Diagnosis of pulmonary and extra pulmonary tuberculosis from Babylon Iraq patient

Israa, K. Obayes[1, 2], Mohammad A. K. Al-Saadi [1], and Hadi, F. AL-Yasari[1*]

[1] Department of Microbiology, College of Medicine, Babylon University, Babylon, Hilla, Iraq.
[2]Department of Medical Laboratory Techniques, Al-Mustaqbal University College, 51001 Hillah, Babylon, Iraq..
Corresponding author: Email: Israa.Khudhair@mustaqbal-college.edu.iq, mbmc.kadhum70@gmail.com, * hadialyasari@gmail.com, ruqayaya89@gmail.com

Abstract:

sputum samples were collected from 110 suspected pulmonary tuberculosis after they were suspected to have tuberculosis according to their clinical manifestations. They were admitted to Babylon Center of Tuberculosis and Chest Diseases during the period January to October 2021.A total of 90 TB patients consisting of 51 males and 39 females were involved in this study. Their age ranged from 11 to more than 70 yearsafter diagnosis the patients there were 50 confirmed pulmonary tuberculosis, also collected 40 samples from extra pulmonary tuberculosis patients on the basis of clinical findings. Evaluation of direct Acid fast bacilli smear microscopy, Gene Xpert and Löwenstein–Jensen culturing of sputum for diagnosis of pulmonary tuberculosis**,** the result showing, the GeneXpert was considered the gold standard test which has a higher sensitivity than AFB smear microscopy in respiratory samples require several minutes to perform diagnosis of patients with high clinical suspicion of PTB, the lymph nodes is most common in Extra pulmonary tuberculosis.

Keywords: Pulmonary tuberculosis, Extra pulmonary tuberculosis.

DOI Number: 10.14704/nq.2022.20.12.NQ77109 **NeuroQuantology 2022; 20(12): 1311-1323**

Introduction

Tuberculosis (TB) is considered one of the most important infectious diseases in the world and its incidence is on rise (Abdalla *et al*.,2016). Tuberculosis is a leading global cause of morbidity and death (Dye,2006). TB infection is acquired by the inhalation of droplets containing *Mycobacterium Tuberculosis* bacilli (MTB). Tuberculosis is an immunological disease and the clinical manifestations of tuberculosis are the functions of the immune status of the host (Singh ,2000). The world health organization (WHO) considered Iraq within a high burden of TB among seven countries of the Eastern Mediterranean Region include 20 thousand represent of new and relapse TB cases (represent 54% of all cases), of these four thousand death recorded annually (WHO,2017). There may be common symptoms of tuberculosis: night sweats, fever, chills, lack of appetite, tiredness and weight loss (Dolin, 2010). If a tuberculosis infection becomes active, the lungs are most often involved (in 90 % of cases) (Behera, 2010). Chest pain and a persistent sputum-producing cough can be symptoms. Around 25 % of individuals will have no signs (they remain asymptomatic) (Vigenschow, 2019). People will sometimes cough up blood in small quantities, and the infection can erode through the pulmonary artery in extremely rare cases, resulting in massive bleeding. Tuberculosis affects the upper lung lobes more often than the lower ones (Dolin, 2010). Tuberculosis primarily affects the lungs, but some of its most devastating clinical consequences arise because of its ability to spread from the lungs to other organs. Extra pulmonary TB constitutes 15–20% of all TB cases. One in every five TB cases presents

eISSN 1303-515

with extra pulmonary TB (Gambhir *et al*., 2017). EPTB can be either primary (at the site of initial infection) or secondary (disseminated), which usually occurs due to hematogenous or lymphatic spread of bacteria from the primary organ, reactivation of latent TB (LTBI), ingestion of infected sputum, or spread locally from adjacent organs (Gopalaswamy *et al*., 2021). The diagnosis was important for detection MTB infection and confirm the presence of bacterium by non-costed conventional techniques in addition to molecular techniques which revealed high specificity and sensitivity compared to conventional methods (Maynard-Smith *et al.,* 2014).The confirmation of MTB infection through the short period by using geneXpert and PCR have a potential role in limitation and termination the incidence of infection by introducing anti-TB drug quickly furthermore limitation of multidrug-resistant MTB (Agrawal *et al.,* 2016).

Materials and Methods

1.Sputum samples

The diagnosis of pulmonary TB requires a respiratory specimen constitute the most common type of specimen submitted for the culture and isolation of mycobacteria, about 2-5 ml sputum of deeply coughing patients collected at early morning in a sterile container for both microscopy and mycobacterial cultures for patients with suspected pulmonary tuberculosis. Patient's mouth, lips, tongue, or fingers should not touch the inside of the container and should rinse his/her mouth with water, the sputum should consist of thick mucoid material, not saliva (Allen *et al.,* 2016).

2. Sputum processing

A-Principle

Sputum is a complex specimen consisting of network mucin molecules, filamentous actin, cell debris, leukocytes, inflammatory mediators and elastin fibers and normal flora bacteria embedded within this matrix. Efficient processing of sputum samples leads to the release of bacteria trapped within the complex sputum matrix. So the sensitivity of diagnostic tests for MTB is largely dependent on the efficiency of sputum processing protocols (Yoshimatsu *et al.,* 2015). Sputum specimens are treated with a mucolytic agent to break down the mucus and a decontaminating agent to suppress the growth of the normal flora present in the sample. If this were not done, the normal flora would overgrow the more slowly growing species of mycobacteria, making their isolation from clinical samples impossible. The initial processes involve homogenization and decontamination of sputum by Modified Petroff's method in which 4% sodium hydroxide (NaOH) is used (Goldman and Green, 2009).

