Urinary 302bmicroRNAs gene expression in recurrent lower urinary tract infection caused by Pseudomonas aeruginosa Isolates.

Bareq A. Allateef[1], Maysaa S. Alshukri[2], Mohammad R. Jodi[3]

[1]Technical Institute of Babylon, Al-Furat Al-Awsat Technical University (ATU),
E.mail: barq10@atu.edu.iq, bareq.as86@gmail.com
[2]Departments of Microbiology, Medicine, College of Medicine, Babylon University, Iraq
E.mail: dr.maysaa.salih@gmail.com
[3]Departments of Microbiology, Medicine, College of Medicine, Babylon University, Iraq
E.mail: mrjudi1965.MJ@gmail.com

***Correspondence author:** Bareq A. Allateef (barq10@atu.edu.iq, bareq.as86@gmail.com)

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Abstract:

The real-time polymerase chainreactions were performed by using specific primers with reference gene GAPDH and the target genes 302b microRNA, A case–control study was done in Babylon city hospital from February 2021 to March 2022. A total of 110 patients with RUTIs withdifferent age and sex, and healthy individuals as control group were enrolled in this study. Midstream urine was taken for culturingand identification of Pseudomonas aeruginosa.The present study found that the expression of Mi302b gene expression increased in the RUTI patient with P. aeruginosa when compared with control group.so the expressiongene is increased more than (%25)fold when compare with control group.which considered as a potential biomarker ,the result show (30) 27.3% isolate were belong to P. aeruginosawhere able to manage chronic and/or recurrent infections represents a significant difference at P<0.05 .Method RNA was extracted from patients group and controls ,then gene expression of miR-302b by relative RT-PCR both in patient and control.

Keywords

Gene expression 302b, microRNA, mRNA gene, Real-time PCR, RUTI.

Recurrent UTIs are defined as two or more symptomatic episodes within 6 months or three or more symptomatic episodes in a timeframe of ⩽12 months. From a pathophysiological point [1].a rUTI may present as a relapsing infection that corresponds to an incomplete clearance of the causative pathogen and occurs within 14 days of completion treatment or as a re-infection presenting after 14 days of treatment completion [2].

Many virulence factors encoded byP. aeruginosa that provide increased fitness and better chances of survival within the host, promote bacterial growth and survival, thereby maneuvering the host cellular machinery by causing devastating injuries, tissue necrosis, evasion and immune system impairment [3].

Bacteria in polymicrobial UTI also have the ability to protect one other from clinically relevant antibiotics through the increase of tolerant/resilient phenotypes in the bacterial community [4].

MicroRNAs (miRNAs) are small single-stranded RNA molecules with24 nucleotides in length that can bind to 3/-

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untranslated region (3/-UTR) of target messenger RNA (mRNA) for enhancing or preventing translation [5]. miRNAs are not translated into proteins and exert keyroles in cellular and biological mechanisms [6].

miRNAs canfunction as potential therapeutic, diagnostic and prognostic factors incancer. As miRNAs can regulate apoptosis, differentiation, migrationand angiogenesis under physiological conditions, dysregulation in miRNA expression results in the development of various pathological events, particularly cancer [7].

It has also been found recently thatmiRNAs can selectively promote or inhibit proliferation and progressionof cancer cells [8].

miRNAs in the host was shown to be related to infectious diseases and associatedwith the eradication or susceptibility of the infection[9].

Material and methods

Patient and Clinical sample

A case-control study was conducted in Hospitals in Babylon city between February 2021 to the March 2022. Individuals admitted these centers with a suspected of Recurrent Urinary tract infection by clinical features.

A total of 110patients with RUTI were obtained with different age and sex, midstream urine were takes from patients for culturing and identification of pseudomonas aeruginosa. for culturing and it identification and diagnosed according to (forbes ,et al.,)[10], and vitek compact system.

Two ml blood samples were obtained from all patient infected with p.aeruginosaand put in EDTA tube, RNA was extracted for gene expressing stud.

Control group: 2 ml of blood were taken from (20) healthy individuals as Control group, RNA extract from thissamplein order to be study later.

Ethical Approval

Agreement from the family and patients forsampling and carrying out this work was obtained . The necessary ethical approval was obtained by verbal consent from patients.

Gene expression of microRNA 302b by Real-time RT-PCR

After collection of blood samples from patients and healthy individuals, the total RNA was collected extracted according to The TriRNA Pure Kit (Geneaid) the manufacturers' protocol.

