Tb Laboratory Diagnosis, a Comparative Study in Baghdad, Iraq

Aljanabi, Yasir M. AL Abdulateef[1], Shehab Ahmed Lafi[2], Haitham Noaman Eyada[3]

[1]LecturerClinical Laboratory Science Department, College of Pharmacy, HOD. University of Anbar, Ramadi, Iraq, [2]Prof. HOD. Microbiology Department, College of Medicine, University of Anbar, Ramadi, Iraq, [3]Assistant Prof. Department of Medicine, College of Medicine University of Anbar, Ramadi, Iraq.

Abstract

Background: Tuberculosis is an infectious contagious disease caused primarily by *Mycobacterium tuberculosis*. The probability of developing TB disease is much higher among people with risk factors. Iraq was classified the seventh country in Middle East Asia that related to the high burden of tuberculosis. Many laboratory tests are used in diagnosis of TB. The study aimed to compare the results of AFB sputum test,sputum culture, Gene-expert automated system PCR and IGRA test for interferon Gamma in TB patients.

Aims of the Study: This study aimed to compare between efficiency of TB diagnostic tests : AFB, IGRA, IgG, IgM, Gen X pert PCR & Culture on LJ. medium used in TB. Patients attended private Clinics In Bagdad, Karch Sector, IRAQ.

Patients and Method: In a Descriptive study, one hundred fifty four (154) suspected TB patients were attending Lagash Private Clinic in Baghdad, Capital of IRAQ during the period from January 2017 to December 2019. All patients were examined by well expert physician's senior specialist, requested clinical, chest x-ray and lab investigations AFB, IGRA, IgG, IgM, Gen X pert PCR & Culture on LJ medium were done .

Results: Patients were showing different rates of positive tests regarding their age, all patients were showing positive IGRA followed by IgM and PCR tests. While culture and AFB tests were showing the lowest rate in the studied patients sample. Gender showed no effect on the tested parameters used for TB study. We recommend depending IGRA, PCR for the rapid diagnosis of TB.

Keywords: *TB, gene x pert, AFB, IGRA,TB IgM.*

Introduction

Tuberculosis is an infectious contagious disease caused primarily by *Mycobacterium tuberculosis*. The probability of developing TB disease is much higher among people with risk factors[(1-4)]. There is an increasing rate of the drug-resistant tuberculosis strains toward the drugs for curing of tuberculosis in large sector of risky groups, the problems of emergency multi-drug resistant TB (MDR) is major conflict in the world[(5-6)]. TB is remaining one of the major contagious bacterial diseases around the World, in 2014, Iraq was classified the seventh in country,in Medial East Asia (MEA) that related to the high burden of tuberculosis[(5,7)]. Symptoms of pulmonary tuberculosis which includes chest pain, cough, bloody sputum, night sweating and fever are highly observed in such cases. And about 10 % of all (PTB) seem asymptomatic[(8,9)]. When *Mycobacterium tuberculosis* spreads outside the lungs producing different type of TB, the most common types of extra-pulmonary tuberculosis are pleural TB, tuberculous meningitis, lymphatic system (especially around the neck), Urogenital tuberculosis and the bones or joints [(4,10)]. AFB sputum test remains a faster screening diagnostic test for TB and dependent by WHO through DOTS TB control program in endemic countries[(11-13)]. The classical cultivation method on (Lowenstein-Jensen medium) remained as a gold standard to diagnose TB.

Bacilli and to estimate the drug susceptibility DST, but this method is time-consuming (weeks to months). So many new laboratory techniques have been developed by World Health Organization (WHO) to fasten the diagnosis and detection of drug resistance of TB bacilli, these new laboratory method included phenotypic method (liquid culture) such as BancTec MGIT 960 and genotypic method (Molecular PCR Technique) by Gene Xpert system and Line Probe Assay[14-16].Although microscopy and culture remain necessary for treatment monitoring, now global use of rapid molecular tests and automated cultivation systems are increasing, many countries are phasing out the use of smear microscopy for diagnostic purposes[17]. So this study is devoted to compare the significance of AFB, IGRA, IgG, IgM, Gene X Pert PCR & culture on LG .medium in diagnosis of TB patients attending private clinics .