B-Method

The method of sputum processing according to (Satapathy *et al.,* 2014)

3.Diagnosis of *Mycobacterium tuberculosis* in PTB

3.1 Direct microscopy identification of acid-fast MTB

A-Principle

Acid-fast mycobacteria contain mycolic acid in their outer membrane, making the cells waxy and resistant to staining with aqueous-based stains such as the Gram stain. The primary stain, carbolfuchsin, is applied to the cells and phenol is used to allow the stain to

eISSN 1303-515

penetrate into the waxy surface of acid-fast microorganisms. The excess stain is removed with treatment by 1% sulfuric acid. A secondary stain, methylene blue, is then applied to the cell (Ali *et al.,* 2013).

B-Method

The procedure of direct microscopy identification by AFB staining was applied according to (Tille, 2015).

3.2 Routine culture of MTB

A-Principle

All the sputum samples which appears AFB+ selected for culturing to confirm MTB diagnosis. Lowenstein-Jensen (LJ) media also called the egg-based media that consist of eggs, salts, potato flour, amino acids, glycerol, and malachite green used in the present study for the cultivation of MTB The media was previously prepared in slant screw cup tube by the laboratory of the chest and respiratory disease center. This medium supplied with antibiotics to prevent the growth of fungi, yeast, and bacteria throughout the long incubation period (Ali *et al*., 2013).

B-Methods

The specimens cultivated based on Betty *et al.,* (2007).

3.3 GeneXpert MTB/RIF assay for molecular detection of MTB

A-Principle (Pandey *et al.,* 2017)

The Xpert MTB/RIF (Xpert) assay produced by (*Cepheid Inc*., Sunnyvale, CA, USA) is a cartridge-based, semi-automated, rapid molecular assay, which permits rapid TB diagnosis through detection of the DNA of MTB and simultaneous identification of a majority of the mutations that confer rifampicin resistance (which is highly predictive of multi-drug resistant TB).The Xpert assay works by detecting MTB and RIF resistance by polymerase chain reaction (PCR) based on integrates and automates specimen processing, nucleic acid amplification and detection of the target sequences (81-bp *rpoB* gene segment that are associated with RIF resistance) in simple or complex specimens and probing for the mutations that are related to RIF resistance. The assay is automated and completes within 2 hours. The Xpert MTB/RIF assay can be applied to sputum samples or processed sputum sediments samples regardless of the specimen smear was AFB positive or negative. The system consists of an instrument, personal computer, barcode scanner, and preloaded software for running tests on collected specimens and viewing the results. The system requires the use of single-use disposable Gene-Xpert cartridges that hold the PCR reagents and Treat clinical specimens, including used cartridges, as if capable of transmitting infectious agents. Use proper precautions, such as wearing protective disposable gloves, laboratory coats, and eye protection, and follow your institution's safety procedures and guidelines (Maynard-Smith *et al.,* 2014).

B-Method

The method of GeneXpert MTB/RIF assay according to(Al-Omeri, 2011) .

3.5.4 Diagnosis of Extra pulmonary tuberculosis

eISSN 1303-515

EPTB diagnosis depends on clinical criteria including X-ray, signs and symptoms.

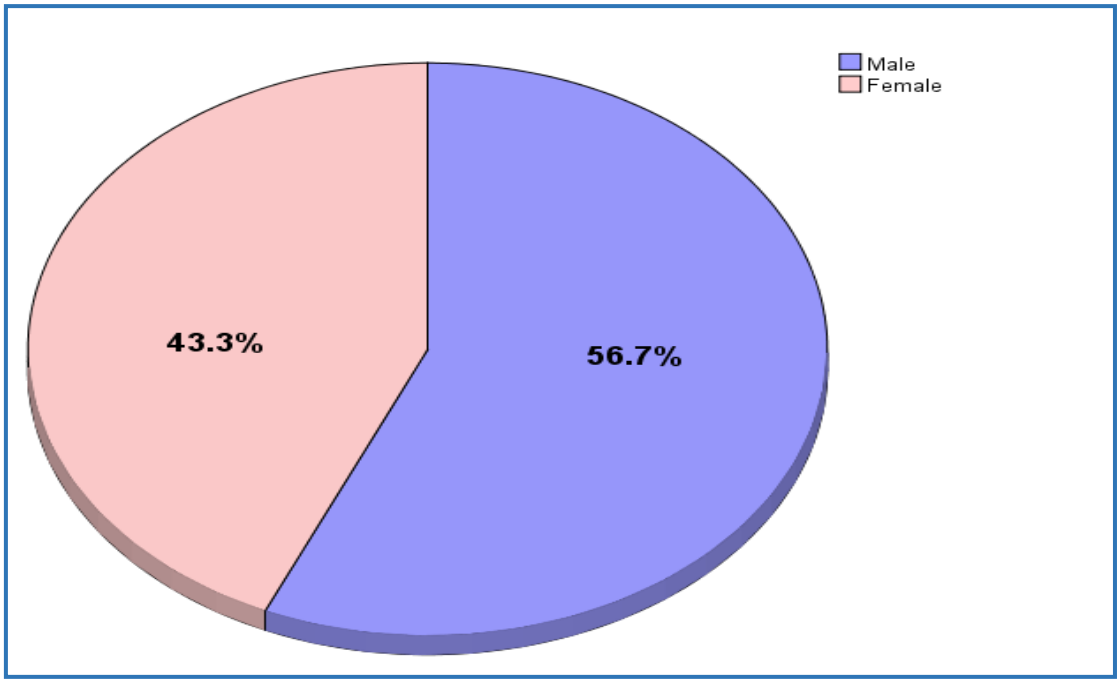
Results and Discussion

1 Demographical data

1.1 Gender distribution of TB patients

A total of ninety patients with pulmonary and extra pulmonary TB cases, there was 51(56.7%) of cases for males and the other 39(43.3%) of cases for females as shown in figure (1).

