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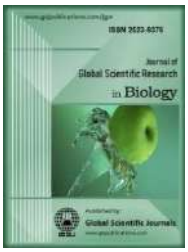

# Molecular Study of Mycobacterium Tuberculosis Infection in TB Centers , Ninava

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ABSTRACT

Tuberculosis (TB) is one of the main health concerns in many countries. Researches on TB care quality and patient survival are a lot but still TB represents a huge health concern. Owing to this, the study evaluates the methods used to detect TB patients as well as the impact of risk factors that are related to the life of patients. The study includes 496 patients whose recorded tuberculosis cases attended Mosul Respiratory infectious Center. Data were analysed using Gene x-pert method ( kits obtained from Sacace) Biotechnologies, and acid-fast stain method (Ziehl–Neelsen stain). Later, these methods are compared among the patients. The comparison held showed that the Specimens collection of 496 were processed for the detection of tuberculosis using GENE X-PERT was more sensitive than Acid-fast stain with a percentage rate (of 26.35%), (22.55%) respectively. A high percentage of tuberculosis can be found in the age group between (10-20 years), (and 90-100 years) and a low percentage can be found in younger people. The demographic distribution of tuberculosis is more in low-income and overcrowded societies which recorded a significant increase of prevalence. It is concluded that the GENE X-PERT technique is fast, accurate, and effective in the detection of tuberculosis for a period not exceeding 4 hours only. In addition, it is an easy-to-use method with a relatively low cost, and it can be performed in low-income areas. electronic document is a “live” template. The various components of your paper [title, text, heads, etc.] are already defined on the style sheet, as illustrated by the portions given in this document. This electronic document is a “live” template. The various components of your paper [title, text, heads, etc.] are already defined on the style sheet, as illustrated by the portions given in this document. This electronic document is a “live” template. The various components of your paper [title, text, heads, etc.] are already defined on the style sheet, as illustrated by the portions given in this document. This electronic document is a “live” template. The various components of your paper [title, text, heads, etc.] are already defined on the style sheet, as illustrated by the portions given in this document.

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## 1. Introduction

Tuberculosis TB is an infectious disease [1] that can affect many parts of the body such as bones, reproductive and urinary system, skin, and mainly the lungs [2]. It is spread by sneezing, coughing or spitting through flying droplets and also through the ear [3], therefore in the case of inhalation of air by a healthy person and contaminated with bacteria, this will lead to infection and the factors that lead to the possibility of infection [4]. However, the infection with some viruses such as HIV can weaken the immune system, as well as poverty and malnutrition are among the most important factors that lead to infection of the person [5].

One of the most important clinical symptoms that one can observe in persons with pulmonary tuberculosis is coughung mostly accompanied with blood , heat with shortness of breath and loss of appetite for food, pain and swelling when injuries outside the lung, especially in the area of joints and lymphocytes [6].

One of the most important methods used in the diagnosis of pulmonary tuberculosis bacteria are using of X-ray for lungs , examining of sputum or saliva samples of the infected person [7]. At least, three samples are considered to detect the tuberculosis germ to confirm the incidence microscopely [8. They are considered one of the traditional methods of the tuberculosis bacterium detection. Bacterial cultures represent one of the important diagnosis that detect the tuberculosis germ. Specific culture is used for this purpose such as the Löwenstein-Jensen medium .It is a good medium for Germ growth, but it takes two to three weeks [9].GENE X-PERT technology is one of the most important up to date fast method that needs no time to show accurate and reliable results [10]. As for health awareness people should not come so close or be in direct or mix with infected people [11], in addition to wearing masks in crowded places, taking the BCG vaccine [12]

The aim of the study is to evaluate the techniques used in testing and diagnosing TB germ. Two methods were assessed; the microscopic test using the dye of Ziehl–Neelsen, a traditional way of testing and an advanced one, the GENE X-PERT method. In addition to studying age and location as variables. Comparison of the different methods being held for detecting , performing a demographic analysis to patients with Tuberculosis.