Patients and Method

In a Descriptive study, one hundred fifty four(154) suspected TB patients were attending Lagash Private Clinic in Baghdad, Capital of IRAQ during the period from January 2017 to December 2019 . Considering research ethics, a written consent was done for each patient. All patients were examined by well expert physician's senior specialist and each patient was submitted to the routine chest exam and requested clinical, chest x-ray and lab required investigations. Inclusion criteria were followed like patients with high risk for TB and patients with extra pulmonary TB were included in this study. Exclusion criteria were followed like patients with other affections like CA lung and tumors of other sites of respiratory diseases, patients with autoimmune diseases like SLE In additions to that patients with any infection causes confusion with TB are excluded in this study). Sputum specimens, Serum,blood were taken from each patient and examined as soon as possible in Lagash Private Clinical Laboratory for Clinical investigations. Each specimen was manipulated following optimal method for each technique; AFB staining technique was done for each sputum and pleural specimen as well as cultivation on LJ. Medium following [12-13]. Test for Interferon Gama (IGRA) ELISA test was done for each serum specimen following instructions of manufacturing company (Sigma). Gene Xpert test (PCR) Test was done for each blood specimen following instructions of the manufacturing company(Gene XpertMTB/RIF, USA). Direct examination (AFB) test was done for each specimen following [12]Guidelines for sputum examination. Data were reported and analyzed using SPSS program Version 2.

Results and Discussion

It is found that IGRA test showed highest ratio (40.5%) of positive result followed by PCR (30.7%) and IgG (28.8%), (Fig-1).

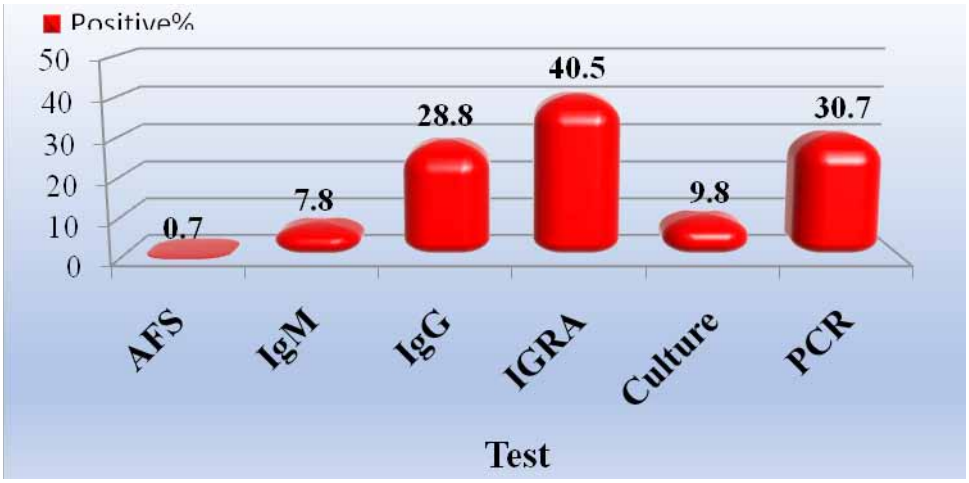

Fig. 1: Rate of positive results of different Laboratory tests.

This was in accordance with the findings of[18], this was might be attributed to the high sensitivity of IGRA test. Non-significant difference ($p \leq 0.5$) was found between IGRA and PCR positive ratio (Fig-1). This was attributed to sensitivity and specificity of these tests. Low ratio of positive IgM and culture (7.8%) and

(9.8%) for each respectively, this can be explained by the fact that the peak of IgM arise in the acute reaction then undergo a decline at the same time IgG undergos an increase and show sharp increase in chronic status[19]. So the low ratio of IgM was found due to the decline of this immunoglobulin in chronic disease like TB without relapse(TB Immunity[20]. Very low ratio(0.7%) positive AFB test was might be attributed to the low bacilli number in sputum specimens and majority of patients were treated so they were showing reduced bacilli number in their sputum specimens. AFB test requires at least 1000-2000 bacterial cells per gram of sputum to give positive results[4,12,13], (Fig-1).

Regarding age group, it was found that significant difference was found between age groups in IGRA & IgG results(P≤0.0001) & (p≤0.017), patients within age group ≥60years old patients were showing highest ratio of IGRA & IgG positive tests results (51.6%) & (48.4%) for each respectively Table This was in accordance with the findings of[20-21].

Table 1: Lab tests results regarding Age groups of patients.

		Age (years)						P value
		<40y		40---59		=>60y		
		No	%	No	%	No	%	
AFS	Positive	1	2.8	-	-	-	-	-
	Negative	35	97.2	55	100	62	100	
IgM	Positive	2	5.6	5	9.1	5	8.1	0.826
	Negative	34	94.4	50	90.9	57	91.9	
IgG	Positive	3	8.3	11	20.0	30	48.4	0.0001*
	Negative	33	91.7	44	80.0	32	51.6	
IGRA	Positive	8	22.2	22	40.0	32	51.6	0.017*
	Negative	28	77.8	33	60.0	30	48.4	
Culture	Positive	2	5.6	7	12.7	6	9.7	0.531
	Negative	34	94.4	48	87.3	56	90.3	
PCR	Positive	7	19.4	21	38.2	19	30.6	0.166
	Negative	29	80.6	34	61.8	43	69.4	
*Significant difference between proportions using Pearson Chi-square test at 0.05 level.								