Figure (1): Gender distribution among TB patients



This result, reveals that the most common TB cases in males as compared to females. This result is supported by previous local result were in Babylon province, Iraq (Mustafa, 2019). Moreover; Shalan *et al.,* (2013) in a local study in Hillah, Iraq showed that out of the 124 TB patients, there were 59% male and 41%. In addition to Manhal, (2009) study, which explains 68% of PTB were males and the other, was females. The WHO report in 2019 indicated that, globally, the male to female ratio was 2:1 among patients with TB (WHO, 2019). This finding could be attributed to the numerous risk factors including: smoking, alcoholism, drug abuse and working in crowding areas, as well as male is more susceptible to microbial infection than females (Schurz *et al*.,2019). Biological mechanisms, such as sex hormones might actually account for a significant part of the difference between male and female susceptibility to infection especially TB (Nhamoyebonde and Leslie, 2014).

eISSN 1303-515

1.2 Age distribution among TB patients

In the current study the age range of all TB cases were ranged from 11 to more than 71 years and differ from male to female in the age range of more frequent cases. There were significantly frequent cases in the age range 21-30 years as shown in the table (1).

Table (1): Age distribution among TB patients

Age group (year)	TB Patients	
	No.	Percentage (%)
11 – 20	14	15.6
21 – 30	23	25.6
31 – 40	13	14.4
41 – 50	13	14.4
51- 60	12	13.3
61 – 70	12	13.3
≥ 71	3	3.3
Total	**90**	**100**

The results in the table (1) showed that tuberculosis affected in all age groups. Although the results indicate there were high tuberculosis infection rate in age group (21-30) in comparison to other age groups. A local study by AL-Jubouri *et al* (2017) found that there is an increase in cases of PTB in Babylon province especially in young age, the infection rate among this age group was (50%). This tendency of high prevalence of young is mainly attributed to Malnutrition can exacerbate the risk of TB disease; other threats such as rising tobacco use and diabetes among younger age also result in increased TB burden (WHO, 2015). The results of this study were in agreement with Al-Kadhimi and Dawood, (2011) where found that TB is significantly more in younger age group; this may be due to high number of young population of Iraq. For interpretation the results, this age range of activity which meet and exposure to too many peoples, in addition, this age range include the most difficult stage of life exposure the individual to tension and psychological problems may be lead to hormonal changes and low immunity of individual which may make the individual low resist the MTB infection (Dhabhar, 2014).Another interpretation of the analytical study of pulmonary tuberculosis in Iraq, mentioned that most cases of PTB in Babylon province in the age group of female (15-24) years and (25-34) years of male because these groups were more active life and mix between each other in school, university, and other work sites to be more exposure to MTB bacterium (Zhang *et al*.,2021).

1.3 Geographic distribution of TB patients

The present study explained that out of 90 confirmed TB case there was 52(57.78%) of cases for rural area populations and the remaining 38(42.22%) of cases for urban area population as shown in figure (2).

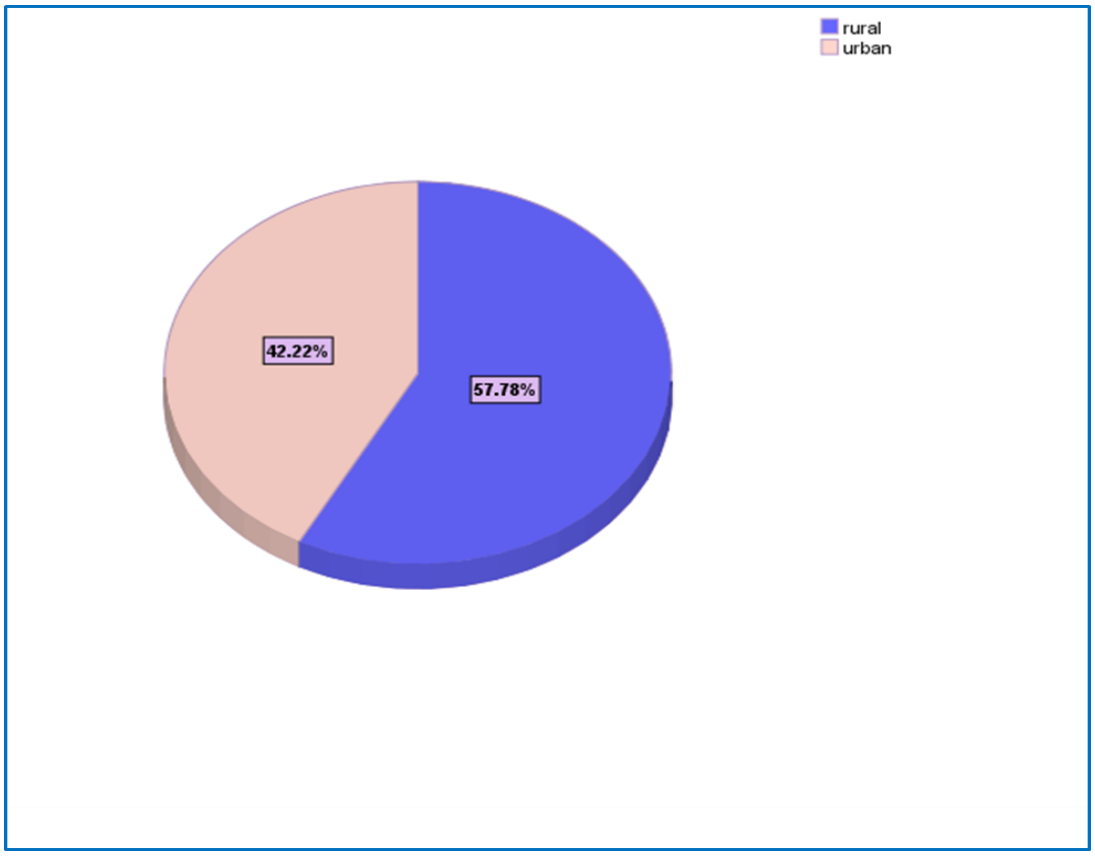


Figure (2): Geographic distribution of TB patients in Babylon province

The significantly increasing, (P<0.05), of TB cases in rural areas compared to urban areas was accordance approximately with that recorded by Bhatt *et al*., (2009) who mentioned that tuberculosis cases were commonly found in rural areas 59%.

