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THE IMPORTANCE OF DIAGNOSING MYCOBACTERIUM TUBERCULOSIS BY REAL-TIME PCR (POLYMERASE CHAIN REACTION IN REAL TIME) COMPARED TO THE DIAGNOSTIC METHODS ADOPTED IN THE CLINICAL LABORATORY

***Khldoun Salah Aslan**

MD, Department of Laboratory, Tishreen University Hospital, Lattakia, Syria.

***Corresponding Author: Khldoun Salah Aslan**

MD, Department of Laboratory, Tishreen University Hospital, Lattakia, Syria.

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ABSTRACT

Background: Tuberculosis remains a serious public health problem worldwide. Ziehl-Neelsen stained smear and culture on LJ media are conventional methods used for the diagnosis of *Mycobacterium tuberculosis* in most developing countries. PCR for the diagnosis of tuberculosis is not evaluated in developing countries**. The Aim of the study:** To compare the ability of Real time PCR to diagnose MTB in pulmonary and extra-pulmonary samples, and compare it with the results of ZN and LJ. **Materials and Methods:** Samples obtained from 96 patients of suspected TB (pulmonary and extra-pulmonary) were processed for detection of MTB by ZN smear examination, LJ medium culture and Real time PCR test**. Results:** The sensitivity of Real time PCR was 97,8% compared to 17, 4% for smear microscopy and 100% for LJ culture. Percentage of TB detection using Real time PCR in pulmonary and extra-pulmonary samples was 96% and 100% respectively. The mean detection time for MTB was 6 weeks by LJ medium culture, less than one day by PCR and 4 days by ZN for 96 samples. **Conclusions:** PCR is a rapid and sensitive method for the early diagnosis of pulmonary and extra- pulmonary tuberculosis.

KEYWORDS: Mycobacterium Tuberculosis, Real time PCR, ZN staining, LJ medium culture, pulmonary TB, extra pulmonary TB.

INTRODUCTION

Tuberculosis is one of the most common infectious bacterial diseases and threatens the lives of people all over the world.[1] Tuberculosis is also a major public health problem due to the high risk of person-to-person transmission, morbidity and mortality. Both HIV epidemic and poor social situations have contributed to an increase in the rate of infection with Mycobacterium Tuberculosis, especially in developing countries.[2]

An early diagnosis followed by adequate treatment is" Essential" to prevent both morbidity and mortality. The microscopic examination of the Ziehl-Neelsen stain and transplantation on the Lowenstein-Jensen are considered to be "traditional" methods used in the diagnosis of Mycobacterium Tuberculosis **TB** in most developing countries.[3]

Although the smear test that removes Ziehl-Neelsen stain is cheap, qualitative and needs 1-2 hours to give a result, it is less sensitive and requires a large number of bacilli (up to 10,000 bacilli/ML) in the sample. It is also not possible to distinguish Mycobacterium Tuberculosis from other mycobacteria, so it is used to investigate the presence of acid-resistant bacilli only. In addition, it requires collecting hay samples on consecutive days, which makes the procedure slow and diagnosis difficult.[4]

This technique is widely used in Syria and other developing countries. The implant has a high quality and is considered the gold standard for diagnosis, but it needs 6-8 weeks to give a result and therefore leads to a delay in the final diagnosis.[5]

Therefore, tuberculosis often remains undiagnosed and even untreated, especially in the case of extrapulmonary samples, as they contain few bacilli, and this leads to low sensitivity for transplantation and smear.[6] Polymerase Chain Reaction (PCR) test in the diagnosis of tuberculosis is not well evaluated in developing countries, including Syria, but it has begun to be actively applied in bacteriological clinical diagnosis.[7][8]

The importance of this study

- Tuberculosis is a global health problem and its importance has increased in Syria under the conditions of war and epidemics.
- The importance of PCR in real time lies in the fact that it is a sensitive, qualitative and fast method of

detecting tuberculosis Bacillus, in addition to the low sensitivity of traditional methods (Nielsen remove staining) and the long time required for transplantation.
- There is no previous similar study confirming the importance of real-time PCR in the diagnosis of tuberculosis in our country Syria.

OBJECTIVES OF THE STUDY

- The problem of diagnosis of Mycobacterium tuberculosis using real-time polymerase chain reaction in pulmonary and extrapulmonary clinical samples.
- To compare the real-time polymerase chain reaction results with the results of implantation on Lowenstein-Jensen and Ziehl-Neelsen stain.
- The aim is to emphasize the importance of using real-time polymerase chain reaction as a rapid diagnostic method in the diagnosis of Mycobacterium Tuberculosis.

Sample Research

The study included 96 samples taken from patients with clinically suspected pulmonary tuberculosis (55 patients) and extrapulmonary tuberculosis (41 patients) visiting Tishreen University Hospitals. The samples were collected during the period from January 2022-2023.

Sample Collection

The samples were divided into two parts: the first part was subjected to microscopic examination and implantation, and the second part was kept in the freezer at -20 degrees Celsius until the polymerase chain reaction (PCR) was performed in real time. Respiratory samples (aspiration, bronchoalveolar lavage) were treated using the method:. NALC(N-acetyl-L-cystein)-NaoH.

