

# Ziehl-Neelsen Technique versus Gene-Expert PCR Method in TB Patients: A Sample Study in Ramadi City, Iraq

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## ABSTRACT

**Background:** Tuberculosis is an infectious contagious disease caused by *Mycobacterium tuberculosis*. The probability of developing TB disease is much higher among people infected with HIV, and also higher among people with risk factors. Iraq was classified the seventh country in Middle East Asia that related to the high burden of tuberculosis. The study aimed to compare the results of AFB sputum test and sputum (Gene-expert automated Polymerase Chain Reaction (PCR) system) in Ramadi TB center, Anbar Governorate, West of IRAQ.

**Patients and Methods:** Four hundred and three (403) TB patients were included in this study. They were attending Thoracic Diseases and TB Central Hospital in Ramadi City, West of Iraq. WHO Sputum specimen was taken from each patient following guidelines. Each sample was manipulated in the lab using AFB and TB Gene expert automated PCR system in Ramadi TB center. Results were reported and analysed using SPSS.

**Results:** The number of patient children was lower than adult patients, one (1) and (102) for each respectively. In adult patients, the highest ratio of TB patients was found among patients within age group

(18-30) years old individuals followed by patients within age groups (31-45) and (45-60) years old individuals, (35.4%), (25.60%) and (23.72%) for each respectively. Further, non-significant difference ( $p \geq 0.5$ ) was found between male and female patients ratio included in this study. Regarding AFB results, only one male child within age group (13-17) was showing positive AFB test result and the same individual was showing positive gene expert result. In adults, patients within age group (18-30) and (46-60) years old were showing highest rate of positive AFB and gene expert results (40.20%) and (35.30%) for each respectively.

**Conclusion:** 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. There is a strong relation ( $P \leq 0.005$ ) between AFB and gene expert results in children and adults.

**Keywords:** TB, Gene-expert automated cultivation, Acid-Fast Bacilli (AFB)

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## INTRODUCTION

Tuberculosis is one of the known global diseases first diagnosed by the scientist Robert Koch who diagnosed *Mycobacterium tuberculosis* (MTB) the causative agent of TB. In March 24, 1882, the probability of developing TB disease is much higher among people infected with HIV (Brooks FG, *et al.*, 2013), and also higher among people affected by risk factors such as mal-nutrition, diabetes, smoking and alcohol consumption (Suryanarayan VK and Chandra P, 1998; Lardizabal A and Bahavaraju R, 2005). TB is remaining one of the major contagious bacterial diseases around the World, in 2014, Iraq was classified the seventh country in Middle East Asia (MEA) that related to the high burden of tuberculosis. During that year the program from WHO in Iraq identified about 20000 new tuberculosis patients and the TB cases death about more than 4000 people. In addition the incidence rate estimation was about 43 per 100000 people (Laura A, 2018; Anderson L, *et al.*, 2015).

In untreated patients, the death rate due to active tuberculosis is more than 66%. Active tuberculosis may become latent TB in most patients infected that lead to producing fibrosis or caseous necrosis in upper or lower part of lung lobes (Crowley LV, 2013).

In latent TB the MTB utilized the granuloma formation to prevent antigen presentation and prevent the destruction of MTB by host immune system (Bozzano F, *et al.*, 2014). Symptoms of Pulmonary Tuberculosis (PTB) 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 (Crowley LV, 2013; Bozzano F, *et al.*, 2014). When *Mycobacterium tuberculosis* penetrates the blood circulation, Military tuberculosis leading to TB lesions will be emerging in another organ of the body such as (bone, lymph node, kidney and brain (Brooks FG,

*et al.*, 2013; Crowley LV, 2013; Velayati AA, *et al.*, 2011). 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 (Brooks FG, *et al.*, 2013; Crowley LV, 2013). There is an increasing rate of the drug-resistant tuberculosis strains toward the drugs for curing of tuberculosis in large sector of risk groups, the problems of emergency Multi-drug Resistant TB (MDR) is major conflict in the world. AccD3 gene is a good drug target in MDR *M. tuberculosis* strains (Asra'a A, *et al.*, 2018). AFB sputum test remains a faster screening diagnostic test for TB and dependent by WHO in TB control programs in endemic countries (Crowley LV, 2013; Vandepitte J, *et al.*, 2003; Forbes BA, *et al.*, 2007). 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 a several new laboratory techniques have been developed by World Health Organization (WHO) to faster diagnosis and detection of drug resistance of TB bacilli, these new laboratory methods included phenotypic methods (liquid culture) such as BancTec MGIT 960 and genotypic methods (Molecular PCR Technique) by gene expert system and line probe assay (Forbes BA, *et al.*, 2007; World Health Organization, 2008). 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 (World Health Organization, 2010; World Health Organization, 2017). So this study is devoted to compare the results of AFB sputum test for patients with the phenotypic sputum cultivation method, (liquid culture), gene expert PCR system used in

Ramadi TB Center, Anbar Governorate, west of Iraq.

