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Estimation of *miR-16* Gene Expression in Iraqi Tuberculosis Patients and Its Association with Response to Therapy

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Abstract: Tuberculosis (TB) is an airborne, infectious disease caused by *Mycobacterium tuberculosis* and commonly occurring in the lungs as pulmonary tuberculosis (PTB), but it can affect any organ in the body. Is considered one of the most common infectious diseases and major causes of morbidity and mortality worldwide. The present study aimed to evaluate the level of *miR-16* in the serum of newly infected TB, Multi Drug Resistance (MDR) of TB patients, and patient's post-therapy. The samples were collected from Iraqi patients with tuberculosis in the unit of the chest and respiratory diseases in the primary health care worker sector in Baghdad city. The total samples were 130 divided into four groups new infection =62 (47.6 %), completed therapy = 27 (20.7%), MDR =21 (16.1%), and control negative =20 (15.38%). Identification of sputum, and blood biomarkers that can be useful for predicting *Mycobacterium*. Diagnose the disease by classical methods [Ziehl-Nilsen (ZN), cultured on Lowenstein Jensen (LJ), molecular method gene x pert, ESR, PCV, Hb, WBCs]. The level of the *miRNA* was estimated by qRT-PCR. The levels of miR-16 were significantly elevated. The levels of *miR-16* returned to those observed in healthy subjects. While In patients with MDR TB, *miR-16* levels were lowest in the serum of MDR TB patients compared to TB new, TB treated group, and healthy controls. In conclusion, *miR-16* in serum may act as a surrogate biomarker for studying TB infection, progression of therapy, and MDR TB. Up to our knowledge this study was the first one that investigated the correlation between the Blood Parameters and *miRNA-16* expression levels, the study revealed that the Hb levels have a positive correlation with the expressions level of this miRNA, which means the Hb levels were elevated in new infection TB causing a lack of hemoglobin.

Keywords - Tuberculosis, microRNA, serum miRNA, drug resistance, therapy, biomarker.

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Introduction

Tuberculosis (TB) is an infectious illness that has been around since ancient times. (1) *Mycobacterium tuberculosis* (Mtb), a systemic infectious illness, causes tuberculosis (TB). TB infection is one of the leading causes of illness and death across the world. According to WHO data, almost one-third of the world's population has been affected. According to current research, 10% of infected persons showed signs of the disease, while 90% have no symptoms. The spread of drug resistance is threatening TB control; Mtb is multi-drug and widely drug-resistant and requires more time and money to cure. With numerous medications, adverse effects are worsened and therapeutic effectiveness is less likely (2). The WHO has worked to develop improved treatment options for drug-resistant Mtb(3). MicroRNAs

(miRNAs) are a type of short RNA molecule that regulates a variety of physiological and pathological processes (4). miRNAs are essential mediators in immunity function and have a determinate role in host–pathogen interactions in multiple infection diseases. The miRNA profile of the bacterial infected cells showed different miRNA expression profiles compared with healthy controls. These observations indicated the important role of miRNAs in immune surveillance and control of infection (5, 6). The metabolic associated miRNAs *miR-155, miR-33, miR-1224,* and *miR-16* increased in Mtb-infected macrophages, according to microarray analysis in clinical samples and in *vitro* investigations, these microRNAs have been shown to have played important roles in macrophage metabolism (7). By regulating autophagy or apoptosis in infected cells and exploiting the host's energy and metabolic pathways, miRNAs play an essential role in the pathogenesis of Mtb infection. The host's modulation is a complex process. The miRNA profile of mycobacterium-infected cells is influenced by the pathogen. However, the mechanism(s) through which bacterial pathogenicity is expressed is unknown. Factors that alter miRNA expression regulation in the direction of prevention it’s uncertain how immunodetection works (8).