## 2. Materıals and Methods

He The Samples collected are from TB Centre / Mosul infectious disease hospital /Mosul, Iraq . Various clinical specimens included respiratory samples (sputum, BAL, endothelial secretions, bone marrow, tissue, CSF).Universal precautions were considered and all samples collected done mainly with the help of Z-N stain and Gene x-pert.

### 2.1 Acid-Fast Bacteria-Ziehl-Neelsen Stain (AFB)

It demonstrates that acid-fast bacilli is classified within *'Mycobacterium'* that includes the main cause of TB.

### 2.2 Z-N Reagents and methods

A variety of reagents and methods are used for Ziehl -Neelsen (Z-N) staining.

A. A traditional Z-A method is convenient for staining one or two slides at a time.
B. A preferred Z-N method is suitable for staining several slides simultaneously. Finally, some modified Z-N methods for staining weakly acid-fast organisms will be described

## 3. Procedure

**Ziehl- Neelsen stains for acid fast bacteria:**

- Clearing the slide wiping it with a dry cotton cloth.
- Labeling l the slide with a marker
- Preparing the smear on the labeled side.

- Drying thoroughly in air or warm if from 20-33 minute above the blue flame of a Bunsen burner.
- Heaing t fix by flaming from below.
- Placing the smear slide on the staining rack or staining rods
- Covering the whole slide with carbol fuchsin.
- Heating the slide from below until steam arises, does not reach boiling point. Kept for 10 minutes with similar intermittent heating.
- Washing the slide with tap water. Remove the stains from below.
- Decolorizing with 20% sulphuric acid till the colour comes out which takes about 2-5 minutes
- Washing the slide well with water to remove traces of acid
- Countering stain with loefflers methylene blue for 1 minute.
- Washing and drying .

**MTB Gene x-pert**

***Samples collection and reagents preparation:***

This study conducted at the center of respiratory infectious disease center /mosul city /iraq for different suspected patients having tuberculosis . A total of 496 samples pulmonary(sputum , Broncho-alveolar lavage) and extra-pulmonary(Cerebrospinal fluid, pleural fluid and other fluids) were collected from patients with symptoms suggestive of tuberculosis. In this study, suspected tuberculosis patients were assigned to sample collection based on the presentation of symptoms and chest radiographic findings. . Patients with special clinical criteria were requested to provide three different sputum specimens within two days. Extra-pulmonary samples of pleural fluid, lymph node biopsy, cerebrospinal fluid, skin, ascitic fluid, sinus swab, and pus were taken. Different steps must be taken depending to the type and location of the sample taken: 1-Sputum samples: Ask the patient to collect three different samples from deeply cough and collected in a sterilized container . The first one must be collected from the patient after arriving at the center; the second specimen must be collected early in the morning before breakfast; the third was collected at any time of the day. 2- Body fluids and other samples: Using a centrifugations the samples of the body fluid concentrated at 3000 rpm for 15 min. The sediment has been processed. All of the above samples were digested and purified with 4% sodium hydroxide (NaOH); Samples were mixed with equal volumes of NaOH in a sterile falcon tube and mixed with a vortex mixer for a few seconds. The tube was then left at room temperature for 15 minutes. The mixture was then centrifuged at 3000 rpm for 15 min. Samples were neutralized by drops of HCL solution containing phenol red as an indicator and shaken until the color changed from red to yellow.

***GeneXpert MTB / RIF assay***

The GeneXpert MTB assay consists of a single-use multi-chamber plastic cartridge with liquid buffers and lyophilized reagent beads required for sample processing and DNA extraction, and real-time polymerase chain reaction interference. Clinical samples or disinfected sputum pellets are treated with sodium hydroxide and a sample reagent containing isopropanol (SR). SR is added in a 2:1 ratio to the sample or sputum pellets and incubated for 15 minutes at room temperature. The treated sample is transferred to the cartridge, the cartridge is loaded into the GeneXpert instrument, and the automatic process completes the remaining assay steps. The assay cartridge also contains lyophilized Bacillus globigii, internal sample treatment and PCR control. Spores are automatically resuspended and processed during the sample processing step, and B. globigii DNA is amplified during the polymerase chain reaction step. The standard user interface indicates the presence or absence of Mycobacterium tuberculosis, the presence or absence of resistance to RIF, and a semi-quantitative estimation of the concentration of Mycobacterium tuberculosis (high, medium, low, very low).

