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Evaluating MicoRNA-21 of Human of Papillomavirus by RT-qPCR

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Abstract

Human samples from Breast Cancer patients were collected from October 2024 to December 2024.Breast Cancer was included (36 – 74 years). The RT-qPCR, Method was used to detect miR-21 of all samples. The population groups studied samples subject groups were distributed into 4 groups including (36-45,46-55,56-65, and 66-75,) years. The samples were isolated from the Hospital of the (Middle Euphrates Cancer Center) this is the first study in the region to explore the relationship between HPV infection and gene expression patterns, of microRNAin breast cancer patients. A significant reduction in the expression of miR-21-5p, was observed in HPV-positive cases, indicating a potential suppressive effect of HPV on key immunological and regulatory pathways within the tumor microenvironment.

Keywords

MicoRNA-21, HPV, RT-qPCR, Breast Cancer

Introduction

Amongst viruses, *(HPV)* was the most widespread, with over 180 strains, 40 of which were frankly linked to anogenital infections. Human papillomavirus was a minor, non-shroud deoxyribonucleic acid (DNA) virus that infect mucosal cells or skin. The spherical, double stranded viral genome was around eight-kb in length. The genome encodes for six premature proteins through for virus imitation and two twilight proteins, L1 and L2, which were the viral basic proteins (Alameedy ,.2019). Based on their oncogenic potential, HPV types are classified into high-risk HPV (such as types 16 and 18) and low-risk HPV (e.g., types 6 and 11) (Feng *et al*,.2025).

The miRNAs are a class of small non-coding RNAs, typically 18–22 nucleotides long, that play crucial roles in gene expression regulation through RNA interference (Petracci et al,.2025). They can recognize their target genes through nearly perfect sequence complementarity, and negatively regulate gene expression post-transcriptionally either by mRNA cleavage or translational suppression (Qin et al,.2025). miRNA can function as oncogenes (oncomiRs) or tumor suppressors. Due to their high stability in body fluids and tissues, miRNAs are promising biomarkers of various diseases, including cancers (Wiśnik et al,.2025).

The MiRNA-21 is recognized as an ―oncomiR‖ due to its widespread deregulation in numerous cancer types. It was first identified as an inhibitor of apoptosis across various cell lines and is now considered one of the most

prominently overexpressed microRNAs in cancers such as breast (Jaksic et al,.2025).

Methodology

Design Of Study

In the case sectional study, the breast cancer without HPV sample was patients suffering from breast cancer without viral infections.

Patients

Whole blood samples used in this study were obtained from one hundred patients with breast cancer of age ranging from 36 year to 74 years who were admitted to Middle Euphrates Cancer Center during the interval between October 2024 until December 2024Serum sample collection. The whole blood samples were collected from patients with breast cancer. The samples (5ml) of whole blood were put into suitable containers, labelled and kept in an icy box. It were then transferred to the laboratory within two hours of collection. In the laboratory, the collected whole blood samples were stored in the freezer at -20 °C until use for molecular study and detection of viruses by (RT qPCR).

Real-time quantitative polymerase chain reaction (RT-qPCR) Technique

This technique was used to amplify the gene of the virus for detection of Human Papillomavirus and gene expression and study miR-21 in all tested samples (Total Blood) by using real time kits (GoTaq® qPCR and RT-qPCRSystems, GoTaq®2-StepRT-qPCR System,AddBio,cat.No.22101,Korea) The mixture was prepared with a final volume 20 µl by mixing all contents. This technique was performed Alamin center for advanced research and biotechnology,in Alnajaf province, by using (Analytik Jena\Qtower3G) and in college of veterinary medicine,university of kufa by using (Agilent technologies\Stratagene Mx3005p) device.

Gene expression

These results explain the use of miR-21 gene expression biomarker of one hundred patients infected with breast cancer according to the type of human (Papillomavirus) positive. RNA, microRNA extraction,and cDNA synthesis were performed according to manufacturing protocols. Expression level(the equation 2^ -ΔΔCt) evaluated and compared with one hundred samples of breast cancer without HPV by RTqPCR.