In an epidemiological study in Iraq clarified that 97% of urban areas can reach to health care centers compared with 79% of rural areas (Korzeniewski,2006). The reasons of prevalence TB in rural people were due to more complicated diagnoses, knowledge about TB, socioeconomic status, education level, malnutrition, contact with domestic animals and farm animals. In addition, rural clinics might experience medication stock outs or shortages than those located in urban settings (Skosana, 2015). In addition, the TB patients suffered more co-morbid mental disorders than the general population because of the length of treatment, loss of income, stigma, and isolation from society (Xu *et al*., 2017).

Global statistics indicate that the pTB burden is more acute in urban areas. However, in countries where large portions of the population are rurally located and reside in extreme poverty, TB is dominant among rural dwellers. Poverty and limited access to health facilities, health workers, diagnosis and treatment are increased the ability of people who reside in rural areas with pTB (WHO, 2015).

2 Diagnosis of MTB infections

2.1 Diagnosis of PTB infections

A total of (110) sputum suspected samples enrolled in the present study from by the physician according to chest X-ray which has PTB-like symptoms may be predicted to have PTB disease. The diagnostic confirmation of PTB in laboratory by bacteriological and molecular methods indicatted that 50(45.4%) were confirmed of PTB.

2.1.1 Bacteriological and Molecular diagnosis of pulmonary tuberculosis

eISSN 1303-515

A total of (110) sputum sample tested, 26(23.6%) were AFB smear positive by ZN stain and the remaining 84 (76.3%) samples were AFB negative, as shown in figure (3). The culturing of sputum given 10(9%) positive growth and 100(90.9%) negative growth,as shown in figure (4-6). The GeneXpert given 50(45.45%) positive and the remaining 60 (54.5%) were GeneXpert negative.

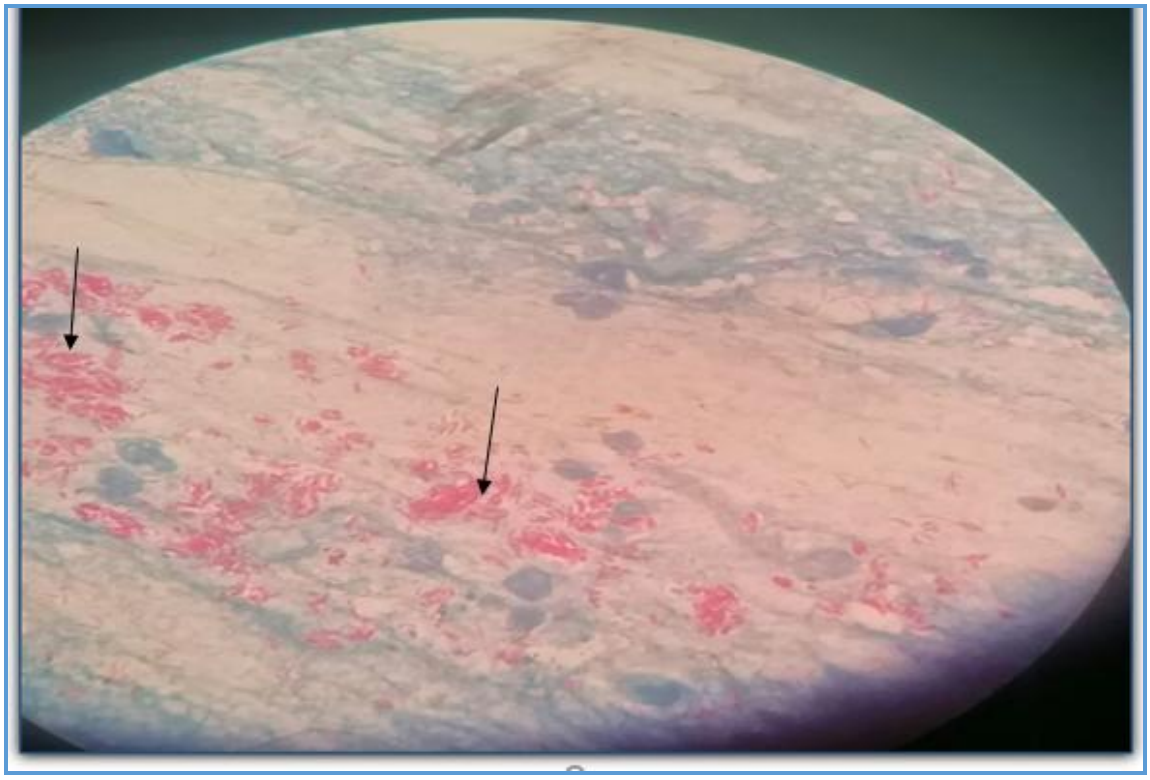


Figure (3): Bacteriological diagnosis of *Mycobacterium tuberculosis.* The field of AFB sputum smear stained with Ziehl Neelsen microscopy under oil immersion lens.MTB stained with red-purple( )