MATERIALS AND METHODS

The following devices were used in the research

- Ziehl-Neelsen stain
- Lowenstein-Jensen
- Optical microscope with 100× lens
- Real Time PCR device
- Frozen samples
- Containerized tubes on anticoagulant
- Micropipette especially for working on a Real Time PCR device

Real Time PCR method[10][9]

This technique is based on performing the amplification of the target DNA and the detection of the amplified output simultaneously in one tube, thereby reducing the time it takes to complete the DNA assay from hours to minutes.

The procedure for working on Real Time PCR is divided into two steps.

Amplification: like normal PCR and includes.

Denaturation: which is carried out by raising the temperature of the reaction mixture up to 94-95°c And the incubation is for two minutes, during which a separation occurs between the two target DNA energies that act as Template.

Annealing: which is carried out by cooling to a temperature of 55-60°C, where a annealing occurs between the two primers that are added to the reaction mixture and their complementary sequences at the two separate energies of the target DNA. The two prefixes specify the two ends of the DNA to be amplified.

Extension: which is done by heating up to a temperature of 72°C, which represents the temperature Optimal for the thermally stable DNA polymerase enzyme, which is added to the reaction mixture. In this reaction, by the action of polymerase, a union is obtained between the triphosphate deoxynucleosides dNTPs.

Detection: based on scintigraphic technology. The sample is exposed to a tungsten or halogen source which causes the substance added to the sample to be scintigraphed and the signal is amplified as the number of copies in the DNA sample is amplified. The emitted signal is detected by a detector and sent to a computer after converting it into a digital signal that is displayed on the monitor. The signal can be detected when it reaches the threshold level (the lowest detection level by the detector).

Statistical Analysis

- ✓ Statistical analysis was carried out with IBM SPSS version 20 program.
- ✓ The sensitivity and specificity of diagnostic tests were calculated as the Gold Standard of transplantation for diagnosis.
- ✓ Chi-Square tests were used to assess the differences in the distribution of categorical variables of the study group.
- ✓ Differences at the P-value threshold less than or equal to 0.05 were considered statistically significant.

RESULTS

Sample Characteristic

Our study included 96 patients who had visited Tishreen University Hospital, and were clinically suspected of having either pulmonary tuberculosis (55 patients) or extrapulmonary tuberculosis (41 patients).

The pulmonary samples included 35 bronchodilators and 20 expectorant samples, the extrapulmonary samples included 25 urine samples, 6 pleural fluid samples, 5 Ascites fluid samples, 4 blood samples and one biopsy.

We recorded the presence of 46 cases of tuberculosis according to the" gold standard for the diagnosis of tuberculosis (transplantation on the center of Lowenstein-Jensen) at a rate of 48%.

Distribution of the sample according to Tuberculosis infection

Tuberculosis infections were distributed among 26 pulmonary tuberculosis infections out of 55 samples at a rate of 47% and 20 extrapulmonary tuberculosis infections out of 41 at a rate of 49%.

When performing transplantation on Lowenstein-Jensen, the growth of Mycobacterium tuberculosis-causing Tuberculosis was found in 46 samples and the isolation rate of non-tuberculous mycobacteria was 2% (two out of 96 samples). The sensitivity of the test was 100%, its quality was 96%, with a positive predictive value 95,8%.

Table (1): TB positive transplant results.

P value	Total	Real injury			Transplantation on Lowenstein-Jensen
		Healthy	Injured		
0,00	48	2	46	Positive	
	48	48	0	Negative	
	96	50	46		

The number of positive lung samples in the transplant amounted to 26 TB samples and the number of exopulmonary samples infected with Mycobacterium tuberculosis was 20 samples compared to two samples infected with non-TB Mycobacterium.

Comparison between ZN and LJ

The number of positive samples with Ziehl-Neelsen stain removes only 8 out of 46 samples with a sensitivity of 17.4%, 100% quality, with a positive predictive value of 100% and a negative predictive value of 43.2%.

Table 2: Comparison between ZN and LJ.

P value	Total	Transplantation on LJ			Ziehl-Neelsen stain
		Healthy	Injured		
0,002	8	0	8	Positive	
	88	50	38	Negative	
	96	50	46		

From the previous table, we note that the P-value is equal to 0,002, which is lower than the significance level of 0,05, and therefore the apparent differences in the classification of patients suspected of having tuberculosis between staining and transplantation are of a statistical significance. The number of positive pulmonary samples by staining amounted to four samples and the number of extrapulmonary samples to four.

Comparison between PCR and LJ

Polymerase chain reaction (PCR) in real time revealed the presence of 45 positive samples of Mycobacterium tuberculosis. The sensitivity was 97.8%, the specificity was 100%, the positive predictive value was 100% and the negative predictive value was 2%.

Table (3): Comparison of PCR results with implantation.

P value	Total	Transplantation on LV			Real time PCR
		Healthy	Injured		
0	8	0	45	Positive	
	88	50	1	Negative	
	96	50	46		

From the previous table we note the absence of significant differences in the classification of patients suspected of tuberculosis between transplantation and polymerase chain reaction. The number of PCR positive pulmonary samples was 25 and the number of extrapulmonary samples was 20.

