Detection of tuberculosis in smear predator pulmonary TB in Fayoum Chest Hospital

Assem F. Al Essawy[a], Randa I. Ahmed[a], Fadwa A.E. Raheem[b], Heba M. Bakri[c]

Background Tuberculosis (TB) is one of the causes of health problem in millions of people annually, and in 2015, it was one of the top 10 reasons of doom worldwide, ranking above HIV/AIDS as one of the important causes of death owing to an intended disease.
A negative smear result in pulmonary TB is believed to be a widespread clinical problem, so early detection of smear-negative pulmonary tuberculosis (SNPTB) is important for TB control and restriction of number of deaths, and it is tricky in these patients.

Aims To detect TB in SNPTB in Fayoum Chest Hospital

Design This was a retrospective study.

Setting Fayoum Chest Hospital and Fayoum University Hospital in Egypt were used for conducting the study between 2015 and 2017.

Patients and methods Fifty patients suspected to have pulmonary TB and had negative sputum smear results were included in the study.
For each patient, full history was taken, and clinical body checkup was done. Then, plain posteroanterior chest radiograph was done. Tuberculin test, direct sputum examination, and other diagnostic methods used for detection such as GeneXpert, bronchoscopy, bronchoalveolar lavage (BAL), transbronchial lung biopsy, Löwenstein–Jensen culture, QuantiFERON, or even open lung biopsy were recorded.

Statistical analysis Coding of the data was done then entered with SPSS (Statistical Package for the Social Sciences) version number 18 windows 7 after that data were summarized using mean, standard deviation, median, minimum and maximum in the quantitative data with using frequency (count) & relative frequency (percentage) for categorization of data.

Results It was found that 42% of patients were diagnosed by GeneXpert and 46% were diagnosed by BAL during bronchoscopy, whereas 68% of patients had positive 'Löwenstein–Jensen culture' result.

Conclusion The GeneXpert MTB/RIF assay is an important test for quick diagnosis of acid-fast bacilli SNPTB.
Flexible fiberoptic bronchoscopy is a beneficial tool in the diagnosis of pulmonary TB in patients whose sputum smear is negative.
Clinical implications are as follows: in patients with SNPTB, microbiological samples should be obtained (through sputum, BAL, or induced sputum), and then radiological investigation should be performed. Thereafter, antituberculous treatment should be started following the diagnosis, with follow-up of the case.
Rapid detection and proper treatment of pulmonary TB, even in smear negative patients, can eliminate spread of the infection to others and may decrease the severity of the disease.

Egypt J Bronchol 2018 12:473–481

Egyptian Journal of Bronchology 2018 12:473–481

Keywords: Fayoum Chest Hospital, other diagnostic methods, smear-negative tuberculosis, tuberculosis

[a]Chest Department, Faculty of Medicine, Fayoum University, [b]Lecturer of Clinical Pathology in Faculty of Medicine, Fayoum university, [c]Fayoum Chest Hospital, Fayoum, Egypt

Correspondence to Randa I. Ahmed, MD Degree of Pulmonology, Faculty of Medicine , Fayoum University, Fayoum, Egypt. Tel: +20 109 894 9345; e-mail: dr.randa80@yahoo.com

Received 10 December 2017 **Accepted** 4 July 2018

Introduction

Tuberculosis (TB) is a public health problem worldwide, with approximately nine million new cases annually [1].

Despite the efforts to develop new diagnostic methods for TB, microscopic examination of direct sputum smear remains the most used diagnostic test in low-income and middle-income countries, including Egypt [2].

Smear-negative pulmonary tuberculosis (SNPTB) is considered as a major problem in countries with a high to a moderate TB prevalence [3].

In 2015, 0.4 million patients with HIV died of TB, from the total of 1.8 deaths in which TB was the cause of death [4].

TB has plagued mankind for millennia, causing disease, deformity, and death since prehistoric times [5].

Target of the study

The aim was to detect TB in patients with SNPTB in Fayoum Chest Hospital.

Patients and methods

This research was carried out on 50 patients with sputum smear-negative TB of 170 patient who

attended outpatient clinics of tuberculosis in Fayoum Chest Hospital, and the rest of the 120 patients were sputum positive for acid-fast bacilli (AFB) staining in the duration from June 2016 to January 2017.

