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Comparison between Smear-Positive and Smear-Negative Pulmonary Tuberculosis

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Abstract

Background: Tuberculosis is a communicable disease that is a major cause of ill health, one of the top 10 causes of death worldwide. The diagnosis of tuberculosis can be strongly inferred by finding acid-fast bacilli (AFB) by microscopic examination. Some patients have clinical and radiological features of pulmonary tuberculosis but AFB negative.

Objectives: To determine the difference between smear positive and smear negative pulmonary tuberculosis regarding clinical features, risk factors, and radiological features.

Patients and Methods A cross sectional study was conducted on 120 patients from different regions in Baghdad at randomly distributed health care centers, from 1st April 2019 to 1st February 2020, through a questionnaire including demographic data, risk factors, symptoms and chest X-rays findings. The patients were divided into two groups; smear-positive (whose sputum tests reveal positive acid fast bacilli in at least one out of the three successive tests) and smear-negative (who are diagnosed to have pulmonary TB despite that their three successive sputum tests give negative results to acid fast bacilli test). The criteria used to diagnose smear negative relied on the WHO guidelines. *P* values < 0.05 were considered statistically significant.

Results: Out of 120 patients, 58% were smear-positive, and 42% were smear negative, the highest age group in smear positive was in those ≥ 64years, while the highest age group in smear negative between 18-40years. Positive smear was significantly statistically positively associated with hemoptysis and consolidation and negatively associated with the use of steroid and cavitation.

Conclusions: Smear positive and smear negative pulmonary tuberculosis have similarities in clinical and radiological features, but hemoptysis and consolidation were higher in positive than in negative smears, while the use of steroids and cavitation were higher in negative than positive smears.

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Key Words: smear-positive, smear-negative, pulmonary tuberculosis

Introduction

Tuberculosis (TB) is a communicable disease that is a major cause of ill health, one of the top 10 causes of death worldwide and the leading cause of death from a single infectious agent.(1) About a quarter of the world's population is infected with M. tuberculosis and thus at risk of developing TB disease(2). 30 countries account for the high TB burden countries and make 87% of the world's cases; Iraq is not listed as one of these countries(3). The development of TB requires infection by Mycobacterium tuberculosis (M. TB)) and inadequate containment by the immune system (1). M. TB is transmitted from person to person via the airborne route (4).

The most common symptom of pulmonary tuberculosis is persistent, productive cough; with duration over 2 to 3 weeks; initially dry later productive. Fever is usually low-grade. Up to 20% of patients may have no fever. Fever is less common in older people. Other symptoms include appetite loss, weight loss, night sweats, and hemoptysis. (1,5).

Diagnostic tests for TB disease include sputum smear microscopy (developed more than 100 years ago), rapid molecular tests (first endorsed by WHO in 2010) and culture-based methods; the latter take up to 12 weeks to provide results but remain the reference standard. TB that is resistant to first-line and second-line anti-TB drugs can be detected using rapid tests, culture methods and sequencing technologies(2). Acid fast staining techniques such as Ziehl-Neelsen (ZN) stain or Auramine O – Rhodamine stain remain the easiest and cheapest methods, although they are less sensitive and not specific for diagnosis of MTBC.(6) It is estimated that up to 50% of Ziehl-Neelsen staining smear were negative, although the samples showed the presence of MTBC by other methods including culture.(7)

The optimum number of sputum specimens to establish a diagnosis has been examined in a number of studies. In a recent review of data from a number of sources, it was stated that, on average, the initial specimen was positive in about 83–87% of all patients ultimately found to have acid-fast bacilli detected, in an additional 10–12% with the second specimen, and in a further 3–5% on the third specimen.(8) Smear-positive cases are the most infectious and most likely to transmit their disease in their surroundings; they are the focus for infection control measures and contact investigations. (9)

Generally, smear negative pulmonary TB is defined, according to WHO (2010), as a clinical suspect with at least two negative AFB smears and a positive culture, OR two negative smears, radiographical abnormalities consistent with active pulmonary TB, no

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improvement with a course of broad-spectrum antibiotic (if HIV-negative), and the decision to treat for TB (10, 11).

The probability of finding acid-fast bacilli in sputum smears by microscopy is directly related to the concentration of bacilli in the sputum. Sputum microscopy is likely to be positive when there are at least 10,000 organisms per milliliter of sputum. At concentrations below 1,000 organisms per milliliter of sputum, the chance of observing acid-fast bacilli in a smear is less than 10%.(12)

Chest X-ray in patients with pulmonary tuberculosis typically presents as fibronodular (consolidation) opacities in upper lobes with or without cavitation (1).

