Original Article

Regulatory Role of microRNA-24 in Breast Infection Caused by *Staphylococcus aureus*

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Abstract

Background: MicroRNAs (miRNA) is detected at high levels in various body fluid secretions, including serum, saliva, urine, milk, and seminal fluid. Mother's milk is one of the richest sources of miRNAs and has ~1400 mature miRNAs. **Objectives:** This study aimed to investigate various *Staphylococcus aureus* isolates that because mastitis and breast abscess and detect the role of miRNA-24 in infection. **Materials and Methods:** A case–control study was carried out on patients admitted to Al-Hilla General Teaching Hospital (breast cancer consultation) and out-patient clinics from August 2023 to January 2024. A total of 80 samples including milk and abscess specimens were collected from patients presenting with specific symptoms such as redness and pain in the breast, which are suspected indications to breast infection. Blood samples were obtained from all patients. RNA was extracted for gene expression study via real-time quantitative polymerase chain reaction. **Results:** A total of 20 blood samples were collected from patients with breast infection, and RNA was extracted to detect the gene expression of microRNA-24. The results in this work show the expression of microRNA was upregulated during breast infection caused by *S. aureus,* which was identified according to main biochemical methods and culturing characteristics; also, the present study shows a highly significant correlation between patients and control according to miRNA-24 expression ($P < 0.001$). **Conclusions:** Expression of microRNA was upregulated in breast infection caused by *S. aureus.*

Keywords: Gene expression, mastitis, miRNA-24, real-time PCR, *S. aureus*

Introduction

Mastitis is an inflammation of the breast and can be classified into lactation and non-lactation mastitis. Lactation mastitis is the most common form of mastitis.[1] *Staphylococcus* spp. is known to cause, especially *Staphylococcus aureus*.[2]

microRNAs (miRNAs) are non-coding RNAs that are highly conserved, short, single-stranded transcripts that regulate the messenger RNA (mRNA; coding gene) expression by degradation, consist of 17–25 nucleotides, and control gene expression.[3] miRNA binds to the −3-untranslated region (−3-UTR) of target mRNA for improving or preventing translation.[4]

The degradation of mRNA and failure in protein translation may lead to pathological variations in the human body. miRNAs may form stable complexes with proteins in peripheral blood and body fluids, and these complexes may be used as biomarkers for diagnosing multiple inflammatory diseases.[5]

The most commonly reported role of microRNAs in human breast milk is immune modulation; these molecules function in immune system development pathways.[6]

miRNA regulates gene expression by targeting the mRNA of protein-coding genes. Dysregulation of microRNA is associated with disease severity and therapeutic outcomes by diverse treatments.[7]

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miR-24 is an important miRNA encoded by a specific gene located in human chromosome 9q22 and 19p13 regions, which is conserved in species and is expressed in normal tissues such as adipose, mammary gland, kidney, and differentiated skeletal muscle.[8]

The proliferation and migration of vascular endothelial cells can inhibit miR-24 expression, deactivating the nuclear factor kappa B (NF-κB) signaling pathway, regulating inflammation in endothelial cells, and preventing the role of the NF-κB signaling pathway in atherosclerosis.[9]

miRNA was found in milk fractions and/or peripheral blood after study of the infection of mammary glands by pathogens such as *S. aureus, Streptococcus agalactiae*, and *Streptococcus uberis*.[10]

Materials and Methods

Patients and clinical sample

A case–control study was carried out in Al-Hilla General Teaching Hospital (breast cancer consultation) and out-patient clinics from August 2023 to January 2024. Patient with mastitis or breast abscess were admitted to the hospital.

A total of 80 samples of milk and abscess specimens were collected from patients with specific symptoms such as redness and pain in the breast, which are suspected indications to breast infection according to the diagnosis by a physician by use of the sterile clean method. Samples were collected from patients and transferred to a microbiology laboratory for culture and identification, according to Al-Awadi *et al*.[11]

Blood samples were obtained from all patients and placed in ethylene diamine tetraacetic acid tubes. RNA was extracted for gene expression study.

Control group

One milliliter of blood samples was taken from 15 healthy individuals (control group) who are devoid of any sign and symptoms for any illness. RNA was extracted from these samples.

Ethical approval

The important ethical approval was taken by verbal consent from patients. This study was approved by the Committee Of Publication Ethics at College of Medicine, Babylon Province, Iraq under reference number BMS/0203/016.

Gene expression of (miRNA-24) by the RT-PCR technique

After collection of blood specimens from patients and healthy individuals, RNA was extracted from whole blood samples according to the manufacturer's instructions (Geneaid Company, Korea).