The research was be approved by the Ethics Committee of Faculty of Medicine, Fayoum University.

Study population comprised 50 patients selected from the general population, with the following criteria of inclusion and exclusion.

Inclusion criteria were as follows:

(1) Age of patient: 17–70 years.
(2) Sputum-negative smear for AFB.
(3) Radiological or clinical suspicion to have pulmonary TB.

Exclusion criteria were as follows:

Sputum positive smear for AFB.

All patients were subjected to the following to reach the proper diagnosis:

(1) Full history taking (name, age, and residence).
(2) History of smoking.
(3) History of chest symptoms (e.g. cough, expectorations, hemoptysis, chest pain, dyspnea, and chest wheezes).
(4) Full clinical examination, including general and local examination.
(5) Routine laboratory tests.
(6) Tuberculin skin test:
0.1 ml (two tuberculin units) of the purified protein derivative RT23 (Statens Serum Institute, Copenhagen, Denmark) was injected intradermally into the anterior surface of the forearm with a standard tuberculin syringe, and then evaluation of the reactions was done within 48–72 h after the injection, where we recorded the transverse diameter of the area of the induration (in mm). The test was considered as positive when induration was 10 mm or more in the transverse diameter [6].
(7) Microbiological examination of sputum was done as follows:
(a) Three consecutive early morning sputum specimens in three successive days were collected and examined for presence of AFB in the smear.
(b) Induction of sputum was performed by nebulized hypertonic saline in the early morning in case the patient was unable to give sputum spontaneously.
(c) For each sputum sample obtained, a smear was performed, air-dried, heat-fixed, and stained using 0.3% auramine for 10 min, 74% alcohol containing 1% hydrochloric acid for 4 min, and 0.1% potassium permanganate for 30 s [7].
(8) Radiological examinations, including plain chest radiograph and high-resolution computed tomography (HRCT) chest.
HRCT chest studies were done with 16-multidetector computed tomography scanner (Light Speed 16; GE Medical Systems, Milwaukee, Wisconsin, USA). Volumetric 1.25-mm slice thickness multidetector computed tomography chest acquisition was done with the patient supine in the cranial to caudal direction over a single breath hold. After that, from the volumetric CT data, a series of contiguous fine collimation 1.25 mm axial HRCT images were reconstructed with high spatial resolution algorithm [8].
Assessment of HRCT scans was done for detection of end bronchial spread of infection, centrilobular nodules, tree-in-bud pattern, larger nodules, lobular consolidations, cavities, bronchoceles, and mediastinal lymph node enlargement.
(9) Examination of sputum by culture:
Decontamination of the fasting morning sputum was done with the modified Petroff method and then cultured on Löwenstein-Jensen (L-J) media [9].

Some patients were subjected to the following procedures:

(1) Examination of sputum by GeneXpert MTB/RIF (21 patients):
The test involves three manual steps:
(a) Some reagent was added to liquefy and inactivate the sputum.
(b) 2 ml of liquefied sputum was transferred to the cartridge.
(c) The device was loaded with the cartridge for the assay.
The results are available within 2 h [10].
(2) Fiberoptic bronchoscopy and bronchoalveolar lavage (BAL) examination (23 patients):
(a) The patient signed an informed consent.
(b) Video-assisted bronchoscopy was performed using a flexible video-bronchoscopy system (EVIS EXERA II video system center CLV-180 bronchoscope, CLV-180 Xenon

light source, and LMD-2140 MD LCD monitor; Olympus, Tokyo, Japan).
(c) Fiberoptic bronchoscopy was performed in a well-equipped respiratory endoscopy unit.
(d) The patient fasted for at least 6 h before the procedure, with an explanation of the procedure to the patient.
(e) Patients had an intravenous cannula.
(f) Patients were premedicated 30 min before bronchoscopy with intramuscular injection of 0.6 mg atropine.
(g) Supplementary oxygen was provided to the patient before bronchoscopy to maintain oxygen saturation above 90%.
(h) Bronchoscopy was performed under local anesthesia.
(i) BAL was done after impaction in the affected segment by about 100 ml saline at room temperature, which was sent for AFB staining and TB culture.
(j) Oxygen saturation on pulse oximetry, heart rate, arterial blood pressure, and ECG were monitored and recorded during procedure [11].