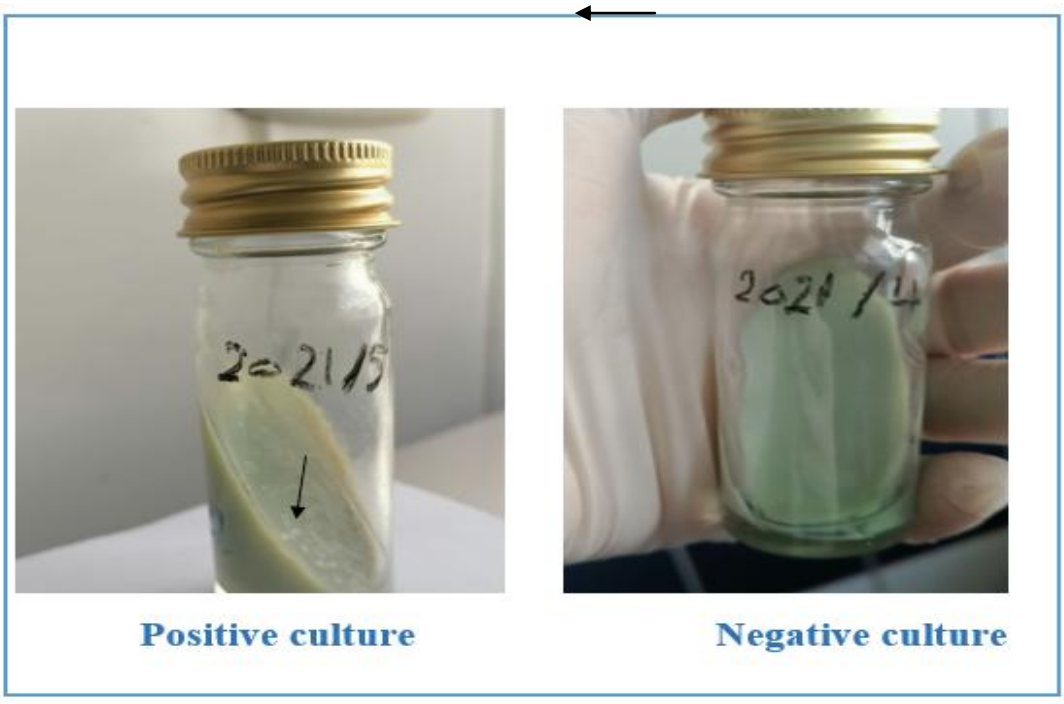


Figure (4): The positive growth ( ) of MTB colonies on slant Löwenstein–Jensen medium for up to 8 weeks .the sample was sputum

The sensitivity, specificity and accuracy of laboratory diagnostic technique were calculated based on the golden standard which was the GeneXpert. The sensitivity of the AFB smear, Gene Xpert and Culture of the clinically conformed 50 pTB cases in this study were 60.4%, 98% and 27%, respectively. The specificity of the AFB, Gene Xpert and Culture methods were 95%,100%,98.3%, respectively . Therefore, the accuracy (98.2%) were obtained from GeneXpert results followed by AFB smear (81%) and culture 72 %, as shown in table (2).

Table (2): Evaluation of direct Acid fast bacilli smear microscopy, Gene Xpert and Löwenstein–Jensen culturing of sputum for diagnosis of pulmonary tuberculosis

Diagnostic Test	Positive No.=110, (%)	Negative No.=110, (%)	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	Accuracy (95% CI)
Gene Xpert	50 (45.45%)	60 (54.5%)	98%	98.3%	98%	98.3%	98.2%
AFB smear by ZN stain	26 (23.6%)	84 (76.3%)	60.4%	95%	89.6%	77.9%	81%
L-J culture	10 (9%)	100 (90.9%)	27%	100%	100%	68.9%	72%

CI: confidence intervals, PPV: positive predictive value, NPV: negative predictive value, AFB: acid fast bacilli, ZN: Ziehl–Neelsen, LJ: Löwenstein–Jensen

Although the culturing of MTB was considered the gold standard test for diagnosis of MTB, it was low sensitivity and high specificity compared to AFB smear microscopy because the culture of MTB require specific laboratory environments incubation reach to 8 weeks until getting colony on slant L-J medium , in addition to the sputum require processing to limit contaminants normal flora that lead to false positive/negative results (Asmar and Drancourt, 2015). The low sensitivity of TB culture in this study can be attributed to the anti-TB antibiotic administration in many TB patients. In a study of Agrawal *et al.,*(2016) clarified the evaluation of AFB smear microscopy in 170 sputum samples of suspected pulmonary tuberculosis the sensitivity and specificity were 22.2%, %, and 78.5% respectively. The sputum smear test is quicker, produces results in about 30 min, it variable to detect from 10% to 75% of TB cases, and requires trained microscopists compared to 33.33% of cases can be detected by L-J culture. The quicker diagnostic test can limit TB spread in a crowded area and limit arise resistance strains of MTB to the drugs used in the treatment of the infection (Chaudhuri *et al.,* 2011).In a near study performed in Kerbala province explained that among PTB cases (602), the smear positivity was approximately (65%) (Mohammed *et al.,* 2018). Al-Omeri,(2011) in Baghdad province, was found low positivity (2.89%) of culturing MTB on L-J media to be inappropriate for routine diagnosis of pulmonary tuberculosis because high negativity of culturing**.**Although lacking sensitivity,