Comparison among the three methods for TB diagnosis

When comparing the ability of the three Tests to detect Mycobacterium Tuberculosis in different clinical samples, it was found that Real time PCR has the same ability of transplantation to diagnose Mycobacterium tuberculosis in different samples, while staining has a very low ability" to diagnose tuberculosis.

Table (4): Comparison among the TB diagnostic methods.

Real time PCR	Transplantation on LJ	ZN Stain	TB	Total	sample type
15 (94%)	16 (100%)	4 (25%)	16	35	Bronchoalveolar lavage
10 (100%)	10 (100%)	-	10	20	Disperse
10 (100%)	10 (100%)	4 (16%)	10	25	Urine
4 (100%)	4 (100%)	-	4	4	Blood
3 (100%)	3 (100%)	-	3	6	Pleural
2 (100%)	2 (100%)	-	2	5	Ascites
1 (100%)	1 (100%)	-	1	1	Biopsy

DISCUSSION

- Ziehl-Neelsen staining is widely used in the diagnosis of tuberculosis. Numerous studies have shown a decrease in its sensitivity, reaching 64%, as well as a decrease in its quality by 98%. While our study showed a decrease in sensitivity and an increase in quality than reported in these studies. It is unclear the main reason for the decrease in sensitivity in this study. A possible explanation for the decrease in sensitivity is Laboratory workload, the difference in the skill of detectives and the variability of abilities between laboratories.
- Transplantation is considered a gold standard for the diagnosis of tuberculosis in developing countries. Based on our study, it was found that the implant was positive in all samples compared to the Ziehl-Neelsen staining and the sensitivity of the implant was higher compared to the staining. Other studies have shown implant sensitivity ranging from 80-85%.
- Real time PCR was positive in all but one of the positive implants, which may be due to the presence of inhibitory substances that hinder its work in the samples.
- When comparing the three Tests in the detection of extrapulmonary tuberculosis [which is difficult to detect due to the nature of the few-Bacillus samples, insufficient sample due to its use in many tests (bacteriological, histological, chemical)], we found that Real time PCR has a higher sensitivity to staining and has the same sensitivity as implantation. The time required to detect Mycobacterium tuberculosis using Real time PCR was also less than the time required for transplantation and staining. Other features of Real time PCR is its ability to detect Mycobacterium tuberculosis even if it is found in small quantities (only two bacilli). It can also detect non-live bacilli in patients who have started treatment or formalin-stabilized samples.
- Our study showed that Real time PCR plays an "important" role in the rapid diagnosis of Mycobacterium tuberculosis in pulmonary and extrapulmonary tuberculosis and has a high sensitivity and quality compared to traditional methods.
- PCR sensitivity and quality were high compared with other countries such as Iran, India, Pakistan, Korea and China.

CONCLUSION

- ❖ It is recommended to use Real time PCR as a sensitive and qualitative rapid diagnostic method in the diagnosis of Mycobacterium Tuberculosis.
- ❖ Suggestion to replace the traditional methods used in the diagnosis of tuberculosis with polymerase chain reaction PCR in real time, especially in the diagnosis of extrapulmonary Tuberculosis.

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REFERENCES

1. Dabernal H, Thves C, Bouakaz C, Nicolaeva D, Keyser C et al. "Tuberculosis epidemiology and selection in an autochthonous Siberian population from 16th-19th century," *PLoS One*, 2014.
2. World Health Organization. 2002. "Global tuberculosis control", WHO report 2002. 295. World Health Organization, Geneva, Switzerland.
3. Pervez MAK, Hasan QN, Rumi MAK, Ahmed S, Salimullah M, Tahera Y et al. PCR can help early diagnosis of pulmonary tuberculosis. Southeast Asian J Trop Med Public Health, 2003; 34: 147-53.
4. Morre DF, Curry JI. Detection and identification of mycobacterium tuberculosis directly from sputum specimen by Amplicore PCR. J Clin Microbiol, 1995; 33: 2686-91.
5. Yam WC. Recent advances in rapid laboratory diagnosis of tuberculosis. MWD Bull, 2006; 11: 6-7.
6. Chakravorty S, Kamal M, Tyagi JS. Diagnosis of Extrapulmonary tuberculosis by Smear, Culture and PCR, Using Universal Sample Processing Technology. J Clin Microbiol, 2005; 43: 4357–62.
7. Tiwari V, Jain A, Verma RK. Application of enzyme amplified mycobacterial DNA detection in the diagnosis of pulmonary and extra-pulmonary tuberculosis. Indian J Med Res, 2003; 118: 224-8.
8. Cegielski JP, Devlin BH, Morris AJ. Comparison of PCR, culture, and histopathology for diagnosis of tuberculous Pericarditis. J Clin Microbiol, 1997; 35: 3254-7.
9. *JORGENSEN, JAMES and PFALLER, MICHAEL. 2015. Manual of Clinical Microbiology 11TH*

EDITION. America. ASM Press.
10. Levinson, warren. 2014. Review of medical microbiology and immunology thirteen edition. United States. McGrow Hill Edication.