House Keeping gene (GAPDH) used for comparative project with patients and breast cancer without HPV

Human total RNA extraction kit (TRIpure Total RNA Extraction Reagent,Lot No.T:86-27-59760950,ELK Biotechnology,UK and European), and according to the instructions provided by the manufacturer as the following steps:

1. Collected fresh human blood in an anticoagulant-treat collection tube.
2. Add 0.75 mL of TRIzol™ Reagent per 0.25 mL of sample (1.5 ml or 2.0 ml tube.
3. Mixed by vortex or Pipet the lysate up and down several times to homogenize Incubated on ice for 10 min, vortex briefly 2 times during incubation.
4. Incubate for 5 minutes to permit complete dissociation of the nucleoproteins complex.
5. Add 0.2 mL of chloroform per 1 mL of TRIzol™ Reagent used for lysis, then securely cap the tube.
6. Incubate for 10 minutes in ice.
7. Centrifuge the sample for 15 minutes at 12,000 × g at 4°C. The mixture separates into a lower red phenol-chloroform, and interphase, and a colorless upper aqueous phase
8. Transfer 0.5 ml of the aqueous phase containing the RNA to a new tube.
9. Add 0.5 mL of ethanol to the aqueous phase, per 1 mL of TRIzol™ Reagent used for lysis.
10. Incubate for 10 minutes in ice.
11. Centrifuge for 10 minutes at 12,000 × g at 4°C. Total RNA precipitate forms a white gel-like pellet at the boϑom of the tube.
12. Discard the supernatant .
13. Resuspend the pellet in 1 mL of 80% ethanol / DEPC Water .
14. Vortex the sample then centrifuge for 5 minutes at 12,000 × g at 4°C.
15. Discard the supernatant & air dry the RNA pellet for 5–10 minutes.
16. Resuspend the pellet in 50–100 µL of RNase-free water & Stored RNA at -70ºC.

Design and preparation of the primers

The primer used in this study were prepared according to the recommendations of the manufactured by dissolving a lyophilized sequences in appropriate volume of nuclease free water to yield 100 pmol/ µl as a stock solution . A working solution was prepared with the final

concentration 10 pmol/ µl by dilution methods. This primer was designed based on the NCBI which is about mixed gen for human HPV-A3B, MicroRNA-21 and GAPDH primer (immunity primers) were obtained from OLIGO (Macrogen)company, in Table 1.

Table (1) the primer design of Human *Papillomavirus*

Name	Sequence	Bases	
MicroRNA-21-F	AACACGCTAGCTTATCAGACTGAT	24bp	
MicroRNA-21-R	GTCGTATCCAGTGCAGGGT	19bp	
Micro-RNA-21-RT	GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACAGCCTG	50bp	
GAPDH-F	AATTCCATGGCACCGTCAAG	20 bp	104 bp
GAPDH-R	ATCGCCCCACTTGATTTTGG	20bp	

Statistical analysis

The un paired T-test test was used to compare the two groups. The results are shown as mean ± SD. The correlation test was done by pearson correlation test.

Results and discussion

This study highlights the role of MicroRNA-21-5p (miR-21-5p) as a pro-inflammatory biomarker in patients with breast cancer. The expression levels of miR-21-5p were found to be significantly reduced in breast cancer patients who were positive for Human *Papillomavirus* (*HPV*), compared to those who were *HPV*-negative.

As shown in figure (1),(2) and table (2) miR-21-5p expression was markedly decreased in *HPV*-positive breast cancer cases ($p < 0.001$), suggesting a potential inverse relationship between *HPV* infection and miR-21-5p regulation. These findings may indicate that *HPV* infection modulates inflammatory and oncogenic signaling pathways through the downregulation of specific microRNAs such as miR-21-5p, warranting further investigation into its mechanistic role in virus-associated breast carcinogenesis