(3) Open lung biopsy was done when indicted (one patient).
Pathological examination of biopsies was done. The excised biopsy was fixed in 10% formalin and then embedded in paraffin wax. Thereafter, the sections 4 μm thick were cut from each block and stained with hematoxylin and eosin and examined under light microscope.

(4) QuantiFERON-TB Gold In-Tube assay (Cellestis Limited, Carnegie, Victoria, Australia) was done in one patient as follows:
One milliliter of blood was collected into each of three colored tubes in the order of gray (negative control, 'nil'), red (test tube), and purple (positive control; mitogen-coated) tubes, and was incubated at 37°C. Following a 16–24-h incubation period, the tubes were centrifuged, and the plasma was removed. Finally, the measurement of the amount of interferon-gamma (IU/ml) was done by enzyme-linked immunosorbent assay (the test was considered positive if the interferon-gamma level was above the cutoff test value >0.35 IU/ml) [12].

Statistical analysis

Collection and coding of data were done and then entered into Microsoft Access. Analysis of the data was performed using statistical package for the social sciences software, version 18, in Windows 7 (SPSS Inc., Released 2009, PASW Statistics for Windows, Version 18.0, Chicago: SPSS Inc.).

Results

Fifty patients with smear-negative pulmonary TB were included in this study, with mean age of 43.5±16.7 years. Regarding sex, 68% of study group were males and 32% were females (Figs 1 and 2).

As for their residence, 48% lived in Fayoum district, 24% in Etsa, 16% in Senoris, 8% in Tamiya, and 4% in Ibshaway (Fig. 3).

Overall, 62% of study group were nonsmokers, 6% were exsmokers, and 32% were smokers.

Regarding smoking methods, 26% of smoked cigarette, 2% smoked shisha, and 4% smoked both, with a mean smoking duration of 23.1±12.8 years (Table 1).

In this study, 90% of patients complained of cough, 80% of expectoration, 58% of night fever, 24% of night sweating, 38% of dyspnea, 20% of hemoptysis, 58% of loss of weight, and 56% of loss of appetite (Table 2).

The mean pulse of the patients in this study was 95.5 ±13.1 mmHg, mean respiratory rate was 23.4±5.9 breaths/min, mean body temperature was 37.6 ±0.65°C, and mean oxygen saturation was 94.4±2.9%.

Regarding blood pressure, the mean systolic blood pressure was 114.4±10.7 mmHg, and the mean diastolic blood pressure was 75.8±8.8 mmHg (Table 3).

Overall, 56% of the cases showed harsh vesicular breathing (HVB) with crepitation, followed by 14% who had HVB only.