Patients within age group 40-59 years old were showing highest ratio (38.2%) of positive PCR test followed by the age group ≥60 years (30.6%).

Patients less than 40 years old were showing the lowest positive results of the above mentioned tests **(**Table-1). This can be explained by the fact that increased rate of TB infection with age increase[6-21] due to the potency of BCG vaccine undergo decrease with higher age so the infective rate will be higher in the age group ≥60year followed by 40-59 years old (Table-1). All patients showed positive IGRA and PCR tests patients were showing positive IgM (100%). Sera from TB culture positive patients were showing more positive IgM than sera from culture negative specimens, (25%) & (8.5%) for each respectively, this was in accordance with the finding of[6] (Table-2).

Adult patients within the age group 46-60 years old were showing the highest rate of positive AFB and gene expert results Followed by the findings of patients within the age group 18-30 years old for AFB and Gene Expert. This was in accordance with the findings of[20] in Baghdad. This is might be due to the reactivation of childhood TB among individuals within age group 45-60 years old patients[4].

Table 2: Relation between IgM Test and other Laboratory Tests

		IgM				P value
		Positive		Negative		
		No	%	No	%	
AFS	Positive	1	8.3	-	-	-
	Negative	11	91.7	141	100	
IgG	Positive	11	91.7	33	23.4	0.0001*
	Negative	1	8.3	108	76.6	
IGRA	Positive	12	100	50	35.5	-
	Negative	-	-	91	64.5	
Culture	Positive	3	25.0	12	8.5	0.065
	Negative	9	75.0	129	91.5	
PCR	Positive	12	100	35	24.8	-
	Negative	-	-	106	75.2	
*Significant difference between proportions using Pearson Chi-square test at 0.05 level.						

This indicates the early stage of infection IgG positive results were showing more IgM positive results patients (Table-2). Results of this study showed that high ratio of positive IGRA, PCR, IgM and culture results were showing higher positive IgG results,(75%, 52%,25%,20%)for each respectively(P≤00 (Table-3). Regarding IGRA test, 75.8% of positive PCR test patients were showing positive IGRA test, while 53.2% of IgG positive patients were showing positive IGRA test.

Followed by culture and IgM positive patients, 24.25 and 19.4% of them were showing IGRA test respectively (Table-3).

Table 3: Relation of IgG and other tests results.

		IgG				P value
		Positive		Negative		
		No	%	No	%	
AFS	Positive	1	2.3	-	-	-
	Negative	43	97.7	109	100	
IgM	Positive	11	25.0	1	0.9	0.0001*
	Negative	33	75.0	108	99.1	
IGRA	Positive	33	75.0	29	26.6	0.0001*
	Negative	11	25.0	80	73.4	
Culture	Positive	9	20.5	6	5.5	0.005*
	Negative	35	79.5	103	94.5	
PCR	Positive	23	52.3	24	22.0	0.0001*
	Negative	21	47.7	85	78.0	
*Significant difference between proportions using Pearson Chi-square test at 0.05 level.						

Regarding IGRA test, all patients with positive culture were showing positive PCR tests. This was due to the high relation between the two tests. All patients with IGRA positive results were showing positive PCR test, this means positive relation between two tests (Table-4).

Table 4: Relation between PCR and other tests.

		PCR				P value
		Positive		Negative		
		No	%	No	%	
AFS	Positive	1	2.1	-	-	-
	Negative	46	97.9	106	100	
IgM	Positive	12	25.5	-	-	-
	Negative	35	74.5	106	100	
IgG	Positive	23	48.9	21	19.8	0.0001*
	Negative	24	51.1	85	80.2	
IGRA	Positive	47	100	15	14.2	-
	Negative	-	-	91	85.8	
Culture	Positive	15	31.9	-	-	-
	Negative	32	68.1	106	100	
*Significant difference between proportions using Pearson Chi-square test at 0.05 level.						

The same interpretation is acceptable for Gene Expert test was the presence of adequate bacterial cells of Mycobacterium tuberculosis in sputum to show positive Gene Expert system DNA test results. Similar results were nearly obtained by Al-Ouqaili et al 2018. Who use PCR test on sputum specimens from patients in Ramadi City, west of Iraq. The study concluded that AFB and Gene Expert tests are suitable tests for the identification of TB infection in adults and children above 6 years due to easy sampling of sputum suitable for these tests. In conclusion, patients were showing different rates of positive tests regarding their age, most of patients were showing positive IGRA followed by IgM and PCR tests. While culture and AFB tests were showing the lowest rate in the studied patient sample. Gender showed no effect on the tested parameters used for TB study. We recommend use depending IGRA and PCR method for the diagnosis of TB. In the same time, dependable test depends on the age and stage of disease. Tb is endemic disease in Iraq like hydatid cyst and brucellosis.[25,26]

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

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