The real-time qPCR relative gene expression (2dd cT) reactions were performed by using specific primers targeting reference gene GAPDH and the target genes microRNA 302b as show in (Table :1-1). Conversion the total RNA to cDNA and amplification of DNA was done according to instructions provided by GoTaq® 1-Step RT-qPCR System (Promega) using BRYT Green® dye, where RT-qPCR Mixture and conditions were summarized in tables (1-2), where the final volume of RT-qPCR reaction was 20µl. Relative expression fold was calculated by delta delta method (2^-(ΔΔCt) according to Livak and Schmittgen, 2001[11].

Table (1-1): RT-qPCRMixture

Component	Volume
GoTaq® qPCR Master Mix, 2X	10µl
GoScript™ RT Mix for 1-Step RT-qPCR (50X)	0.5 µl
Forward primer (20X)	1µl
Reverse primer (20X)	1µl
RNA Template	5 µl
Nuclease-Free Water	2.5 µl

Table (1-2): Primerssequence and condition used in qRT-PCR.

Primer name	Sequence 3'-5'		step	Temp/Time/ cycles
miR-302b-F	GCGTAAGTGCTTCCATGTT	[12]	-Revers transcription	-37c_o for15min.
miR-302b-R	TCCAGGGACCGAGGA		-Reverse transcriptase inactivation and GOTaq DNA polymerase activation	95$c_o$for10min 1 cycles -10 $c_o$for30sec.

GAPDH-F	GTCTCCTCTGACTTCAACAGCG	-Denaturation Annealing and data collection -Extension	37 $c_o$for30sec. 40 cycles -72c $c_o$for 30sec.
GAPDH-R	ACCACCCTGTTGCTGTAGCCAA		

Statistical Analysis

Molecular results was done by using Chi-square(x2) test. P values less than (0.05) is considered. Statistical analysis was performed by using SPSS 19 version. Data were expressed as (mean ± SD).

Result and Dissection

Distribution of recurrent urinary tract infection associated with pseudomonas aeruginosa.

A total of 110 clinical specimen were collected from patient were admit tohospitals in Babylon city, the result show (30) 27.3% isolate were belong toP. aeruginosawith a significant difference at P<0.05. as show in table (1-3). Which identified according to culture characters biochemical test and vitek compact system.

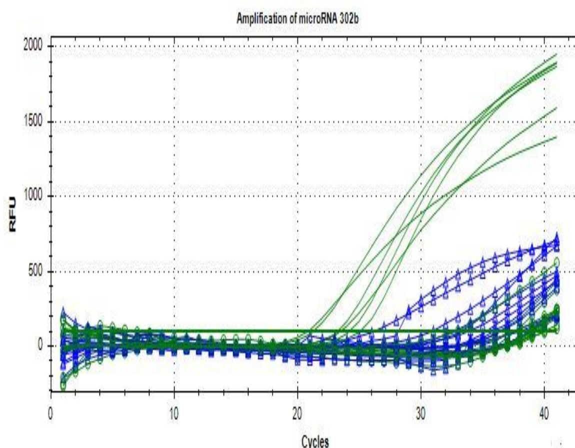

Table (1-3) Distribution of pseudomonas aerogenosastrains isolated from patients with RUTI diseases.

Results	No.	%	P value
Positive	30	27.3	<0.0001*
Negative	80	72.7	
Total	110	100	

* represents a significant difference at P<0.05.

Levels of microRNA 302b Gene expression

A total from 30 bloodpatient sample, RNA was extracted to detect the gene expressing of microRNA302b by RT-qPCR(Relative gene expression[2ΔCt] methods ,in this method the expression levelof micro302b gene in test sample as well as in control sample normalized with house-keeping geneGAPDH as show in figure(1-1)

Results described using PCR efficiencies and mean crossing point deviations between samples and controls, represent a significant difference at p⩽ 0.05.

Fig (1-1): microRNA 302b Gene expression level. This is the first run for 15 samples, represents amplification of Reference gene (GAPDH), represents amplification of samples RUTI Patients, and represents amplification of control samples.

Table (1-4: microRNA 302b Fold Gene Expression in Control and Patients versus the reference gene (GAPDH).

Groups	N	Expression levels (2^-(ΔΔCt))		
		Mean	SD	SE
Control	20	1.26	0.99	0.22
Patient	30	14.01	5.36	0.98
P value		<0.0001*		

* Represent a significant difference at p⩽ 0.05.

Table (1-5): microRNA 302b (C T) Expression in Control and Patients versus the reference gene (GAPDH).

Groups	N	Ct		
		Mean	SD	SE
Control	20	39.72	0.90284	0.20188
Patient	30	36.10	0.90290	0.16485
P value		<0.0001*		

* represent a significant difference at p⩽ 0.05.