Materials and methods

One hundred and thirty blood samples were taken from patients infected with tuberculosis, including newly infected patients, who completed treatment after being infected with tuberculosis, and multi-drug resistant (MDR), from December of the year 2022 until April of the year 2022. Healthy subjects without tuberculosis were taken, at age and gender matched the patient's group, Descriptive information was obtained from each patient and control subjects to fill up a questionnaire present for this purpose. Two ml of whole blood took from patients and put in an ethylene diamine tetra acetic acid (EDTA) tube to estimate PCV, HB, ESR, and level of gene expression. Sputum was collected from patients in accordance with Iraq's National Tuberculosis Program. Each patient had three specimens obtained: one when he initially arrived at the clinic, one before breakfast, and one at any other time during the day (5). Two sputum samples are used in Ziehl – Nelseen staining (ZN) and culture, with the third sample being used in Gene Xpert. Diagnose the disease a classical method [Ziehl-Nilsen (ZN), cultured on Lowenstein Jensen (LJ), molecular method gene Xpert, ESR, PCV, Hb, WBCs]. The levels of the *miRNA-16* were estimated by qRT-PCR. The levels of *miR-16* in the serum of TB patients as compared to uninfected controls. The Statistical Analysis System- SAS (2018) program was used to detect the effect of different factors on study parameters. Least significant difference –LSD test (Analysis of Variation-ANOVA) was used to significantly compare between means. The Chi-square test was used to significantly compare between percentage (0.05 and 0.01) probability.

Results and discussion

Study group categorization

The samples were collected from Iraqi patients with tuberculosis in the unit of the chest and respiratory diseases in the primary health care worker sector in Baghdad city. The total samples were 130 divided into four groups new infection =62 (47.6 %),

completed therapy = 27 (20.7%), MDR =21 (16.1%), and control negative =20 (15.38%). The results in table 1 showed that the most prevalent age groups were (20-39) and (40-59) years old of TB patients, and the lowest recorded age group was observed in <20, which may be due to the BCG vaccine which is given at young age, vaccination gives good protection for children however this immunity decline with age.

Table (1): Patient's distribution according to Age and their TB types

Variables	new infection		completed therapy		MDR		control negative	
Age Group (years)	No.	%	No.	%	No.	%	No.	%
< 20	6	9.67%	2	7.40%	0	0%	1	5%
20-39	30	48.38%	13	48.14%	5	23.80%	4	20%
40-59	13	20.96%	6	22.22%	11	52.38%	10	50%
≥ 60	13	20.96%	5	18.51%	5	23.38%	5	25%
Total	62	100	27	100	21	100	20	100

Tuberculosis and gender

Figure (1) shows the distribution of TB cases according to gender. The percentage of Males subjects was more innumerable than females in TB cases according to results, completed therapy The result was men 66.67 % and females 33.33%.