**MATERIALS AND METHODS**

Four hundred three (403) patients were included in this study; they were attending Thoracic Diseases and TB Central Hospital in Ramadi, West of Iraq during the period extended from January 2017 to February 2018. 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. Sputum specimens were taken from each patient to perform the required tests. Sputum examination was done on the early morning sputum samples. Direct examination (AFB) test was done for each specimen following WHO Guidelines for sputum examination (Vandepitte J, *et al.*, 2003; Forbes BA, *et al.*, 2007). Gene part MTB/RIF Automated PCR was done for each sputum specimen using gene pert automated system (USA) following materials and method described by the same manufacturing company. Data were reported and analyzed.

**Statistical analysis**

Analysis of data was accomplished using the available statistical package of SPSS-22 (Statistical Packages for Social Sciences-version 22). Data were

presented in simple measures of frequency, percentage, mean, standard deviation, and range (minimum-maximum values). The significance of the difference of different means (quantitative data) was tested using Students-t-test for the difference between two independent means or Paired-t-test for difference of paired observations (or two dependent means), or ANOVA test for difference among more than two independent means.

**RESULTS**

The study result revealed that the number of child patients were lower than adult patients one (1) and (102) for each respectively. In adult patients, highest ratio of TB patients was found among adult patients within age group (18-30) years old individuals followed by patients within age groups (31-45) and (45-60) years old individuals, (35.4%), (25.60%) and (23.72%) for each respectively. Non-significant difference ( $p \leq 0.5$ ) was found between male and female adult patients ratio included in this study (*Table 1*). Regarding AFB results, it showed that only one male child within age group (13-17) was showing positive AFB test and the same individual was showing positive gene expert result (*Table 2*) (*Figures 1 and 2*). In adults, patients within age group (18-30 and 46-60) years old were showing highest rate of positive AFB and gene expert results (40.2%) and (35.30%) for each respectively (*Table 2*) (*Figures 1 and 2*). There was a strong relation ( $P \leq 0.005$ ) between AFB and gene expert results in children and adults.

**Table 1: Number of patients (children and adults) included in the study**

Age group (year)	Male	Female	Total no (%)
A-Children			
1-6	1	1	2, 6.25%
7-12	5	4	9, 28.12%
13-17	9	12	21, 65.62%
Total children	15	17	32, 100%
B-Adults			
18-30	72, 37.11%	58, 32.76%	130, 35.04%
31-45	52, 26.80%	43, 24.30%	95, 25.60%
46-60	42, 21.65%	46, 25.98%	88, 23.72%
≥ 61	28, 14.43%	30, 15.81%	58, 15.63%
Total adults	194, 52.3%	177, 47.70%	371, 100%
Total patients	209, 51.86%	194, 48.13%	403, 100%

**Table 2: Ratio of Acid-Fast Bacilli (AFB) and gene expert positive results among studied patients**

Age group (year) (Children)	AFB test results		Gene expert result		Total
	Male	Female	Male	Female	
1-6	0	0	0	0	0
7-12	0	0	0	0	0
12-17	1	0	1	0	1
Total children	1	0	1	0	2
B-Adults					
18-30	11	8	12	10	41, 40.2%
31-45	2	4	2	4	12, 11.76%
46-60	9	9	8	10	36, 35.3%
≥ 61	2	3	4	4	13, 12.74%
Total adults	24	24	26	28	102, 100%
Total patients	25, 23.58%	25, 23.58%	27, 25.47%	29, 27.58%	106, 100%

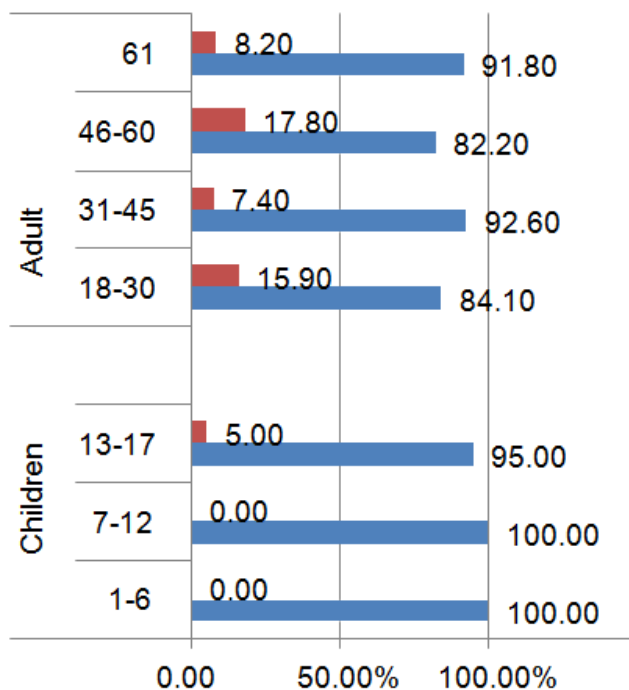


Figure 1: Acid-Fast Bacilli (AFB) results among Genders and age groups of patients. Note: Red indicates people with AFB positive and blue indicates people with AFB negative