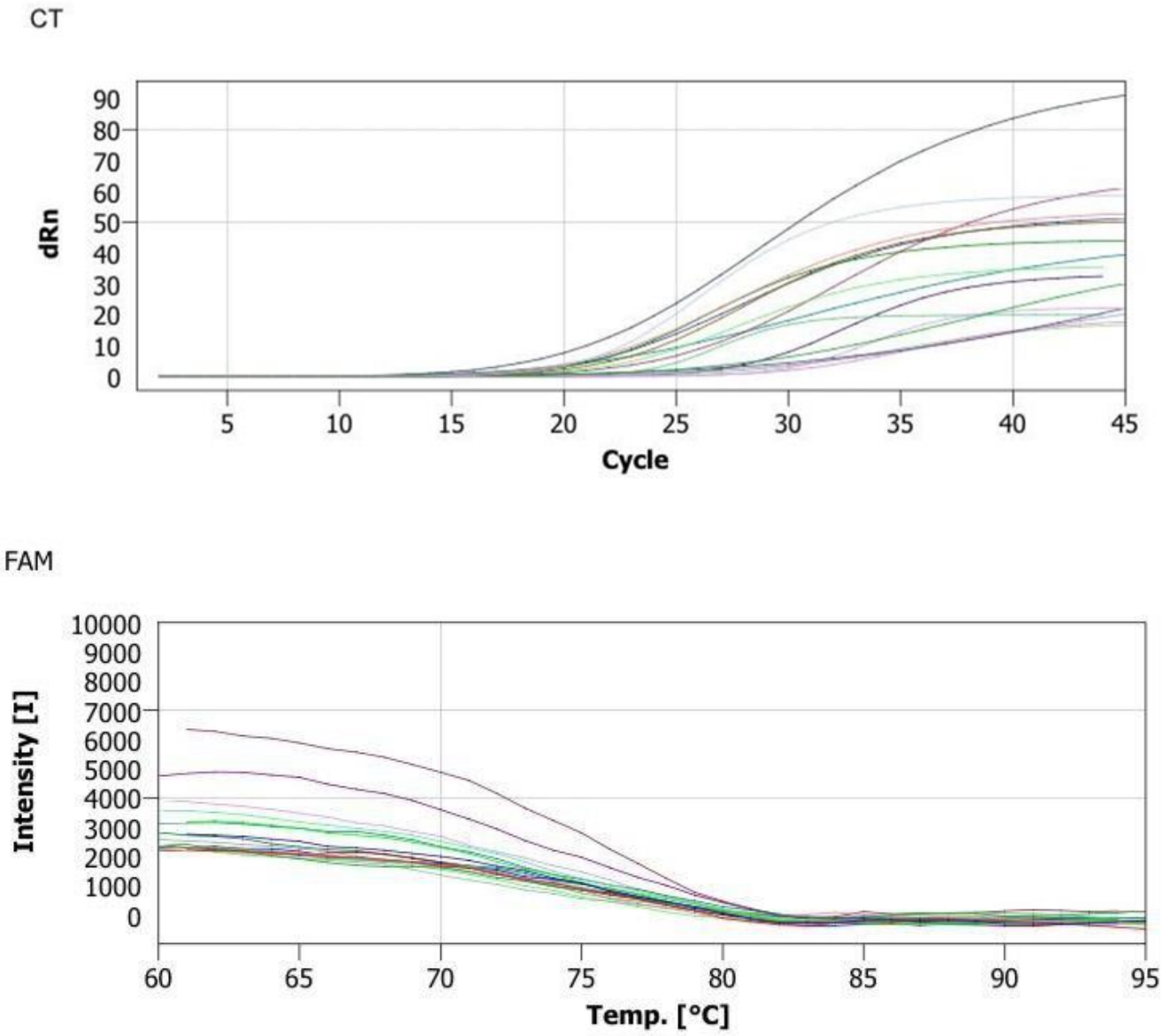

Figure1: Determined of MicroRNA and Houskeeping (GAPDH)expression for Breast Cancer with HPV and breast cancer without HPV by RT-qPCR