sputum smear microscopy is inexpensive, relatively simple to perform and a rapid diagnostic test (Allen *et al.,* 2016). The waxy cell wall of MTB may decrease the sensitivity of AFB method because the can resist Carbol fuchsin stain to enter the bacterium (Kurup and Chester, 2014).Many global studies were reported the sensitivity, specificity, positive predictive value (PPV) and negative predictive value compared between the diagnostic methods such a study of Sutay *et al*., (2017) which were compared between GeneXpert and smear microscopy for detection of MTB in children and revealed that 100% specificity and PPV for both genexpert and AFB smear microscopy and the sensitivity of GeneXpert was approximately three times higher than AFB smear microscopy and NPV of GeneXpert is also higher as compared to AFB smear microscopy. Their study concluded that the genexpert was a better diagnostic test as compared to smear microscopy and can significantly reduce false negatives and the delay in treatment initiation (Pandey *et al*., 2017; Sutay *et al*., 2017).According to the present finding, the GeneXpert was considered the gold standard test which has a higher sensitivity than AFB smear microscopy in respiratory samples require several minutes to perform diagnosis of patients with high clinical suspicion of PTB and agreed with Agrawal *et al*., (2016).The accuracy of TB diagnostic test requires a quick test with high specificity and sensitivity (sensitivity 100%, specificity 98.8%) and easy to perform such as the molecular test like GeneXpert which had been used as gold standard test to compare with other methods for routine diagnosis (Elhassan *et al*., 2016).

2.2 Diagnosis of Extra pulmonary tuberculosis infections

A total of 40 patients suffering from extra pulmonary tuberculosis cases on the basis of clinical findings in the present study that found the specimens type were: 18(45%) lymph nodes, 7(17.5%) pleural effusions, 6(15%) bone and joints and 9(22.5%) others,as shown in figure (5).

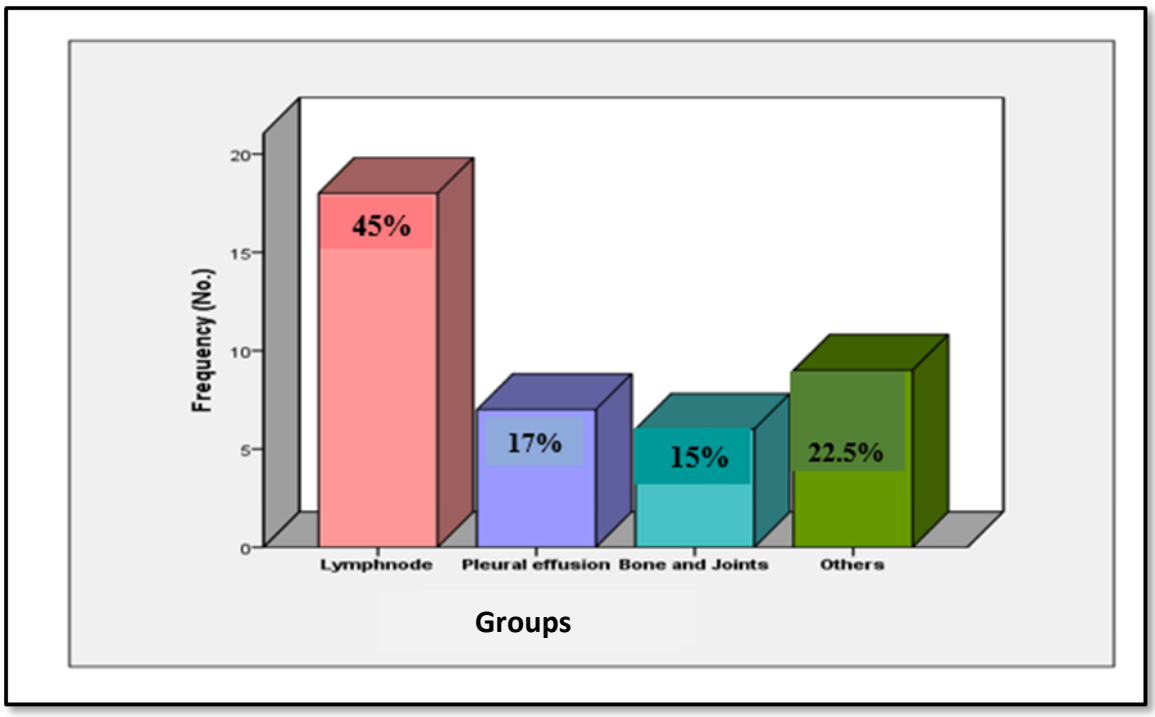


Figure (5): Clinical distribution of Extra pulmonary tuberculosis

Figure (5) Showed that lymph nodes is most common in Extra pulmonary tuberculosis, this result agreement with (Khosravi *et al*., 2017) that found The lymph nodes were the most frequent specimens (26%), followed by bone biopsies.In other a study of Al-Ghafli *et al.,* (2019) that found the Lymph node (58.1%) was the most commonly reported site of infection followed by gastrointestinal system (18.7%), central nervous system (9.6%), bone and joints (5%) and urogenital systems (4.5%) respectively. There were different rare infection-sites such as blood, pericardial, oral, skin and soft tissues that together comprised 4.5% of the total enrolled cases.Along with most previous studies, we found that the two main infection sites were lymph nodes and pleural. In a study in the USA, lymph nodes followed by pleural and osteoarticular were the main infection sites. Lymph nodes were also the most common site of EPTB in several studies from Saudi Arabia (Al-Hajoj *et al.,* 2015). In the European Union, most countries showed a higher prevalence of lymphatic TB as the first site including The Netherlands (39%) and the United Kingdom (37%), whereas pleural TB was seen in Poland (36%) and Romania (58%) as the most prevalent form (WHO,2020).However, compromised host-immune system (e.g. due to synergism of HIV and MTB infections) increases the risk of more serious TB manifestations such as concurrent extra-pulmonary TB (EPTB) (a clinical presentation of TB that entitles the dissemination of mycobacterial bacilli in both pulmonary and extra-pulmonary sites). This is largely due to failure of host-immune system to enclose *Mycobacterium tuberculosis* (MTB) bacilli within lung parenchyma. EPTB diseases may emerge at one particular body site or at multiple body sites even with no sign of PTB-concurrency (e.g due to re-activation of previous TB infection). Non-concurrent EPTB is very challenging at diagnosis and treatment, albeit of its lower infectious potential (Forssbohm *et al*., 2008). Probably, due to individual weakness of the immune system and macrophage dysfunction, the bacteria can spread between tissues through body fluids such as blood and lymph **(**Krishnan *et al*., 2017).