**Detection of *Mycobacterium* by various methods**

The study was done in the TB center / Mosul infectious disease hospital/Mosul city, Iraq, 496 clinical specimens, both pulmonary and extra-pulmonary (like CSF, and other body fluids, etc.), were processed for detection of *M. tuberculosis* using Ziehl Neelsen stain/Acid-fast stain and GENE X-PERT (Smartcycler, Cepheid) using kits obtained from Sacace Biotechnologies,

Out of a total of 496 clinical specimens, positivity was observed by different methods just as follows: maximum by Gene x-pert (78/ 296), and Ziehl-Neelsen (85 / 377).

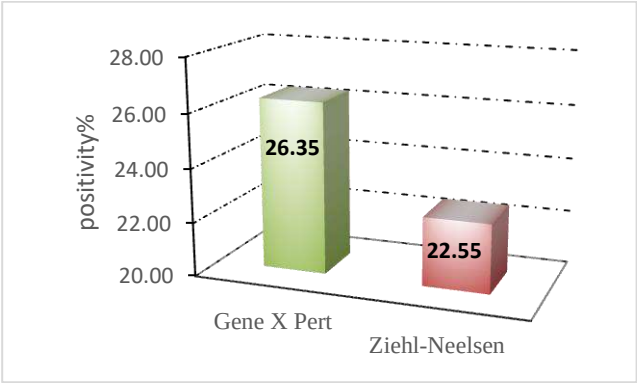


**Figure 1: Comparison of detection of Mycobacterium by different methods**

Amongst the specimens, maximum positivity was observed in sputum 41(48.24% ) for AFB stain,and33(42.31%) for GENE X-PERT followed by bronchoalveolar lavage 29 ( 34.21 ) for AFB, and 14(17.95 %) for GENE X-PERT and pus 6 (7.06 %) for AFB, and 7(8.97%) for GENE X-PERT. Urine and body-fluids like pericardial fluid did not yield any positivity.

Table. 1: Prevalence (%) of Mycobacterium in different clinical specimens.

| Total specimen | AFB- SMEAR POSITIVE | P value | GENE X-PERT POSITIVE | P value |
|---|---|---|---|---|
| 1. Sputum n=66 | 41 (48.24%) | 0.000 ** | 33 (42.31%) | 0.000 ** |
| 2. CSF n=78 | 2 (2.35%) | | 7 (8.97%) | |
| 3. BAL n=164 | 29 (34.12%) | | 14 (17.95%) | |
| 4. pus n=86 | 6 (7.06%) | | 7 (8.97%) | |
| 5. Tissue n=48 | 1 (1.18%) | | 8 (10.26%) | |
| 6. Other fluid n=50 & FNAC | 1 (1.18%) | | 5 (6.41%) | |
| 7. Bone marrow n=15 | 4 (4.71%) | | 4 (5.13%) | |
| 8. Plerural fluid n=64 | 1 (1.18%) | | 0(%) | |
| 9. Pericardal n=4 | 0(00%) | | 0(00%) | |
| 10. urine n=24 | 0(00%) | | 0(00%) | |
| Total clinical specimen n=496 | Total positive=85 Out of 377( 22.5%) | | Total positive =78 Out of 296 (26.35 %) | |