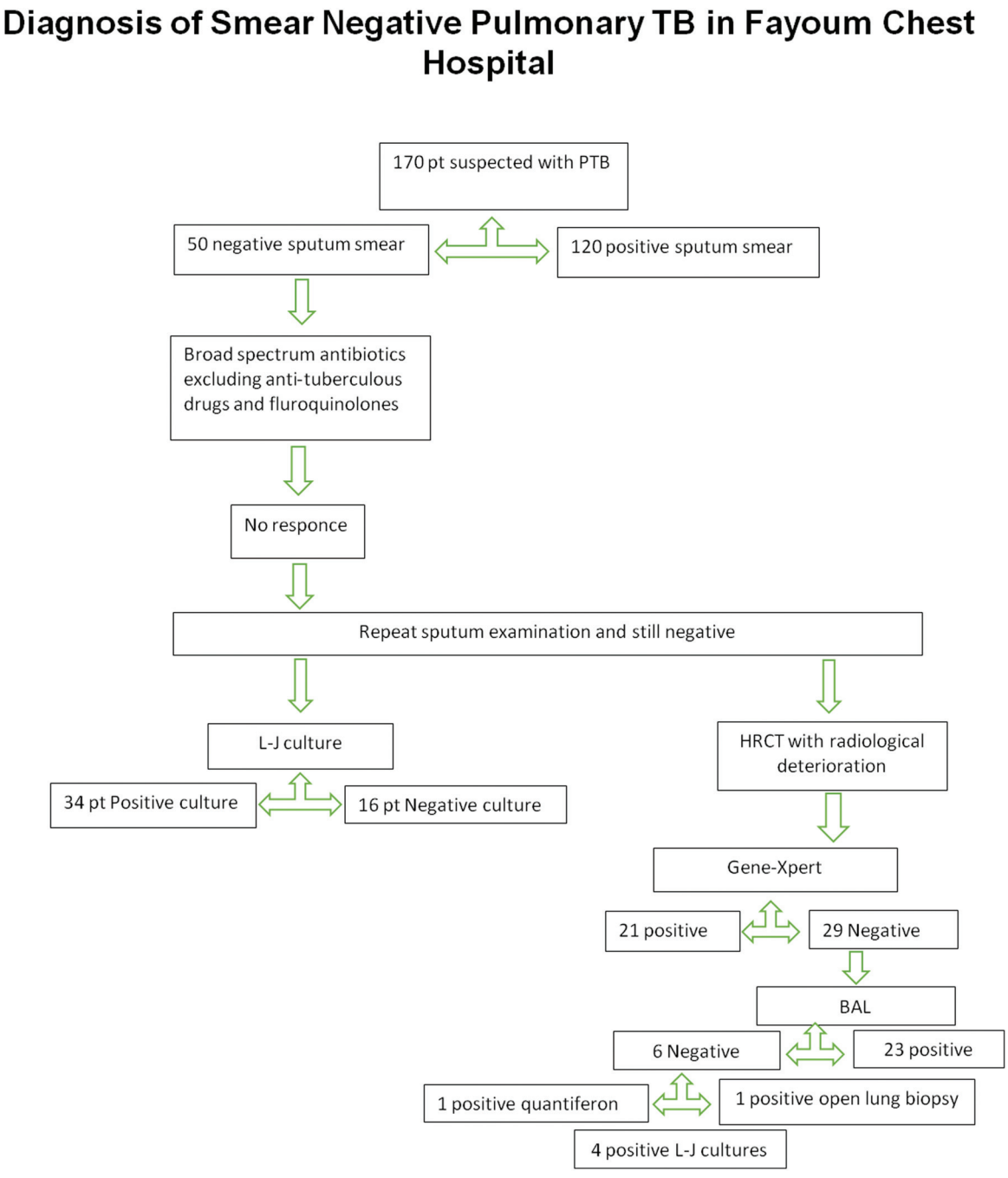
Regarding the site of auscultation finding, 66% showed these finding bilaterally, followed by 24% on right side and 10% on left side (Fig. 4).

Overall, 62% of the patients received BCG vaccine, and 90% had positive tuberculin test result, with mean reaction size of 16.4±3.6 mm (Fig. 5).

Regarding radiological finding, 72% of the patients had consolidation, 34% had cavitation, 12% had plural effusion, 8% had emphysema and 6% had cystic changes (Fig. 6).

We requested sputum culture 'L-J medium culture' for all patients of the study.

Figure 1



Sex of the patients in the study group.

Overall, 32% had negative culture and 68% had positive L-J culture (Table 4).

Regarding the different ways of diagnosis used in this study, 46% of cases were diagnosed by BAL for AFB, 42% were diagnosed by GeneXpert, and 2% by both QuantiFERON and open lung biopsy (Table 5).

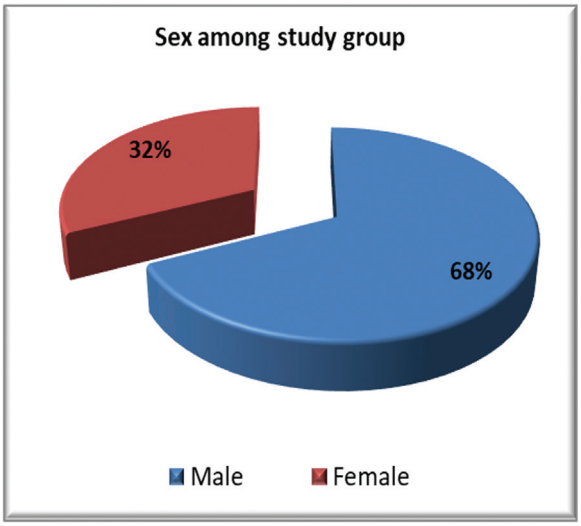
Discussion

Smear-negative pulmonary TB is a common problem in clinical practice and causes transmission in approximately one in six patients with pulmonary affection [13].