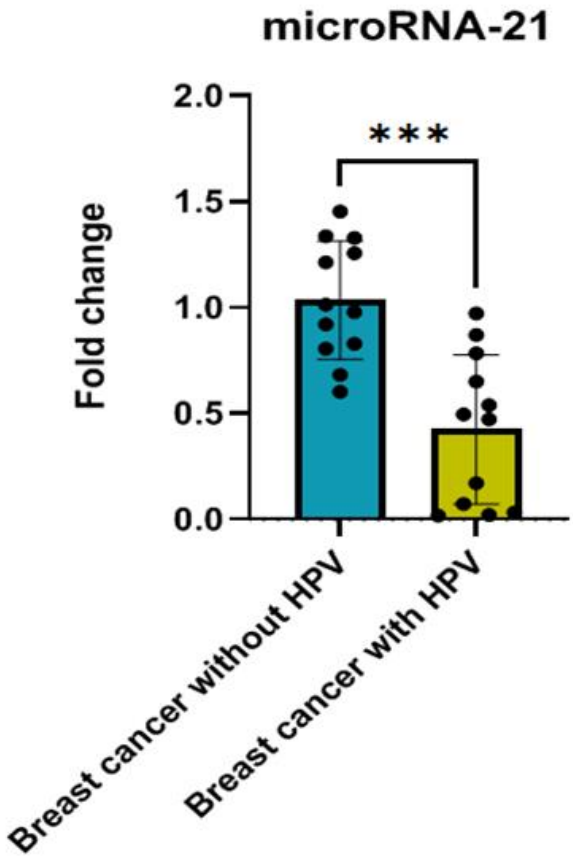

Figure (2). Comparing level of Micro-RNA-21-5p gene expression of breast cancer patients with HPV and breast cancer without HPV by RT-qPCR

Table (2). Alterations in the levels of miR-21-5p of Breast cancer patients with HPV and breast cancer without HPV.

microRNA	**Breast cancer (positive for *HPV*) mean (SD)**	**Breast cancer (negative for HPV)**	***P* value**
miR- 21-5p	0.42(0.35)	1.03(0.28)	P< 0.001

According to Abbas *et al*. (2024), quantitative RT-PCR analysis revealed a significant upregulation of miR-21 expression, particularly in high-grade breast tumors. Their findings suggest that miR-21 expression increases in parallel with tumor progression, reinforcing its utility as a molecular biomarker for breast cancer development and advancement. These findings are broadly consistent with the current study, which also supports miR-21 as a marker of disease progression. However, a key distinction in our results is that miR-21 expression was significantly decreased in *HPV*-positive breast cancer cases compared to *HPV*-negative ones.

As a researcher, this observed downregulation of miR-21 in *HPV*-associated breast cancer may be attributed to the immunomodulatory functions of viral oncoproteins E6 and E7. These proteins are known to interfere with host immune responses, and may inhibit miR-21 expression

to evade immune detection. This suggests that *HPV* may alter the tumor microenvironment and gene regulatory networks in a manner distinct from *HPV*-negative tumors, indicating a divergent pathophysiological behavior.

To date, no published studies have directly examined the correlation between **miR-21** expression and **HPV status** in breast cancer, making this observation novel and potentially significant (Papagianni et al., 2021). Mechanistically, miR-21 is known to target the 3' untranslated region (3'UTR) of the **PTEN** gene, suppressing its expression (Meng et al., 2007). PTEN is a tumor suppressor that regulates the cell cycle, and its inhibition by miR-21 leads to enhanced cellular proliferation (Wang et al., 2019). Moreover, overexpression of miR-21—often observed in advanced breast tumors—has been associated with increased **TGF-β** signaling, which promotes tumor invasion and expansion (Asangani et al., 2008; Zhao et al., 2008).

Furthermore, miR-21 has been shown to activate STAT3, which in turn enhances miR-21 transcription, forming a positive feedback loop. This regulatory axis may play a pivotal role in maintaining the oncogenic state. Additionally, miR-21 influences the IGF-1 signaling pathway, contributing to cell proliferation and activation of the PI3K/AKT cascade (Yang *et al*., 2024).

According to Abdulmalek *et al*. (2024), miR-21 also acts as a positive feedback regulator of the MAPK/ERK1/2 pathway. Its expression is upregulated by ERK1/2 activation, while concurrently suppressing negative regulators of this pathway, thereby perpetuating oncogenic signaling.

Conclusions

A significant reduction in the expression of miR-21-5p, was observed in HPV-positive cases, indicating a potential suppressive effect of HPV on key immunological and regulatory pathways within the tumor microenvironment.

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