**Refer to high significant differences between groups at (P<0.01).

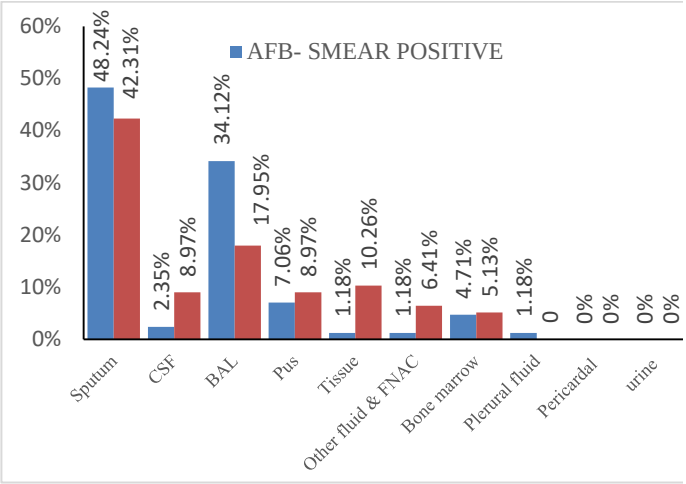


**Figure 2. Prevalence (%) of Mycobacterium in different clinical specimens.**

Table 2. TB occurrences according to age groups

| S. No. | Age Group (in years) | % Number of Positive AFB | % of Positive GENE X-PERT |
|---|---|---|---|
| 1. | 0-10 | 33.33% | 37% |
| 2. | 11-20 | 45.45% | 40.90% |
| 3. | 21-30 | 29.29% | 25.70% |
| 4. | 31-40 | 17.24% | 17.80% |
| 5. | 41-50 | 19.70% | 16.00% |
| 6. | 51-60 | 20.00% | 19.60% |
| 7. | 61-70 | 20.00% | 17.60% |
| 8. | 71-80 | 22.86% | 20.10% |
| 9. | 81-90 | 22.22% | 22.30% |
| 10. | 91-100 | 33.33% | 45% |

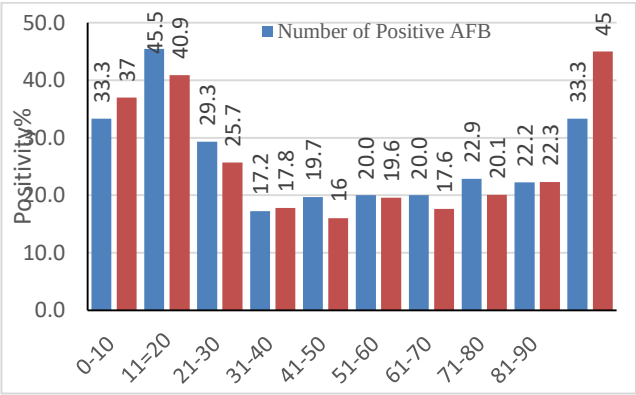


**Figure 3. Prevalence of Tuberculosis in different age groups**

With reference to the above data, it was concluded that the age groups of (11-20) years and (91-100) years showed the highest percentage of positivity. This may be due to the impairment of the immune system.

**Comparison between Ziehl-Neelsen staining and GENE X-PERT methods**

Direct Ziehl-Neelsen staining and GENE X-PERT were used for the detection of

*Mycobacterium*. A total of 110 samples were processed by the two methods.