SNPTB is an important cause of transmission in societies. More importantly, the lateness of diagnosis and initiation of treatment often leads to irreversible damage of lungs in the infected individuals [14].

WHO (2013) defined a case of SNPTB by a case with at least two negative AFB smears but a positive TB culture, or two negative AFB smears and radiographical abnormalities consistent with active

Figure 2



Residence of the patients in the study group.

Table 1 Smoking history in the study group

Smoking	*N*=50 [*n* (%)]
No	31 (62)
Exsmoker	3 (6)
Cigarette	13 (26)
Shisha	1 (2)
Both cigarette and shisha	2 (4)
Smoking duration (mean±SD) (years)	23.1±12.8

Table 2 Different clinical manifestations in the study group

Variables	*n* (%)(*N*=50)
Cough	45 (90)
Expectoration	40 (80)
Night fever	29 (58)
Night sweating	12 (24)
Dyspnea	19 (38)
Hemoptysis	10 (20)
Loss of weight	29 (58)
Loss of appetite	28 (56)

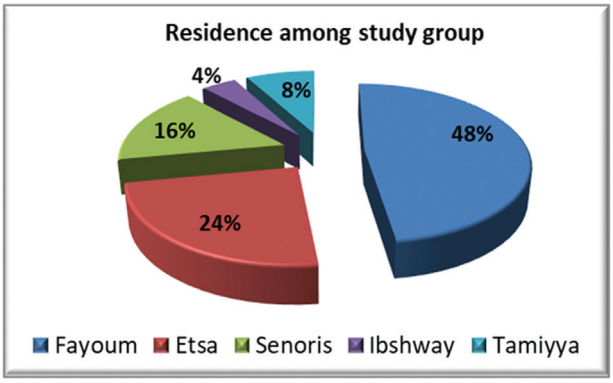
Table 3 Description of vital signs in the study groups

Variables (*N*=50)	Minimum	Maximum	Mean±SD
Pulse (beat/min)	65	120	95.5±13.1
RR (/min)	16	45	23.4±5.9
Temperature (°C)	36.5	39.5	37.6±0.65
So2%	85	99	94.4±2.9
Blood pressure			
Systolic	90	140	114.4±10.7
Diastolic	60	90	75.8±8.8

pulmonary TB and treatment for TB with clinical response [15].

As the diagnosis of TB may be missed in patients with sputum-negative smear, so we need other diagnostic modalities for proper diagnosis of such cases.

Figure 3



Auscultation finding in the study group.

The study was carried out on 50 patients with sputum smear-negative result and high clinical suspicion of TB from the 170 patients who attended the outpatient clinics of tuberculosis in Fayoum Chest Hospital, and the rest of the 120 patient were sputum positive for AFB staining in the duration from June 2016 to January 2017. The study different methods for diagnosis of TB according to the scheme of diagnosis are stated in Fig. 6.

The age of our study patients ranged between 17 and 70 years, with a mean age of 43.5±16.7 years. Regarding sex, 68% (*n*=34) were male patients and 32% (*n*=16) were female, with male to female ratio of 2.1 : 1.

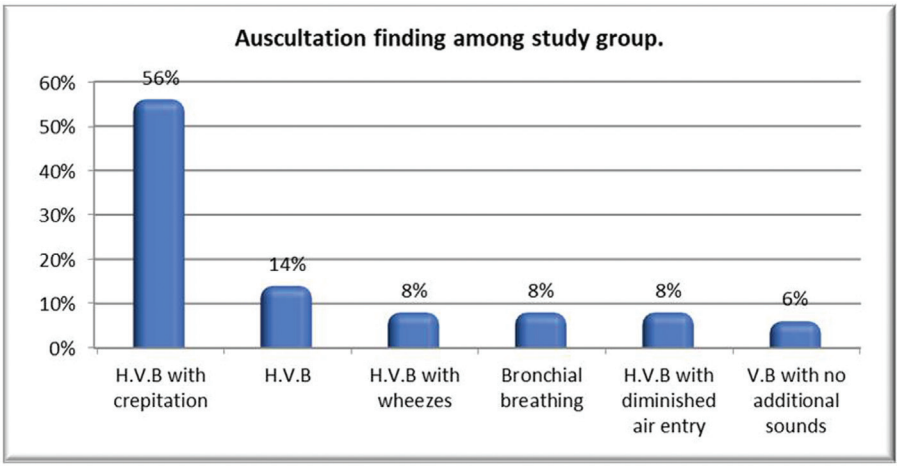
This was in agreement with the result of (Rao S) whose study was on patient gender and its role in tuberculosis control. He reported that the ratio of male to female was 2:1 among 446 pulmonary tuberculosis patients [16].