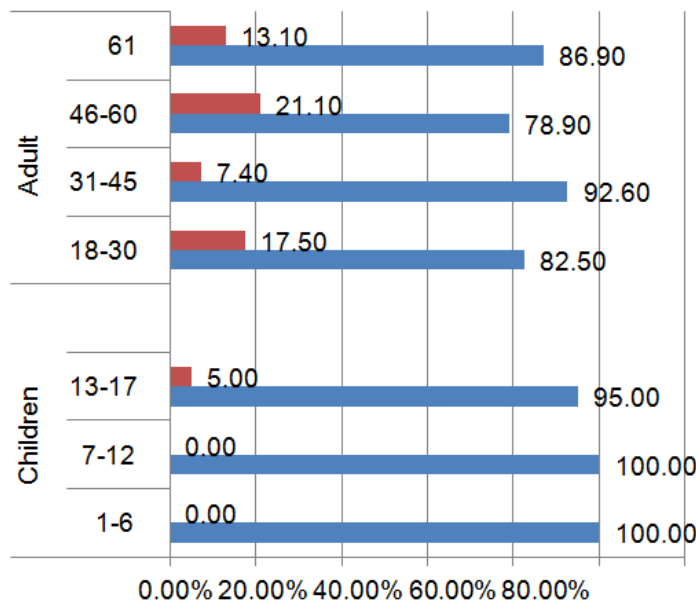


Figure 2: Gene expert results among genders and age groups of patients. Note: Red indicates people with gene expert positive and blue indicates people with gene expert negative

## DISCUSSION

The lower number of children than adult patients was might be attributed to the difficulty in recognition and diagnosis of TB in children particularly lower than five years (Suryanarayan VK and Chandra P, 1998; Crowley LV, 2013). Children with TB do not have the symptoms commonly associated with pulmonary symptoms tuberculosis in adults, cough is not prominent and hemoptysis is very rare and reaching the fact that 25% of children with TB disease have extra-pulmonary TB, especially TB meningitis, miliary

TB adenitis and bone and joint infections (Suryanarayan VK and Chandra P, 1998; Laura A, 2018; Selekman J, 2006).

The highest rate of TB infection was found among young adults within age groups (18-25) and (31-45) years old individuals, this was in accordance with the findings of (Laura A, 2018; Ali LG, *et al.*, 2013; Talib AAL, 2010). This was might be attributed to the high-risk factors (addiction and smoking) within these age groups such risk factors increase probability of TB infection in such age groups (Yang Z, *et al.*, 2004). TB infection in other

age groups above 60 years might be due to reactivation of old calcified TB lesions of childhood TB as well as the relapse of TB infections caused by strains resistant to anti TB drugs (Laura A, 2018; Velayati AA, *et al.*, 2011; Talib AAL, 2010). Young adult males were showing higher rate of infection than females, this was might be attributed to the predisposing factors which imposed an increase of TB infection among young adults and teen-aged individuals such as smoking, alcohol and drug addiction. However, epidemiologic studies mentioned that male to female TB infection ratio in young adults was 2:1 (Laura A, 2018).

#### Acid fast staining versus gene expert

AFB results revealed that only one male child within the age group (13-17) years was showing positive AFB test result and the same individual was showing positive gene expert result, this was might be attributed to the same fact mentioned above which stated that children were showing low rate of AFB while tuberculin test has a definite role in the diagnosis of TB in children particularly children lower than 5 years old (Suryanarayan VK and Chandra P, 1998; Selekman J, 2006). 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 Al-Hasani (Talib AAL, 2010), in Baghdad. This is might be due to the reactivation of childhood TB among individuals within age group 45-60 years old patients (Brooks FG, *et al.*, 2013; Yang Z, *et al.*, 2004).

Regarding young adults age group 18-30-year-old individuals, the high ratio of AFB and gene expert positive results cause was might be due to the predisposing factors facilitated TB infection among them like smoking alcohol consumption, drug addiction and other affections leading to lowered immune status (World Health Organization, 2017) who mentioned that 20% of people in house hold develop infection micro-epidemics have occurred in closed environments. Gene expert test was showing high sensitivity and specificity in TB diagnosis in both children and adults (Vandepitte J, *et al.*, 2003; Forbes BA, *et al.*, 2007; Dunn JJ, *et al.*, 2016; Causse M, *et al.*, 2011). There was a strong relation ( $P \leq 0.005$ ) between AFB and gene expert results in children and adults. This was attributed to the presence of adequate number of *Mycobacterium tuberculosis* cells in specimens necessary for positive AFB results (Vandepitte J, *et al.*, 2003; Forbes BA, *et al.*, 2007; Dunn JJ, *et al.*, 2016). 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 (Al-Ouqaili MT, 2018), who use PCR test on sputum specimens from patients in Ramadi City, west of Iraq.

#### CONCLUSION

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. There is a strong relation ( $P \leq 0.005$ ) between AFB and gene expert results in children and adults.

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