Table. 3: Identification of TB according to specimens types

| S.NO. | SPECIMEN | Z.N. STAINING | GENE X-PERT |
|---|---|---|---|
| 1. | CSF | -VE | +VE |
| 2. | BAL | +VE | -VE |
| 3. | PUS | -VE | -VE |
| 4. | SPUTUM | +VE | +VE |
| 5. | CSF | -VE | -VE |
| 6. | CSF | -VE | +VE |
| 7 | CSF | -VE | +VE |
| 8. | PUS | -VE | +VE |
| 9. | PUS | -VE | +VE |
| 10. | CSF | -VE | +VE |
| 11 | SPUTUM | +VE | +VE |
| 12. | BAL | -VE | -VE |
| 13. | BAL | -VE | -VE |
| 14. | CSF | -VE | +VE |
| 15. | BAL | -VE | -VE |
| 16. | BAL | -VE | -VE |
| 17. | BAL | +VE | +VE |
| 18. | BAL | +VE | +VE |
| 19. | BAL | +VE | +VE |
| 20. | SPUTUM | +VE | +VE |
| 21. | BAL | +VE | +VE |
| 22. | BAL | -VE | -VE |
| 23. | CSF | +VE | +VE |
| 24. | CSF | +VE | -VE |
| 25. | CSF | -VE | +VE |
| 26. | CSF | -VE | +VE |
| 27. | SPUTUM | -VE | +VE |
| 28. | BAL | +VE | +VE |
| 29. | BAL | -VE | +VE |
| 25. | BAL | +VE | +VE |
| Total +ve (110) | | 12/110 ( 10.91%) | 21/110 ( 19.09%) |

Again, with reference to the above data, it was concluded that the technique of Gene x-pert was highly accurate and reliable for the identification of *Mycobacterium, whose* its maximum number of sample was 21(19.09%)

It was observed that Ziehl-Neelsen staining had a low sensitivity for the identification of *Mycobacterium,* It was only 12(10.09% ) of the total samples.

Again, with reference to the above data, it was concluded that the technique of Gene x-pert was highly accurate and reliable for the identification of *Mycobacterium, whose* its maximum number of sample was 21(19.09%)

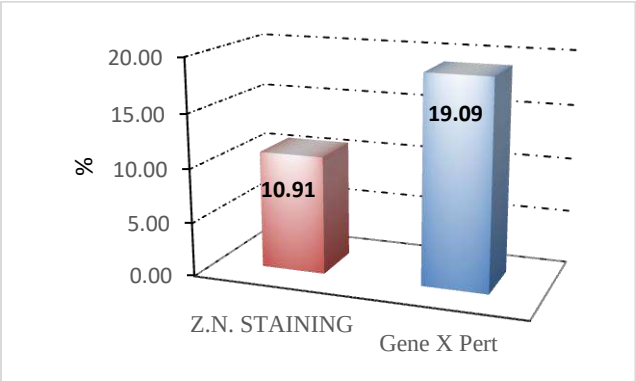


**Figure 4. Comparison between the Ziehl-Neelsen and GENE X-PERT methods in most positive specimens of Tuberculosis**

Table 4.: Detection of *Mycobacterium tuberculosis* in different locations around Ninava province

| Location | AFB SMEAR | GENE X-PERT |
|---|---|---|
| 1.SUMMER (45) | 20 (11.49%) | 25 (11.85%) |
| 2.ALNOOR (21) | 13 (7.47%) | 8 (3.79%) |
| 3.ALINTISAR (55) | 22 (12.64%) | 33 (15.64%) |
| 4.ALRASHEDIAYA (64) | 28 (16.09%) | 36 (17.06%) |
| 5.ALHADBA (16) | 8 (4.60%) | 8 (3.79%) |
| 6.GOGJALI (12) | 4 (2.30%) | 8 (3.79%) |
| 7.ALHARAMAT (27) | 18 (10.34%) | 9 (4.27%) |
| 8.HAMAM ALALEEL (30) | 20 (11.49%) | 10 (4.74%) |
| 9.ZUMMAR (24) | 5 (2.87%) | 19 (9.00%) |
| 10.RAJIM HADEED (21) | 7 (4.02%) | 14 (6.64%) |
| 11.ALNAHRAWAN (18) | 7 (4.02%) | 11 (5.21%) |
| 12.BADOUSH (12) | 5 (2.87%) | 7 (3.32%) |
| 13.HAY ALTANAK (10) | 4 (2.30%) | 6 (2.82%) |
| 14.TAL ALRUMAN (6) | 2 (1.15%) | 4 (1.90%) |
| 15.ALESLAH ALZIRAEE (8.) | 3 (1.72%) | 5 (2.37%) |
| 16.SINA QADEMA (2) | 2 (1.15%) | 0 (0.00%) |
| 17.ALFAYSALYIA (14) | 6 (3.45%) | 8 (3.79%) |