The study revealed that the largest number of patients were from Fayoum district, with 48% (*n*=24), where the Fayoum Chest Hospital is located; 24% of patients were from Etsa; 16% from Senoris; 8% from Tamiya; and 4% from Ibshaway.

Our study shows that 62% (*n*=31) of patients were nonsmokers and 32% were smokers: 13 patients were cigarette smokers, one patient was a shisha smoker, and two patients smoked both. Moreover, 6% (*n*=3) patients were exsmokers.

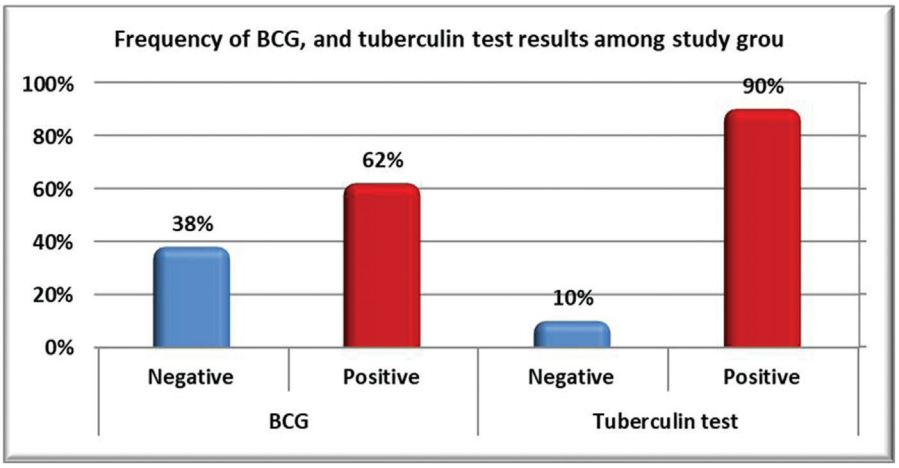
These results were partially supported by the results of Fatma *et al.* [17] who reported that the percentage of smokers among their patient's study was 54.2% and nonsmokers were 45.7%, with no significant difference

Figure 4



Frequency of BCG and tuberculin test results in the study group.

Figure 5



Radiological finding in the study group.

Table 4 Löwenstein–Jensen culture results in the study group

L-J culture	*N*=50 [*n* (%)]
Negative	16 (32)
Positive	34 (68)

L-J, Löwenstein–Jensen.

Table 5 Different diagnostic methods in the study group

Positive test	*N*=50 [*n* (%)]
BAL for AFB	23 (46)
GeneXpert	21 (42)
QuantiFERON	1 (2)
Open lung biopsy	1 (2)

AFB, acid-fast bacilli; BAL, bronchoalveolar lavage.

detected between smokers and nonsmoker patients regarding complaints, with great significant differences in relation to sex of the patients.