This study aimed to evaluate the clinical and radiological characteristics of patients with smear negative pulmonary TB in comparison with patients with smear positive TB

Patients and methods

This is a cross sectional study which was performed on 120 patients from different regions in Baghdad at randomly distributed health care centers that responsible for diagnosis, treatment and follow up of patients with tuberculosis, from 1st April 2019 to 1st February 2020.

Ethical and legal permission was taken from the training and research department, public health directorate, ministry of health, Iraq. Data collection was performed by a preformed questionnaire regarding demographic data (age, gender, address, occupation), risk factors (systemic steroid use(equivalent to 15 mg prednisolone or more for at least one month, the causes to use steroid by these patients were other respiratory diseases, chronic joint illness or dermatological symptoms.), positive family history of TB, smoking, diabetes mellitus and history of prison), symptoms (cough sputum, hemoptysis, fever, sweating, anorexia, weight loss) and chest X-rays findings (consolidation, cavitation, hilar adenopathy, pleural effusion and miliary changes).

The patients were divided into two groups; smear-positive and smear-negative. Smear-positive group included patients whose sputum tests reveal positive acid-fast bacilli in at least one out of the three successive tests.Smear-negative group included patients who are diagnosed to have pulmonary TB despite that at least two successive sputum tests give negative results to acid fast bacilli test. The criteria used to diagnose smear negative TB relied on the WHO guidelines which stated that if the patient has clinical picture suggestive of pulmonary tuberculosis with highly suggestive imaging findings and negative smear results on sputum AFB test and did not respond to a two-week trial of antibiotics that do not include levofloxacin or antituberculosis medications, these patients can be considered to have pulmonary tuberculosis and given treatment course of antituberculosis. [10,13]

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Inclusion criteria: Age above 18 years**,** rural and urban areas**,** patients with clinical features of pulmonary tuberculosis
Exclusion criteria: Patients with extrapulmonary TB**,** patients with incomplete data

Statistical analysis

New Microsoft Excel Worksheet 2013 and Statistical package for social sciences (SPSS-25) were used for the data collection, table formation and chart creation. Chi square test was used to measure the significance of the collected and compared variants. The accepted statistically significant difference is P value of or below 0.05.

Results

The total number of patients included in the study was 120; 69 (58%) patients were smear-positive and 51 (43%) were smear-negative as shown in figure 1.

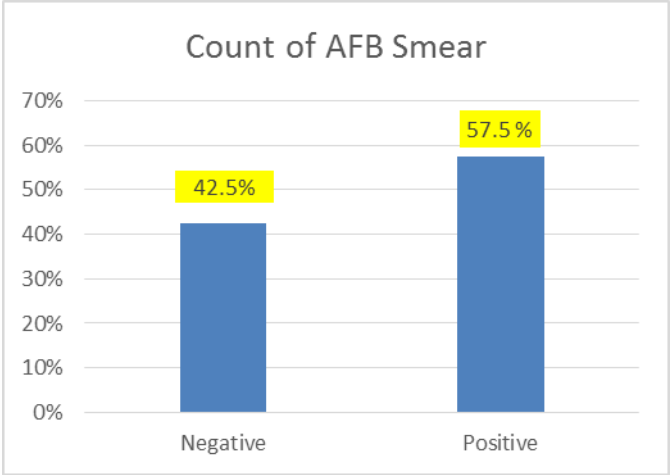

Figure 1: the percentage of patients involved in each group

Figure 2 illustrates that 54% (65) of the patients were female while males counted 46% (55) of total number of the studied sample.

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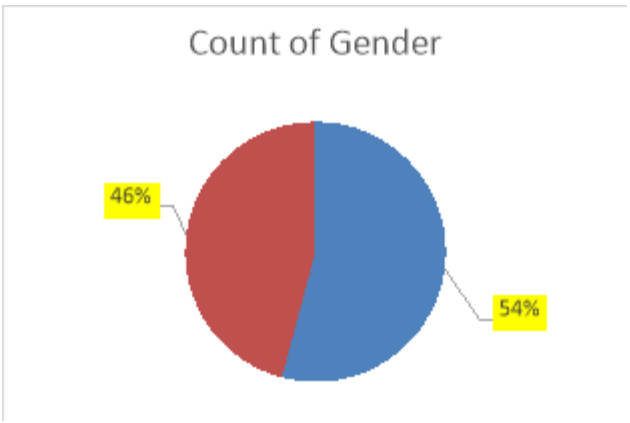

Figure 2: the gender percentage

As shown in table 1, around 65 percent of smear negative patients aged 64 years or more, while 25% and 10% aged 18-40 years and 41-63 years respectively. On the other hand, 46% of smear positive patients were 18-40 years old and 43% were 64 years or older and 10 percent were between 41-63 years old.