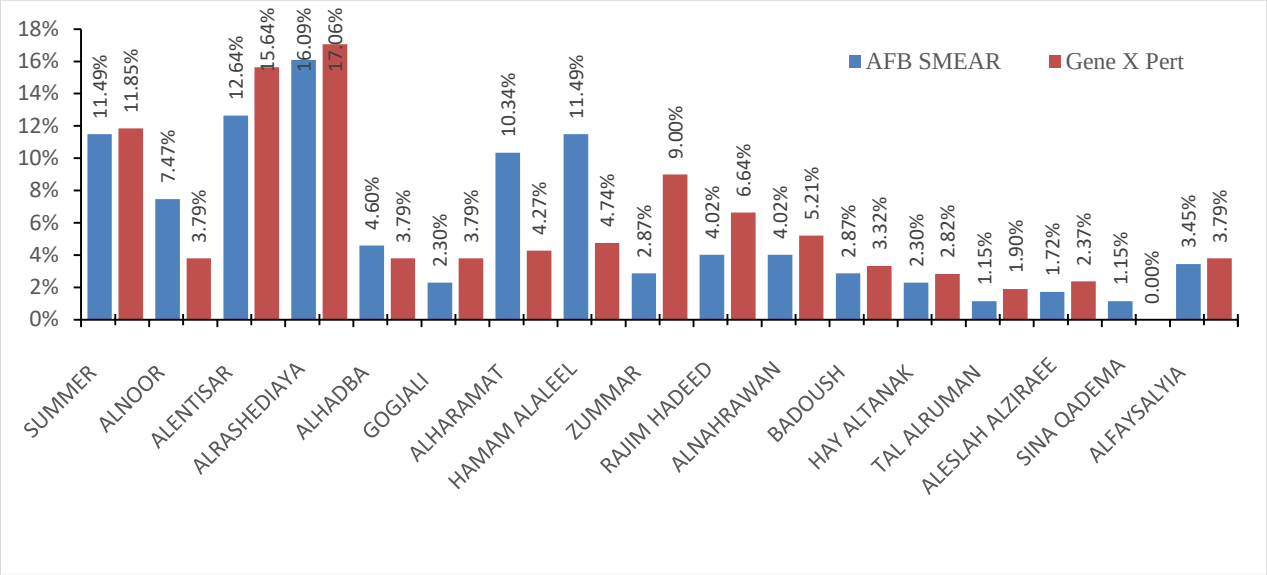


Figure 5:Distribution of tuberculosis according to the location

The data studied for distribution of tuberculosis showed that people who reside in Alrashediya Quarter have been more exposed to be infected of TB with a percentage rate (16.09%) by AFB STAIN and(17.06%) by GENE X-PERT, followed by Al- intisar Quarter with a percentage rate ( 12.64%) detected by afb and (15.64%) detected by GENE X-PERT while people reside at Senaa Alqadema Quarter have less infection with TB with a percentage rate (1.15%) .

## 4. Discussion

As developing countries have the largest share in the prevalence of tuberculosis, modern diagnostic methods such as GENE X-PERT technology, which is characterized by the identification of TB products in diagnostic samples, can be used. A study published in the *New England Journal of Medicine*, has demonstrated a similar test [13],[14], if it is an adjunct test in diagnosing MDR-TB infections. Traditional diagnoses of the disease lack require dedicated tools, technicians and experts .The culture method takes 2-6 weeks and is highly sensitive and it consumes specialized substances to support harmful bacteria on the culture. Sputum screening is quick to show results, which may take about 30 minutes, but the results of this test reveal 10%-75% of all TB patients and require specialized microscopy technicians. So in developing countries, the disease is not diagnosed correctly due to the lack of experts in which increases the prevalence of infection [15], [16].