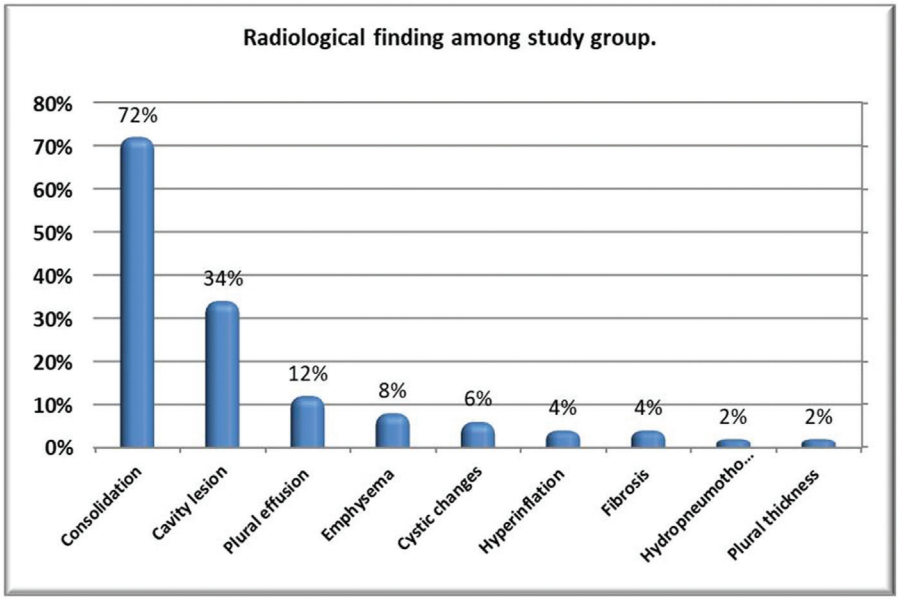
Our results were in disagreement with Leung and colleagues, who studies 404 patients from 16 to 64 years of age and 447 patients more than 64 years of age for analyzing the relationship between smoking and TB in Hong Kong. They stated that there was a consistent association between smoking and TB within different segments of population in Hong Kong [18].

In recording of chest symptoms, we found in this study that 90% of our patients had chronic cough, 80% had expectoration, 58% had night fever, and 58% had loss of weight.

Moreover, 56% of patients experienced loss of appetite, 38% from dyspnea, 20% from hemoptysis, and 24% from night sweating.

On examination, 19 patients of 50 had high temperature, 13 patients had tachycardia, and 56%

Figure 6



Algorithm for research in the study sample.

(*n*=28) of patients had vesicular breathing, with inspiratory crepitation by auscultation.

This was in agreement with Fatma *et al.* [17], who reported that the main chest complaint was cough in 100% of patients accompanied by hemoptysis in 38.6%, toxic symptoms in 38.6%, and chest pain in 22.8% [19].

Moreover, Shin *et al.* [20] studied the role of fiberoptic bronchoscopy in the rapid diagnosis of SNPTB in 145 patients and reported that the main symptoms were cough in 63% of the patients with TB, expectoration in 33.3%, fever in 24.1%, and chest pain in 24.1%, hemoptysis in 13%, and weight loss in 5.6%.

Unlike our study, Lee and colleagues, who studied 84 patients for rapid diagnosis of SNPTB by HRCT and whole-blood interferon-gamma assay, reported that 48% of the tuberculosis patients lacked expectoration. The lack of sputum was a sensitive predictor of SNPTB [21].

Regarding tuberculin skin test (TST), we found a strong relationship between TST and SNPTB. Overall, 90% (*n*=45) of patients had positive TST with mean induration size of 16.4±3.6 mm, with 10% (*n*=5) having no reaction to TST.

Moreover, we took history of BCG vaccination from our patients, as well as examined the BCG scar, and we found that patients with BCG scar were 62% (*n*=31), and 38% (*n*=19) had no BCG scar.

These results agree with Sohair *et al.* [12], who compared between using QuantiFERON and tuberculin skin test in diagnosis of pulmonary TB, and found that 36 (90%) of 40 patients of her study had a positive TST finding.

Similar to our study, Kanaya *et al.* [22] studied TST results for identifying pulmonary TB in patients with sputum smear negative, and they found that TST was positive in 81% of patients.

Egypt is considered as an endemic area for TB, so BCG vaccine is mandatory in national vaccination programs. This causes a false-positive TST result, but this effect continues for several years after the vaccination [23].

In our study, there were significant HRCT findings. Overall, 72% of patients had lobular consolidation, 34% had cavities, 12% had plural effusion and 6% had cystic changes. Although HRCT provides additional information for diagnosing pulmonary TB, HRCT alone has limited value for diagnosing pulmonary TB in smear-negative patients.

This coincides with Nakanishi *et al.* [24], who used HRCT to examine 116 patients with sputum smear-negative pulmonary TB and described different radiological pictures in HRCT, such as lobular consolidation, tree-in-bud appearance, and large nodules, and commented on their relation to active infection in SNPTB, but the diagnostic accuracy was not favorable.