Table 1: distribution of patients according to age

	AGE no. (%)		
	18-40	41-63	≥ 64
Smear Negative	13(25%)	5(10%)	33(65%)
Smear Positive	32(46%)	7(10%)	29(43%)
Grand Total	45(38%)	12(10%)	63(53%)

Taking into account the living area for the patients, 88% of smear-positive patients were living in urban areas while 76% of the smear-negative patients lived in urban areas, with no statistically significant difference between the two groups ((p value 0.083). As in table 2

Table 2: comparison between the groups according to living areas

	Rural	Urban	Total	P Value
Positive	8(12%)	61(88%)	69(100%)	0.083
Negative	12(24%)	39(76%)	51(100%)	
Total	20	100	120	

Seven major symptoms were listed in this study and most commonly encountered symptom was cough for both smear-positive and smear-negative patients, 94% and 90% respectively. There was no significant difference in percentage of fever 84% versus 82%,

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sweating 77% versus 78% and sputum production 62% versus 65% for smear-positive and smear-negative cases respectively. On the other hand, anorexia and weight loss were noticeably higher in smear-positive patients compared to smear-negative patients, 75% of smear- positive patients have had both anorexia and weight loss while 65% of smear-negative patients lost appetite and 63% lost weight. Finally, although the rate of hemoptysis was much lower than all other symptoms, it was also higher in smear-positive cases (26%) than smear-negative ones (18%). However, all of these differences are statistically insignificant except hemoptysis, which showed statistically different values between the groups. As in table 3

Table 3: comparison between the groups regarding symptoms

Symptom	no. of patients (percentage)			
Cough				
	Yes	No	Total	P Value
Positive	65(94%)	4(6%)	69(100%)	0.410
Negative	46(90%)	5(10%)	51(100%)	
Sputum				
	Yes	No	Total	P Value
Positive	43(62%)	26(38%)	69(100%)	0.789
Negative	33(65%)	18(35%)	51(100%)	
Hemoptysis				
	Yes	No	Total	P Value
Positive	20(29%)	49(71%)	69(100%)	0.048
Negative	7(14%)	44(86%)	51(100%)	
Fever				
	Yes	No	Total	P Value
Positive	58(84%)	11(16%)	69(100%)	0.804
Negative	42(82%)	9(18%)	51(100%)	
Sweating				
	Yes	No	Total	P Value
Positive	53(77%)	16(23%)	69(100%)	0.834
Negative	40(78%)	11(22%)	51(100%)	
Anorexia				
	Yes	No	Total	P Value
Positive	52(75%)	17(25%)	69(100%)	0.204
Negative	33(65%)	18(35%)	51(100%)	
Weight loss				
	Yes	No	Total	P Value
Positive	52(75%)	17(25%)	69(100%)	0.136
Negative	32(63%)	19(37%)	51(100%)	

Regarding the risk factors, 19 (37%) patients out of the total 51 in the smear negative group have given history of using steroids (equivalent to 15 mg prednisolone per month

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within three months before diagnosis of TB). This was significantly different in comparison with smear positive patients where only 7 (10%. The P value was <0.001. On the other hand, there were no significant differences in terms of other well established risk factors for TB like diabetes mellitus, smoking, positive family history and history of prison, p value ≥ 0.05. As in table 4

Table 4: risk factors compared between the groups

Steroids	**no. (percentage)**			
	Yes	No	Total	P Value
Positive	7(10%)	62(90%)	69	0.000
Negative	19(27%)	32(63%)	51	
Family Hx				
	Yes	No	Total	P Value
Positive	20(29%)	49(71%)	69	0.359
Negative	11(21%)	40(79%)	51	
Smoking				
	Yes	No	Total	P Value
Positive	29(42%)	40(58%)	69	0.236
Negative	27(53%)	24(47%)	51	
DM				
	Yes	No	Total	P Value
Positive	20(29%)	49(71%)	69	0.959
Negative	15(30%)	36(70%)	51	
Prison history				
	Yes	No	Total	P Value
Positive	12(17%)	57(83%	69	0.128
Negative	4(8%)	47(92%	51	

Regarding the imaging findings, the two significantly different changes were consolidation and cavitation, 78% (54) of the smear positive patients have obvious consolidation on their chest x ray at the time of diagnosis whereas the consolidation was seen in 61% (31) of smear negative patients. Cavitation was significantly more commonly

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recorded in smear negative patients (73%) than in smear positive patients (48%). The P value of consolidation was 0.037 and the P value of cavity was 0.007. While the other three findings (hilar adenopathy, pleural effusion and miliary changes) have had no significant difference between the two groups of patients, p value ≥ 0.05. As in table 5

