV infection status, whereas they displayed no statistically significant correlation with inflammatory status. This suggested that changes in the expression of these two microRNAs were more directly related to specific HPV-related molecular alterations than to general inflammatory states. These findings suggest that the circulating *miR-23b-5p* and *miR-375-5p* may be complementary markers of HPV infection. Further studies involving larger populations are highly recommended to confirm the diagnostic utility of the circulating *miR-23b-5p* and *miR-375-5p* genes in humans with high risk HPV infections.

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Novelty Statement

This study provides novel evidence on the diagnostic potential of the circulating *miR-23b-5p* and *miR-375-5p* genes as non-invasive biomarkers for high-risk human papillomavirus (HPV) infection. Unlike previous studies that primarily focused on tissue-based analyses, this research highlights the clinical utility of the serum-derived microRNAs, demonstrating their considerable upregulation in HPV-positive women and their strong diagnostic performance based on ROC analysis. Furthermore, the study establishes a positive correlation between miRNA expression levels and HPV infection status, supporting their role in early detection and molecular screening strategies. These findings contribute to advancing the minimally invasive diagnostic approaches for the HPV-related conditions.

Author's Contribution

Ansam Dawod Salman: Conceptualization, study design, data collection, laboratory experiments, data analysis, interpretation of results, and manuscript writing. The author reviewed and approved the final version of the manuscript.

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Ethical approval

The study protocol was reviewed and approved by the Institutional Research Ethics Committee of University of Diyala, College of Science, Department of Biology under approval number 26- ASJ 178. Written informed consent was obtained from all participants prior to sample collection. All procedures were conducted in accordance with the ethical principles outlined in the Declaration of Helsinki.

Generative AI and AI-assisted technology statement

The author declares that no generative AI and AI assisted technology was used in the creation of this manuscript.

Conflict of interests

The author has declared no conflicts of interest.

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