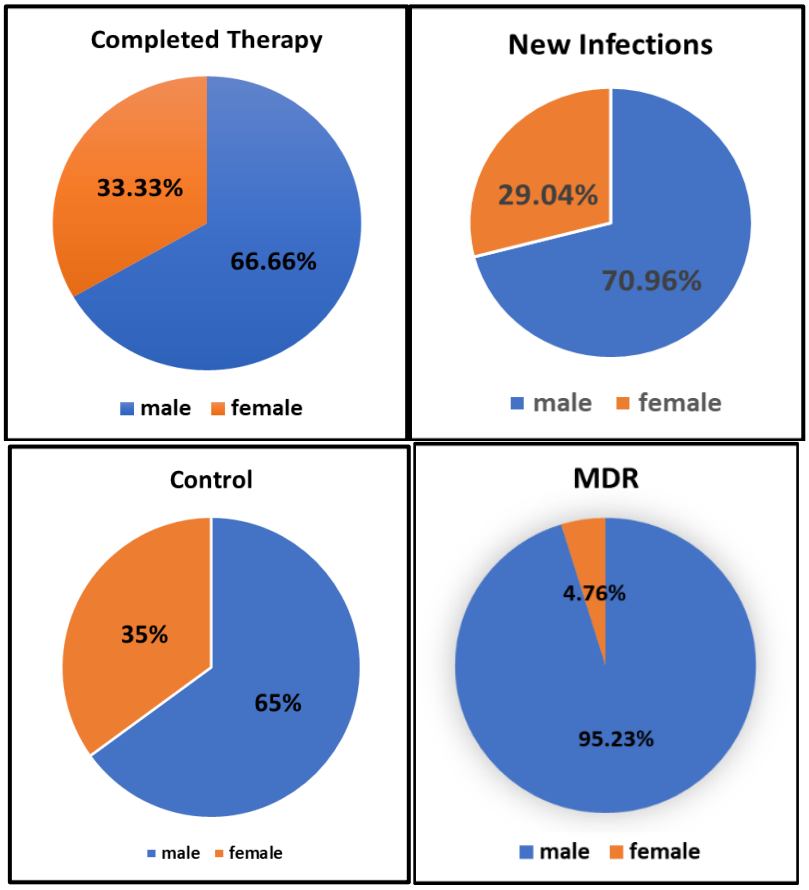

Figure (1): Shows the TB cases according to gender in four groups (complete therapy, MDR, New infections, and healthy controls).

In MDR, the result was men 95.24 % and females 4.76%, while in new infection result was men 70.96 % and females 29.04 %. Healthy controls result was men 65 % and females 35 %. of the results above all, males were more affected than females due to differences in the immune response, behavioral habits, physiological differences, and susceptibility to tuberculosis, This agrees with the researcher(9).

Comparison between different groups according to blood parameters

Table (2) showed the results describing the comparison between four groups according to ESR, Hb, PCV, and WBC. The comparison between four groups according to ESR, results indicated that healthy control (32.85 ±5.79), MDR (69.71 ±8.80), completed therapy (35.52 ±4.29), and new infection TB (65.87 ±4.17), the results showed high significant in *p- Value* (0.0001) and it was significant. While the comparison between four groups according to Hemoglobin (Hb), the results showed the healthy control (13.44 ±0.38), MDR (12.89 ±0.38), completed therapy (12.96 ±0.29), and new infection TB (12.14 ±0.23), the results was significant in *p- Value* (0.0154) and it was significant. The patient may be susceptible to tuberculosis and this study agrees with Abay F, Yalew A (2018) (10). In the comparison between four groups according to PCV, the results showed the healthy control (42.85 ±1.46), MDR (40.47 ±1.23), completed therapy (40.78 ±1.15), and new infection TB (38.45 ±0.73), the results was significant in p- Value (0.0272), and it was significant. The PCV parameter may be useful indices in assessing response to therapy and drug compliance in pulmonary tuberculosis patients living in developing countries. This study agrees with (11). In the comparison between four groups according to WBCs, the results showed control (7.15 ±0.45), MDR (5.19 ±0.55), completed therapy (6.95 ±0.39) and new infection TB (5.02 ±0.26), the results was significant in *p- Value* (0.0001) and it was highly significant. this result is important because WBC counts are usually evaluated in TB patients at baseline, Therefore, they are helpful to point of-care tools to help clinicians identify which patients might be less responsive to treatment (12).

Table (2): Comparison between different groups in blood parameters

Group	Mean ± SE			
	ESR	Hb	PCV	WBC
Control	32.85 ±5.79 b	13.44 ±0.38 a	42.85 ±1.46 a	7.15 ±0.45 a
MDR	69.71 ±8.80 a	12.89 ±0.38 ab	40.47 ±1.23 ab	5.19 ±0.55 b
Completed therapy	35.52 ±4.29 b	12.96 ±0.29 ab	40.78 ±1.15 ab	6.95 ±0.39 a
New infection TB	65.87 ±4.17 a	12.14 ±0.23 b	38.45 ±0.73 b	5.02 ±0.26 b
LSD value	17.059 **	0.945 *	3.216 *	**
P-value	0.0001	0.0154	0.0272	0.0001
This means having the different letters in the same column differed significantly. * (P≤0.05), ** (P≤0.01).				

Gene expression of *miRNA-16 -5p*

Table (3) describes the gene expression of Complete therapy *miRNA - 16* in the control and patient's groups showed that the value of folding for patients (1.69 ±0.39) was more than in

control (1.35 ±0.23). Consider the value of *GAPDH* for patients (27.44) and for control (27.80) which means high gene expression in patients in compression with control with non-significant differences. These results agree with the study of (13), who found that the levels of miR-16 were significantly elevated in the serum of TB patients in compression with uninfected controls.