Caliskan *et al.* [25] also studied HRCT findings in 78 patients of SNPTB according to their culture status and reported that HRCT findings were micronodules in 87% of patients, large nodules in 63%, tree-in-bud pattern in 41%, consolidation in 28%, cavitation in 26%, and bronchiectasis in 32% of patients.

Lee *et al.* [21] also reported significant differences between TB and non-TB cases with respect to HRCT findings of tree-in-bud pattern, nodules and cavities, although 29% of patients with suspected TB based on HRCT were diagnosed as non-TB, and 21% patients thought to be non-TB cases based on CT were diagnosed with TB.In the current study, 42% (n=21) of patients of SNPTB were diagnosed by GeneXpert.

This in agreement with Opota *et al.* [26], who studied 71 patients to compare between GeneXpert MTB/RIF and smear microscopy, and found that the sensitivity was 91.5% and specificity was 99.6% for the GeneXpert MTB/RIF, which were higher than specificity and sensitivity of smear microscopy, which were 94.2 and 64.8%, respectively.

Our results are in line with the study conducted by Boehme *et al.* [27], who evaluated the performance of the GeneXpert MTB/RIF among patients with culture-positive TB smear negative, and found that the total sensitivity of the MTB/RIF test was 97.6%.

We requested sputum culture 'L-J medium culture' for all patients of our study, and the results showed that 68% (n=34) of patients had positive 'L-J culture' and 32% (n=16) had negative 'L-J culture.'

Unlike our study, Affolabi and colleagues reported in their study, which was done on 214 TB suspects, that culture shared in only 2.6% of the global number of proven cases bacteriologically. The development of culture for routine diagnosis therefore does not appear to be a priority [28].

Mitchison [29] stated that the examination of smear of multiple specimens from each patient is almost as efficient as examination of culture in developing countries.

On analysis of the study results, we found that 40% (n=20) of patients had positive L-J culture and positive GeneXpert at the same time, and 20% (n=10) of patients had positive L-J culture and positive BAL smear for AFB at the same time.

Overall, 46% (n=23) of patients in this study had positive BAL in the direct smear for AFB.

This result was similar to the study by Altaf Bachh and colleagues, which enrolled 75 suspected sputum SNPTB cases, and bronchial wash via fiberoptic bronchoscopy was performed for the detection of *Mycobacterium tuberculosis*. They reported that 35% of patients has positive bronchial wash for AFB [30].

Danek and Bower [31] as well as Purohit *et al.* [32] proved the presence of AFB in BAL in 34 and 42%, respectively.

Another study by Kulpati and Heera [33], which studied the role of fiberoptic bronchoscopy and transbronchial biopsy in establishing diagnosis of pulmonary TB in 33 patients, reported that 40% of patients had positive BAL for AFB in the direct smear.

Of the 50 patients, only one patient needed to undergo open lung biopsy for diagnosis and his history was as follow: a 32-year-male patient presented with right upper lung mass associated with bronchopleural fistula, which was removed, and the pathology finding reported caseating granulomatous inflammation of both lung and pleura. BAL examination for AFB was done and was negative.

We also found in our study that one patient was diagnosed by positive QuantiFERON test. A 34-year-oldmale patient who complained of cough, night fever, and hemoptysis with sputum smear negative for AFB but TST was positive (20 mm), and his HRCT showed right pneumonic patch in apical segment of lower lobe with no response to antibiotics.

The limitations in our study are as follows:

(1) The small sample of the study.
(2) The lack of control patients.
(3) The financial supply is limited as our patients were very poor and there was lack of available investigations in our hospital so patient was referred to do investigations on their coast or from available donations.

Conclusion

Gene expert has an important role in the diagnosis of SNPTB followed by BAL of the affected segment.

We should have a good illustrating approach for early diagnosis of SNPTB to prevent spread of infection and its hazardous effect on health.

Acknowledgements

The author express sincere gratitude to Professor Dr Assem Fouad Al Essawy for the continuous support for this study and Dr Randa Ibrahim Ahmed for her patience and motivation. Her guidance helped the author at all times during the work.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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