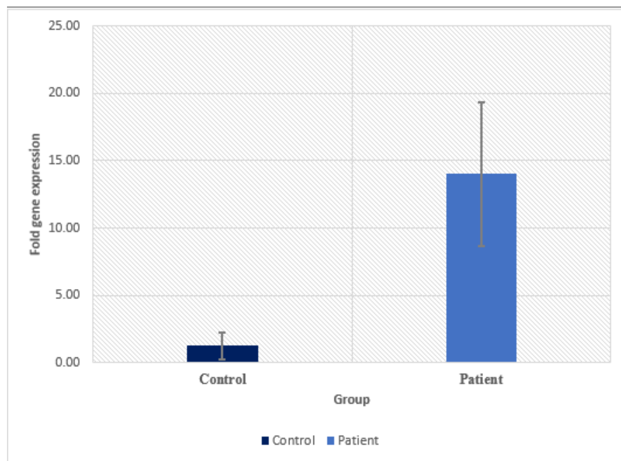

Fig. (1-2): microRNA 302b fold Gene Expression among Control and RUTI Patients versus the reference gene (GAPDH).

Discussion

Distribution of recurrent urinary tract infection associated with pseudomonasaeruginosa

Pseudomonas aeruginosa, a bacteriumconsideredan extracellular pathogen, which can cause chronic infections to many human site, includingthe urinary tract [13].

Penaranda, et al. [14] show that P. aeruginosacan survive inside bladder epithelial cells and becomes tolerant to antibiotic treatment,the bacteria quickly adapt to the intracellular environment and inducing recurrence urinary infection .

Many strain-specific bacterial virulence factors may contribute to the recurrence of UTI, such as flagella/pili, adhesins, extracellular polysaccharides, toxins lipopolysaccharides,ureases, proteases and iron-scavenging siderophores, This behavior has been reported in Pseudomonas aeruginosa[15],and may even be facilitated by polymicrobial interactions during infection[16].

Thänert,et al.,[17],found that patients with hospital readmission was higher inpatients with P. aeruginosacUTI and causing recurrent infection.the ability of P. aeruginosato survive plays an important role in contributing to the chronicity and recurrence of P. aeruginosainfections and that targeting host urinary tract pathways.

Mirna- 302b Geneexpression:

The present study found that the expression of Mi302b gene increased in the RUTI patient with P. aeruginosa when compared with control group.so expressionthe gene is increased more than (%25) fold in compare with control groupas show in figure (1-1).

Huang et al.,[18], found that mi302b expression was significantly elevated with P. aeruginosa.at 3h and 6h, these finding suggest that both mi302a andmi302b may be involved in the process of host defense during P. aeruginosainfection .

miR-302b is a crucial regulator of inflammatory response in host defense against P. aeruginosa invasion and the result agreement with [19],That show this type of microRNA correlated with in host defense against P. aeruginosa.These findings characterize a new crosstalk between host microRNA response and mitophagic activityrequired for defending pathogens' invasion, suggesting that P. aeruginosa induced miR-302/367 cluster targets NF-kB, the specific role of miRNAs in regulation of mitophagy in bacterial infection[20]. Also, it was found that Gram-negative bacterial infection could induceUpregulation of miR-302b expression.

in Gram-negative bacterialinfection could induce upregulation of miR-302b expressionin epithelial cells through TLR/NF-kB-dependentpathways. Then, have proved that miR-302b negativelyregulatesbacteria-triggered proinflammatory cytokine production, thus indicating a new mechanism to counteract thebacterial evasion of innate immune, bacterial infection can alsoupregulate theexpression of miR-302b which in turn inhibits innate antibacterial immune response by blocking TLR signaling and TLRtriggered proinflammatory cytokines production [21].

Study has demonstrated thatmiR-302b regulates the production of proinflam matorycytokines in macrophages in response to P. aeruginosa invasion [22].

As show in table (1-4)and (1-5) of the result overexpression of miR-302b justify the regulated NF-κB and caspase-1 signaling, leading to significantly attenuate induced IL-1β. By genetic analysis, miR-302b exhibited inhibitory function on interleukin-1 receptor-associatedkinase 4 (IRAK4) [23].

As one of the most essential cell types in the antibacterial immunity, macrophages function as the predominant cell for making response to P. aeruginosa infection [24]. The cells uptake and subsequently kill

invasive bacteria by regulating key factors of pattern recognition receptors (PRR) in recognition of PAMP, but also initiating an inflammatory response against P. aeruginosa infection [25].

Therefore, precise modulation of macrophage activity is crucial for immune defense against P. aeruginosa infection. indicating that miR302s might be required for the macrophage-mediated immune defense against infection. In the present study, immune response to P. aeruginosa. The results demonstrate that miR-302 mediated bacterial elimination [26].

Conclusions

It was found that theexpression of miR-302b was significantly increased in patients when compared with control group.in recurrent urinary tract infection associated with pseudomonas aeruginosa .

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