The 496 clinical samples collected from different sites (like CSF, lungs and other body fluids etc.) were processed for detection of *M. tuberculosis* by GENE X-PERT (Smartcycler, Cepheid) using kits obtained from Sacace Biotechnologies, Italy. . It was found that out of a total of 496 clinical specimens, positivity was observed by different methods as follows: maximum by GENE X-PERT (78/ 296, followed by Ziehl-Neelsen (85 / 377).

Mycobacteria were identified in various clinical samples by different methods. GENE X-PERT (26.35%) and then by Ziehl-Neelsen stain (22.25%). Respiratory specimens yielded the maximum positivity (48.24%) for AFB smear (42.31%) for GENE X-PERT amongst the clinical specimens. GENE X-PERT method was found to be more reliable for the diagnosis of *Mycobacterium,* its maximum number of samples 21(19.09%). Ziehl-Neelsen staining was found to be the least sensitive among the three methods conducted where detection of *Mycobacterium* was only 12(10.09%).

A molecular technique has been developed for mycobacterial identification, which is highly accurate and specific when compared to Ziehl–Neelsen technique and phenotypic methods. Molecular methods like Gene x-pert are advantageous in gaining time and guiding anti-mycobacterial therapy. However, culture methods using Löwenstein–Jensen medium are also essential in studying the detailed morphology of Mycobacteria, performing biochemicals and for extensive susceptibility testing. The GENE X-PERT has created new area for research and revolutionary waves in the field of molecular biology and also in life science. Gene x-pert system is extremely more sensitive than other conventional methods. GENE X-PERT is an advanced and quick method for the amplification of the desired gene. This method is very useful as it diagnoses the germ in its early stages and has a specific treatment.

Age is one of the variables that influencing the increase in tuberculosis incidence, as many studies have proven that infections caused by tuberculosis are highly prevalent in elderly people and that the percentage of deaths from pulmonary tuberculosis increases as age increases, due to the presence of some chronic diseases that can weaken the immune system such as acquired immunodeficiency disease, heart disease, diabetes, and lymphocyte infections have a major role in the spread of incidence of pulmonary tuberculosis infection [17].

This study has shown that the incidence rate is lower in middle-aged people, and conversely, where the mortality rate is observed significantly in people aged 80-90 years (22.22%) by AFB, and (22.30%) by GENE X-PERT, The incidence is widespread in the age group 1-20 years (33.3%) by AFB, and (37%) by GENE X-PERT, and that statistically similar with other studies [18-20]. Also, the study showed an increase in the incidence rate in the areas of Al-Intisar (12.64%) by AFB, and (15.64%) by GENE X-PERT and Rashidiya (16.9%) by AFB (17.0%) by GENE X-PERT being poor and densely populated areas and suffering from lack of nutrition and close contacts in contrast to the areas of the Senaa Alkadima (1.15%) by AFB, (0.00) by GENE X-PERT and Tal-Ramman (1.15%) by AFB and (1.90%) by GENE X-PERT as being the areas of the largest area relative to the population, so the infection rate of tuberculosis is directly proportional to the population density, lack of income, education and malnutrition and that results is emphasizing previous studies [21],[22].

## 5. Conclusion

Mycobacteria were diagnosed in various clinical samples by different methods.The most sensitive one was GENE X-PERT as it was detected, the maximum positivity, followed by an Acid-fast stain (Ziehl–Neelsen smear) .The rate of survival of TB patients treared at Respiratory Infectious Disease Centre/ Mosul /Iraq was simiar tother global rates where the elder patients who suffer from chronic diseases were more exposed to TB infections since they suffer from immunity weakness. These results need more studies and researches to lessen the TB infections at the Centre specifically and at the city of Mosul generally.It was found that the incidence of TB was highly existed in crowded areas owing to the poverty and lack of education among these residential areas. The study suggests relying heavily on GENE X-PERT technology in the Mosul infectious Center other than the traditional techniques followed to detect TB infection rapidly which increases the opportunity of fast healing.

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