The real-time quantitative polymerase chain reaction (qPCR) reactions were performed by using specific primers targeting reference gene U6 and expression of the target gene microRNA-24. Conversion of total RNA to cDNA and amplification of DNA were done according to instructions provided by GoTaq® 1-Step RT-qPCR System (Promega, Korea) using BRYT Green+ dye, and the RT-qPCR mixture and conditions are summarized in Table 1, where the final volume of the RT-qPCR reaction was 20 μL. Relative fold gene expression was calculated by the delta–delta CT method (2^2–(ΔΔCt).This method was used in other studies to measure the gene expression according to Al-lateef *et al*.[12]

Data analysis of qRT-PCR statistics analysis

Statistical studies were carried out by using (SPSS, IBM Corp., Chicago, IL, USA) version 26., and the means, standard error (SE), standard division (SD), and significant variances were analyzed with one-way analysis of variance using (least significant difference) test at ($P \leq 0.001$).

Results

Isolation of *S. aureus* from breast infection tissues

In a total of 80 samples, the mastitis rate was 35 (44%),while the abscess rate was 45 (56%), while 20

Table 1 : Primer sequence and conditions used in qRT-PCR mi24 gene expression

Primer name	Sequence 3′–5′	Steps	Temp/time/cycles	References
miR-24-F	GCCTACTGAGCTGATATC	-Reverse transcription -Reverse transcriptase inactivation and GOTaq DNA polymerase activation -Denaturation -Annealing -Extension and data collection	37ºC/15 min/1 cycle 95ºC/10 min/1 cycle 95ºC/10 min/45 cycle 58ºC/30 sec/45 cycle 72ºC/30 sec/45 cycle 60ºC–95ºC/3 min/1 cycle	Design in this study[13]
miR-24-R	GAACATGTCTGCGTATCTC			
U6- F	GCTTCGGCAGCACATATACTAAAAT			
U6- R	CGCTTCACGAATTTGCGTGTCAT			

isolates (25%) obtained from total samples were positive cultures to *S. aureus* after identification by main biochemical methods and culture characteristics, as shown in Table 2.

Gene expression of miRNA 24 level using real-time PCR

Out of 20 blood samples obtained from patients with mastitis, RNA was extracted to detect the gene expression of microRNA-24 by RT-PCR (relative gene expression; 2 ΔCT) methods, where the expression level of the miRNA-24 gene in test samples as well as in control samples was normalized with the housekeeping gene (U6). The results in this work show that expression of microRNA was upregulated during breast infection caused by *S. aureus*; also, the present study show a highly significant correlation between patients and controls according to miRNA-24 expression ($P < 0.001$) as shown in Tables 3–5; Figure 1.

Discussion

S. aureus is considered the important cause of acute mastitis, producing toxins which contribute to systemic symptoms.[14]

The ability of *S. aureus* to invade mammary epithelial cells during mastitis plays a role in the pathogenesis of internalized bacterial cells that can escape from the immune system. Mammary epithelial cells have a component called fibronectin binding protein that can bind to fibronectin on mammary epithelial cells.[15]

Many virulence factors with adhesion, invasion, and speeding properties were produced by *S. aureus,* which can aid in colonization, attachment to host tissue, and evasion of the human defense mechanisms. Tuchscherr *et al.*[16] found that the isolate rates of predominant pathogens of mastitis such as *Staphylococcus aureus* and *S. epidermidis* were 23.6% and 10.7%, respectively.[17]

miR-24 is a type of miRNA found to be transcriptionally suppressed by transforming growth factor beta (TGF-B) signaling, and its expression increased during myogenesis. *S. aureus* is a Gram-positive bacteria with many virulence factors and changes the rate of TGF-B signaling, which can restrict the inflammation and tissue injury of the host during infection in miR-24. It was detected in *S. aureus* infection.[18]

Study done by Zhao *et al.*[19] assumed that miRNA-24 molecules have a regulatory role in several signaling pathways, such as binding at the 3-untranslated region;

Table 2: Percentage of mastitis and abscesses among *S. aureus* isolates from patients

Total number of samples	Mastitis	Abscesses
80	35 (44%)	45 (56%)
	S. aureus 5 (25%)	*S. aureus* 15 (75%)

Table 3: miRNA-24 expression in patients with breast infection and healthy controls

miRNA-24 expression	Case–control comparison		*P* value
	Patients $n = 20$	Healthy controls $n = 15$	
Mean± SD	7.82 ± 1.08	1.04 ± 0.33	<0.001 † HS
Range	6.58–10.12	0.63–1.79	

n: number of cases, SE: standard division, †: independent samples *t* test, HS: highly significant at $P \leq 0.001$