Table 5: Association between imaging findings and smear's results

Imaging finding	no. (Percentage)			
Consolidation				
	Yes	No	Total	P Value
Positive	54(78%)	15(22%)	69	0.037
Negative	31(61%)	20(39%)	51	
Cavity				
	Yes	No	Total	P Value
Positive	33(48%)	36(52%)	69	0.007
Negative	37(73%)	14(27%)	51	
Hilar Adenopathy				
	Yes	No	Total	P Value
Positive	21(30%)	48(70%)	69	0.063
Negative	24(47%)	27(53%)	51	
Pleural Effusion				
	Yes	No	Total	P Value
Positive	11(16%)	58(84%)	69	0.328
Negative	5(10%)	46(90%)	51	
Miliary				
	Yes	No	Total	P Value
Positive	5(7%)	64(93%)	69	0.902
Negative	4(8%)	47(92%)	51	

Discussion

The total number of patients included in this study was 120, more than a half (53%) of the studied patients were above 64 years old and there were slightly more females than males involved in our study (54% versus 46%). These two findings could be correlated

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because life expectancy above 60s for females is more than males in Iraq.[14] Iraq has suffered from wars, economic sanctions, and internal displacement more than other countries in the eastern Mediterranean region in last three decades. Rural areas recorded more smear negative TB than smear positive TB, 60% and 40% respectively but there was no statistical significance between living in rural or urban areas according to smear's results (p=0.08). This could be because the sample size is not fully representative of total cases and because they cannot give fair amount of sputum. This study revealed higher positive smear among patients above 40 years old (53% of overall positive smear), this is agreed by Pourostadi M *et al* done in Northwest of Iran [15].

This study showed that hemoptysis was statistically significant (p 0.048) with positive smear than negative smears (27% vs. 13%). These results are highly correlated with the Brazilian study done by Campos LC *et al* [16], in which hemoptysis was more frequent in positive smear than negative smears (with p 0.013). Neither cough nor sputum were of any statistical significance in this study (p >0.05), this result goes with that of Range et Al (17) in which cough nor statistically significant difference between the two groups (p 0.456), but this against the studies from India, Brazil, Congo and Nepal (18, 16, 19, 20) in which cough more in smear positive than smear negative may be a result of different sampling technique, weather effect during sampling and\or study duration. Fever and sweating had no statistically significant differences between the positive and the negative groups (p= 0.8). These results are similar with the findings of the Indian, Brazilian, Congo and Nepali studies (18, 16, 19, 20) in which fever had no statistically significant differences between the positive and the negative groups, might be due to the fact that both of the symptoms are general constitutional and are not specific to either group. Anorexia and weight loss showed no statistical significance in this study (p > 0.05) which is similar to the Brazilian and the Nepali Study (16, 20) while it differs from the findings revealed by the Indian (18), Sengali (21) and Congo (19) studies. These variations in the results can be explained by some differences in the studied population, the sample size, the duration of the study, the environmental factors, the nutritional status and economies of the population as well as the HIV infection.

This study resulted with strong statistical significance of steroid use and increased negative smears (p<0.01), which means steroid usage was reported more frequently by patients in the smear negative group than patients in the smear positive group, 27% versus 10% respectively. This is agreed with the American study (22). This could be explained by the fact that steroid use might suppress patient's immune and inflammatory response toward smear's test and it might decrease sputum production or corticosteroids indeed accelerate sputum culture conversion. This result is contradicted with the South Indian study (23) that showed no effect of steroid on AFB smear.as a result of longer study duration, different size and different sampling technique. Family History was of no statistical importance in this study (p=0.34) same as the Ethiopian Study (24). Smoking was of no importance statistically in this study (p =0.24) which is agreed by the Brazilian and Kazakhstani (16, 25) Studies, but rejected by the Ethiopian study (24) this discrepancy might be associated with different sampling method, different sample size

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and different inclusion criteria. Diabetes was also of no value statistically (p=0.96), which is in line with the Brazilian Study (16). But it is against both the Iranian and the Kazakhstani studies (15, 25). These variable results might be because of different type of study and \or duration. Prison history revealed no statistical significance (p=0.13) this contradict the study of North West Iran (15) in which imprisonment was statistically significant it might be explained by different age group, different population size, culture and possible different nutritional and environmental conditions affecting sputum production.

In this study, positive smears were statistically affected by Lung's consolidations (p =0.04). This finding is in the line of Iranian, Sengali, Gambian and American studies [26, 21, 27, 28]. Cavitation was higher in the smear negative group than the smear positive group (p = 0.07). This finding is correlated with van Cleeff the study [29] but not the same as with Iranian, Sengali, Gambian and American studies [26, 21, 27, 28], in which the cavitations are positively associated with positive smear's result. In our study may be due to the higher age range of the studied patients compared to other studies. For the Sengali study the patients involved in this study were HIV patients (in our study there was no evaluation for HIV status).

In conclusion age affects smear's results positively. Hemoptysis positively correlated with smear's results. Steroids negatively affects smear results. Lung consolidation was associated positively with smear results. Cavitation was negatively affecting smears results.

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