Table (3): Gene expression of complete therapy *miRNA -16* in control and patients group

Group	*GAPDH*	Ct Mean +SE	ΔCt	ΔΔCt	Folding
Control	27.80±SE	29.90±SE	2.10	-0.00044	1.35 ±0.23
Patients	27.44±SE	29.74±SE	2.29	0.351	1.69 ±0.39
T-test	--	--	--	--	1.0042 NS
P-value	--	--	--	--	0.493
NS: Non-Significant.					

Table (4) described the gene expression of MDR *miRNA-16* in the control and patients group showed that the value of folding for patients (3.84 ±0.69) was more than in the control (1.35 ±0.23). The folding value was high for patients other than the control. Consider the value of *GAPDH* for patients (28.81) and for control (27.80) means with Highly-significant differences.

Table (4): Gene expression of MDR *miRNA-16* in control and patients group

Group	*GAPDH*	Ct Mean +SE	ΔCt	ΔΔCt	Folding
Control	27.80	29.90	2.10	-0.00044	1.35 ±0.23
Patients	28.81	28.04	0.761	-1.418	3.84 ±0.69
T-test	--	--	--	--	1.516 **
P-value	--	--	--	--	0.0020
** (P≤0.01).					

Table (5) mentioned the gene expression of new infection *miRNA16* in the control and patients' groups showed that the value of folding for patients (4.39 ±0.55) more than in control (1.35 ±0.23) was more highly for patients other than in control. Consider the value of *GAPDH* for patients (28.84) and for control (27.80) which means a higher value of gene expression for patients other than control with significant differences.

Table (5): Gene expression of new infection *miRNA-16* in control and patients group

Group	*GAPDH*	Ct	ΔCt	ΔΔCt	Folding
Control	27.80±SE	29.90±SE	2.10	-0.00044	1.35 ±0.23
Patients	28.84±SE	31.45±SE	-0.856	2.78	4.39 ±0.55
T-test	--	--	--	--	2.181 *
P-value	--	--	--	--	0.0294
* (P≤0.05).					

Many researches provide insight into the role of expressed miRNAs in TB, but a detailed understanding of the role of these miRNAs in the anti-mycobacterial response is a matter of highest interest for future investigations. These studies also provide a good foundation for the development of reliable biomarkers and therapeutic targets for TB, Many of

these studies provide insight into the role of these differentially expressed miRNAs in TB, but a detailed understanding of the role of these miRNAs in the anti-mycobacterial response is a matter of highest interest for future investigations. These studies also provide a good foundation for the development of reliable biomarkers and therapeutic targets for TB (14, 15, 16, 17,18).

Correlation coefficient between gene expression and parameters of patients

Table (6) showed the correlation coefficient analysis for mean values of the study parameters with respect to *miRNA- 16* gene expressions showed non-significant correlations ESR. Showed *miRNA- 16* gene expressions showed non-significant correlations HB, except with new infection TB showed significant correlations. MiRNA-16 gene expression with significant correlations with all study parameters. These results of all microRNAs may be due to binding microRNA with specific promoter sites on the genes of the parameters, causing a significant correlation between these parameters and the microRNAs .These results are shown in Table 6. To our knowledge this study was the first one that investigated the correlation between the Blood Parameters and MiRNA-(16, 155) expression levels, this study found that the HB levels have a positive correlation with the expressions level of these MiRNAs , which means the HB levels was elevated in new infection TB causing a lack of hemoglobin.

Table (6): Correlation coefficient between *miRNA-16* gene expression and Blood parameters of patients

Fold of genes	Mean ± SE			
	ESR	Hb	PCV	WBC
Complete therapy *miRNA-16*	-0.05 NS	-0.03 NS	0.09	-0.09
MDR *miRNA-16*	-0.04 NS	0.08 NS	0.02	0.04
New infection *miRNA-16*	0.08 NS	0.003 NS	0.10	0.05
* ($P \leq 0.05$), NS: Non-Significant.				

Conclusions

The present study demonstrated ESR measurement as an inflammatory indicator, but a rather non-specific inflammatory marker for TB patients. HB levels have a positive correlation with the expression level of these *MiR-16*, More TB cases were found in the reproductive age group (20-59) year-old. And found serum levels of *miR-16* as a diagnosis and prognosis marker for TB. If validated in a larger population, believe this could act as a valuable tool for TB management.

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