Conclusions

The comparison between advance technique Gene X-pert and classical technique AFB and culture for the diagnosis of MTB, show that genetic technique is the best with high sensitivity, and specificity.

References

Abdalla AE, Lambert N, Duan X, Xie J (2016) .Interleukin-10 Family and Tuberculosis: An Old Story Renewed. Int J Biol Sci 2016;12(6):710-717.

Agrawal, M., Bajaj, A., Bhatia, V., and Dutt, S. (2016). Comparative study of GeneXpert with ZN stain and culture in samples of suspected pulmonary tuberculosis. Journal of clinical and diagnostic research: JCDR, 10(5): 09-12.

Al- Jubouri, A. M. S. (2017). Study of HLA: DRB1, Vitamin D3 and Some Immunological Factors in Pulmonary Tuberculosis in Hilla City. Ph.D. Adissteration. College of Medicine, University of Babylon. Iraq.

Al-Ghafli, H., Varghese, B., Enani, M., Alrajhi, A., Al Johani, S., Albarrak, A., and Al Hajoj, S. (2019). Demographic risk factors for extra-pulmonary tuberculosis among adolescents and adults in Saudi Arabia. *PloS one*, *14*(3), e0213846.

Al-Hajoj, S., Shoukri, M., Memish, Z., AlHakeem, R., AlRabiah, F., and Varghese, B. (2015). Exploring the sociodemographic and clinical features of extrapulmonary tuberculosis in Saudi Arabia. *PLoS One*, *10*(2), e0101667**.**

eISSN 1303-515

Ali, A. A., Razzaq, M.A. and AL-Saadi, M.A. (2013). Incidence of tuberculosis in Babylon Province-Iraq. *Int J Curr Microbiol App Sci*, *2*(9): 1-4.

Al-Kadhimi H. and Dawood H. (2011). The Effect of Age on Clinical and Radiological Presentation in Patients with Pulmonary Tuberculosis in Baghdad. *The Iraqi postgraduate medical journal*.10 (1):125-129.

Al-Khafaji, J. T. K. (2014). Characterization of Mycobacterium tuberculosis and predisposing factors associated with tuberculosis among risk population in Baghdad City. *J Babylon Univ/Pure Applied Science*, *22*, 2547-58.

Allen, V., Nicol M. P., and Tow L. A. (2016). Sputum Processing Prior to Mycobacterium Tuberculosis Detection by Culture or Nucleic Acid Amplification Testing: A Narrative Review. *Research & Reviews: Journal of Microbiology and Biotechnology, 5*(1): 96-108.

Asmar, S., and Drancourt, M. (2015). Chlorhexidine decontamination of sputum for culturing Mycobacterium tuberculosis. *BMC microbiology*, *15*(1): 155.

Behera, D. (2010). Textbook of Pulmonary Medicine (2nded.). New Delhi: Jaypee Brothers Medical Pub. p.457

Betty, A. F., Daniel, F. S., Alice, S. W., and William, R. B. (2007). Bailey and Scott's diagnostic microbiology. *International edition, ed12, chap. 7*: 93-119.

Bhatt, CP.; Bhatt, AB. And Shrestha, B. (2009). Tuberculosis patients opinion for Directly Observed Treatment short-course (DOTs) program of Nepal. Journal Tuberculosis and Lung Diseases, **1**(1): 39-45

Chaudhuri, A. D., Bhuniya, S., Pandit, S., Dey, A., Mukherjee, S., and Bhanja, P. (2011). Role of sputum examination for acid fast bacilli in tuberculous pleural effusion. *Lung India: official organ of Indian Chest Society*, *28*(1): 21.

Dart, R. K. (1996). *Microbiology for the analytical chemist*. Royal Society of Chemistry.

Dhabhar, F. S. (2014). Effects of stress on immune function: the good, the bad, and the beautiful. *Immunologic research*, *58*(2), 193-210.

Dolin. (2010). Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases, edited by: Gerald, L. P. (7th ed.). Philadelphia, PA: Churchill Livingstone/Elsevier. Chapter 250

Dye, C. (2006). Global epidemiology of tuberculosis. Lancet. Geneva . 367: 938-940.

Elhassan, M. M., Elmekki, M. A., Osman, A. L., and Hamid, M. E. (2016). Challenges in diagnosing tuberculosis in children: a comparative study from Sudan. *International Journal of Infectious Diseases*, *43*: 25-29.

Forssbohm, M., Zwahlen, M., Loddenkemper, R. A.,and Rieder, H. L. (2008). Demographic characteristics of patients with extrapulmonary tuberculosis in Germany. *European respiratory journal*, *31*(1), 99-105.

Gambhir S, Ravina M, Rangan K, Dixit M, Barai S, Bomanji J (2017) Imaging

Goldman, E., and Green L H. (2009). Practical Handbook of Microbiology: Second Edition. *Journal of Chemical Information and Modeling*, *53*.

Gopalaswamy, R., Dusthackeer, V. N., Kannayan, S.,and Subbian, S. (2021). Extrapulmonary Tuberculosis—An Update on the Diagnosis, Treatment and Drug Resistance. *Journal of Respiration*, *1*(2), 141-164.