Table 4: *miRNA-24 (CT) expression* in patients with breast infection and healthy control

Parameters	*n*	CT		
		Mean	SD	SE
miRNA-24 expression				
Patients	20	26.03	**3.09**	**0.69**
Control	15	19.69	1.42	**0.37**
P value		> 0.001*		

n: number of cases, SE: standard division, †: independent-sample *t* test
*: significant at $P \leq 0.001$

Table 5: Sensitivity and specificity of miRNA-24 expression (> 1.65 fold) in breast infection

miRNA-24 expression	Patients $n = 20$	Healthy controls $n = 15$
> 4.18	20 (%)	0 (%)
> 4.18	0 (%)	15 (%)
Sensitivity (%)	100.0	
Specificity (%)	100.0	
PPV (%)	100.0	
NPV (%)	100.0	
AUC (95% CI)	1.000 (1.000–1.000)	

PPV: positive predictive value, NPV: negative predictive value, CI; confidence interval, AUC; area under the curve

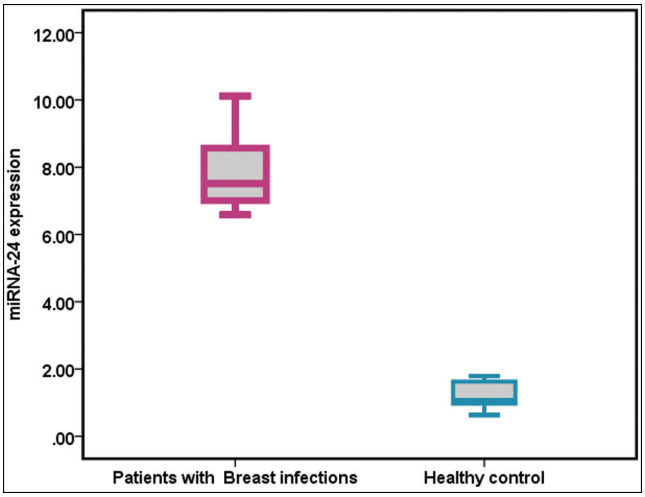

Figure 1: Mean miRNA-24 level of patients and healthy controls

also, micro-24 inhibited the CH13L1 (chitinase-3 like 1), a target for miR-24 molecules. CH13L1 has been suggested to have a role in antibacterial reaction, bacterial clearance, and host tolerance. Thus, the role of micro-24 molecules in the polarization of macrophages is encouraged by *S. aureus* with CH13L1. Hence, miR-24 overexpression downregulated the CH3L1 expression and MAPK signaling pathway in macrophage polarization induced by *S. aureus*. A total of 47 miRNAs specific to human breast milk were also recognized to have a different type of miRNA expression, in contrast to blood plasma.[20]

The exists a correlation between development, metastasis of breast cancer, and miR-24 expression which has an effect on genes regulation and signaling pathways belonging to the progression of the cell cycle, DNA repair, and drug resistance.[21]

The function of micro-24 is increased proliferation of the targets. This indicates the involvement of miRNA-24-3p in cancer progression during regulation of the cyclin-dependent kinase after being transcribed.[22] miR-24-3p expression is upregulated in breast cancer tissues in comparison with benign tissues, proving that miR-24 had an important role in invasion and metastasis of breast cancer. Moreover, they detect the capability of miR-24 on the levels of phosphorylated epidermal growth factor receptor.[23]

Expression of microRNAs vary through different cancer stages. This suggests their oncogenic and anti-oncogenic roles. miRNAs may exist during development and during breast cancer.[24]

Conclusions

MicroRNA is upregulated during breast infection caused by *S. aureus.*

These functions are important to better understand how malignancies can arise from their dysregulation.

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Conflicts of interest

There are no conflicts of interest.

References

1. Blackmon MM, Nguyen H, Mukherji P. Acute Mastitis. 2020 Jul 21. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2020.
2. Li T, Lu H, Wang X, Gao Q, Dai Y, Shang J, *et al*. Molecular characteristics of Staphylococcus aureus causing bovine mastitis between 2014 and 2015. Front Cell Infect Microbiol 2017;7:127.
3. Chauhan D, Davuluri KS. microRNAs associated with the pathogenesis and their role in regulating various signaling pathways during Mycobacterium tuberculosis infection. Front Cell Infect Microbiol 2022;12:1009901.
4. Zhao Y, Zeng Y, Zeng D, Wang H, Zhou M, Sun N, *et al*. Probiotics and MicroRNA: Their roles in the host–microbe interactions. Front Microbiol 2021;11:604462.
5. Ling J, Xie X, Wang Y, Huang W, Luo J, Su J, *et al*. Differential expression profiles of miRNA in granulomatous lobular mastitis and identification of possible biomarkers. Exp Ther Med 2022;24:1-12.
6. Kondracka A, Gil-Kulik P, Kondracki B, Frąszczak K, Oniszczuk A, Rybak-Krzyszkowska M, *et al*. Occurrence, role, and challenges of MicroRNA in human breast milk: A scoping review. Biomedicines 2023;11:248.
7. Li C, Hu X, Li L, Li JH. Differential microRNA expression in the peripheral blood from human patients with COVID-19. J Clin Lab Anal 2020;34:e23590.
8. Kang M, Xiao J, Wang J, Zhou P, Wei T, Zhao T, *et al*. MiR-24 enhances radiosensitivity in nasopharyngeal carcinoma by targeting SP 1. Cancer Med 2016;5:1163-73.
9. Tan H, Qi J, Fan BY, Zhang J, Su FF, Wang HT. MicroRNA-24-3p attenuates myocardial ischemia/reperfusion injury by suppressing RIPK1 expression in mice. Cell Physiol Biochem 2018;51:46-62.
10. Tzelos T, Ho W, Charmana VI, Lee S, Donadeu FX. MiRNAs in milk can be used towards early prediction of mammary gland inflammation in cattle. Sci Rep 2022;12:5131.
11. AL-Awadi SA. Molecular characterization of the bacterial diversity in women with breast infection. Thesis, 2018; College of Medicine, University of Babylon.
12. Al-lateef BA, Al-shukri MS, Judi MR. Expression of circulatory interleukin-6 concentration associated with Pseudomonas aeruginosa persistence in recurrent urinary tract infections. Med J Babylon 2023;20:201-5.
13. Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) method. Methods 2001;25:402-8.
14. Boix-Amorós A, Hernández-Aguilar MT, Artacho A, Collado MC, Mira A. Human milk microbiota in sub-acute lactational mastitis induces inflammation and undergoes changes in composition, diversity and load. Sci Rep 2020;10:18521.
15. Vaughn JM. Virulence characteristics and genetic diversity of Staphylococcus aureus isolates from cases of bovine mastitis in eastern Tennessee. M.Sc Thesis, The University of Tennessee, Knoxville; 2018.
16. Tuchscherr L, Pöllath C, Siegmund A, Deinhardt-Emmer S, Hoerr V, Svensson CM, *et al*. Clinical S. aureus isolates vary in their virulence to promote adaptation to the host. Toxins 2019;11:135.
17. Salmanov AG, Savchenko SE, Chaika K, Vitiuk A D, Beketova HV. Postpartum mastitis in the breastfeeding women and antimicrobial resistance of responsible pathogens in Ukraine: Results a multicenter study 2020.
18. Mirzaei R, Mohammadzadeh R, Mirzaei H, Sholeh M, Karampoor S, Abdi M, *et al*. Role of microRNAs in Staphylococcus aureus infection: Potential biomarkers and mechanism. IUBMB Life 2020;72:1856-69.
19. Zhao H, Huang M, Jiang L. Potential roles and future perspectives of Chitinase 3-like 1 in macrophage polarization and the development of diseases. Int J Mol Sci 2023;24:16149.
20. Dysin AP, Barkova OY, Pozovnikova MV. The role of microRNAs in the mammary gland development, health, and function of cattle, goats, and sheep. Non-Coding RNA 2021;7:78.
21. Kang H, Rho JG, Kim C, Tak H, Lee H, Ji E, *et al*. The miR-24-3p/p130Cas: A novel axis regulating the migration and invasion of cancer cells. Sci Rep 2017;7:44847.
22. Rezaei Z, Sadri F. MicroRNAs involved in inflammatory breast cancer: Oncogene and tumor suppressors with possible targets. DNA Cell Biol 2021;40:499-512.
23. Khodadadi-Jamayran A, Akgol-Oksuz B, Afanasyeva Y, Heguy A, Thompson M, Ray K, *et al*. Prognostic role of elevated mir-24-3p in breast cancer and its association with the metastatic process. Oncotarget 2018;9:12868-78.
24. Wu D, Thompson LU, Comelli EM. MicroRNAs: A link between mammary gland development and breast cancer. Int J Mol Sci 2022;23:15978.