Khosravi, A. D., Alami, A., Meghdadi, H., and Hosseini, A. A. (2017). Identification of Mycobacterium tuberculosis in clinical specimens of patients suspected of having

eISSN 1303-515

extrapulmonary tuberculosis by application of nested PCR on five different genes. *Frontiers in Cellular and Infection Microbiology*, *7*, 3.

Korzeniewski, K. (2006). The epidemiological situation in Iraq. *Przeglad epidemiologiczny*, *60*(4): 845-848.

Krishna, M., and Gole, S. G. (2017). Comparison of Conventional Ziehl–Neelsen Method of Acid Fast Bacilli with Modified Bleach Method in Tuberculous Lymphadenitis. *Journal of Cytology, 34*(4): 188-192.

Kurup, R. and Chester, K. (2014). Comparative evaluation of Ziehl Neelsen staining and knowledge, attitudes and practices of laboratory personnel in relation to Ziehl Nielsen. *West Indian Medical Journal*, *63*(1): 34-38.

Manhal, F. (2009). Allelic distribution of human leukocyte antigen in patients with pulmonary tuberculosis in Baghdad city. *Iraqi postgraduate Medical Journal*, *8*, 253-58.

Maynard-Smith, L., Larke, N., Peters, J.A. and Lawn, S.D. (2014). Diagnostic accuracy of the Xpert MTB/RIF assay for extrapulmonary and pulmonary tuberculosis when testing non-respiratory samples: a systematic review. *BMC infectious diseases*, *14*(1): 709.

Maynard-Smith, L., Larke, N., Peters, J.A. and Lawn, S.D. (2014). Diagnostic accuracy of the Xpert MTB/RIF assay for extrapulmonary and pulmonary tuberculosis when testing non-respiratory samples: a systematic review. *BMC infectious diseases*, *14*(1): 709.

Mohammed, S. H., Ahmed, M. M., Al-Mousawi, A. M., and Azeez, A. (2018). Seasonal behavior and forecasting trends of tuberculosis incidence in Holy Kerbala, Iraq. *International journal of mycobacteriology*, *7*(4): 361-367.

Mustafa J.A.K. (2019). Effect of *IL-17* gene polymorphism in tuberculosis patients in Babylon province. PhD. Thesis. College of Medicine, University of Babylon. Iraq.

Pandey, P., Pant, N.D., Rijal, K.R., Shrestha, B., Kattel, S., Banjara, M.R., Maharjan, B. and Rajendra, K.C. (2017). Diagnostic accuracy of GeneXpert MTB/RIF assay in comparison to conventional drug susceptibility testing method for the diagnosis of multidrug-resistant

S**atapathy, P., Das, D., Murmu, B. N. and Kar, S. K. (2014).** Decontamination of sputum for longer time in sodium hydroxide for isolation of *Mycobacterium tuberculosis*. *International journal of mycobacteriology*, 3(4): 290-292.

Schurz, H., Salie, M., Tromp, G., Hoal, E. G., Kinnear, C. J., and Möller, M. (2019). The X chromosome and sex-specific effects in infectious disease susceptibility. *Human genomics*, *13*(1), 1-12.

Shalan, A. A., Naher, H. S. and Al-Saadi, M. A. (2013). The Association between HLA-DRB Alleles with Pulmonary Tuberculosis in Babil Province, Iraq. *Journal of Biology, Agriculture and Healthcare 3*(14): 1-4.

Singh , M. M. (2000). Immune Response in Tuberculosis . Indian J. Allergy Appl Immunol 14(2) : 79-82.

Skosana,N(2015). Global plan to End TB and key populations 1-19.

Sutay, N., Jha S., and Chaudhary D. (2017). A Comparative Study between Genexpert and Smear Microscopy for Diagnosis of Tuberculosis in Paediatric Patients. *International Journal of Science and Research, 6* (8): 2015-2018.

Tille, P. (2015). Bailey & Scott's Diagnostic Microbiology-E-Book. *Elsevier Health Sciences*. 81-105 transformation artist. RoFo 183(11):1019–1029.

Vigenschow, A. (2019). Structured assessment of healthcare facilities and knowledge, attitudes and practices of healthcare workers regarding tuberculosis infection control in Moyen-Ogooué, Gabon (Doctoral dissertation, Universität Tübingen).

WHO, World Health Organization. (2017). Global Tuberculosis Report 2017.

World Health Organization. (2015). Iraq tuberculosis report. Eastern Mediterranean Health Journal. (8):19–28.

World Health Organization. (2020). Tuberculosis elimination in the WHO European Region: review of key actions, with a special focus on management of tuberculosis infection.

World Health Organization. WHO Global Tuberculosis Report 2019. Geneva: World Health Organization; 2019

Xu, M., Markström, U., Lyu, J. and Xu, L. (2017). Survey on Tuberculosis Patients in Rural Areas in China: Tracing the Role of Stigma in Psychological Distress. *International journal of environmental research and public health*, *14*(10): 1171.

Yoshimatsu, S., Kato-Matsumaru, T., Aono, A., Chikamatsu, K., Yamada, H. and Mitarai, S. (2015). Factors contribute to efficiency of specimen concentration of *Mycobacterium tuberculosis* by centrifugation and magnetic beads. *International journal of mycobacteriology*, *4*(3): 2

Zhang, F., Jabeen, S., Fu, Y., Hussain, S., Tahseen, S., Qadir, S. M., and Prakash, O. (2021). Tuberculosis and Its Predictors in Pakistani Population Transmission Dynamics and Drug Resistance of Mycobacterium.

eISSN 1303-515