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Bacteriological Study for TB bacteria of tuberculosis patients in AL-Rifai District

Sura Qusay Ali [1]*

1 Department of biology, College of education, Al-Iraqia, University, Baghdad, Iraq.

Abstract:

This study aimed to investigate the presence of Mycobacterium tuberculosis in patients with tuberculosis in the district of Rifai. During the study period, a total of 300, 149 males and 151 females of clinically suspected patients were subjected to sputum exam at Consulting Clinic for Chest Diseases in Al-Rifai Health Sector. The sputum examination by Acid fast staining showed 14 cases were positive smear for pulmonary tuberculosis. Age wise analysis of the data shows 4 cases in the age group (20-29) years and (50-59) years, 1 case in the age group (60-69) years old, and no infections in the age group (30-39), worth noting there were no patients in the age group (40-49). The severity of infection was high (+++) in one male, whereas (+) and (++) were seen in females with 2:1 in males Moreover, the prevalence of pulmonary TB was observed in among different seasons Summer had the highest prevalence compared to other seasons of the year.

Key word: TB, *Mycobacterium tuberculosis* .

دراسة بكتريولوجية لبكتريا تي بي لمرضى السل الرئوي في منطقة الرفاعي

سرى قصي علي
الجامعة العراقية / كلية التربية - قسم علوم الحياة

مستخلص:

هدفت هذه الدراسة إلى التحقق من وجود بكتريا المايكوبكتريوم لدى مرضى السل في منطقة الرفاعي. خلال فترة الدراسة ، خضع ما مجموعه 300 من المرضى المشتبه بهم سريريًا كانوا 149 من الذكور و 151 من الاناث، خضعوا لفحص البلغم في العيادة الاستشارية للأمراض الصدرية في قطاع صحة الرفاعي. أظهر فحص البلغم بصبغة أسد فاست أن 14 حالة كانت مسحة إيجابية لمرض السل الرئوي. أظهر التحليل العمري للبيانات 4 حالات في الفئة العمرية (20 – 29) سنة ومثلها أيضا للفئة (50 – 59) سنة. وأظهرت حالة واحدة في الفئة العمرية (60 – 69) سنة ، وعدم وجود إصابات في الفئة العمرية (30 – 39)، مع ملاحظة عدم وجود مرضى في الفئة العمرية (40 – 49). شدة الإصابة كانت عالية (+++) في ذكر واحد، بينما شدة الاصابة (+) و(++) شوهدت بنسبة 2 للاناث و 1 للذكور. علاوة على ذلك ، لوحظ انتشار السل الرئوي في مواسم مختلفة كان الصيف أعلى معدل انتشار مقارنة بفصول السنة الأخرى.

الكلمات المفتاحية : السل ، مايكوبكتريوم .

Introduction

Tuberculosis (TB) is a long-standing global health issue, affecting millions of people each year and causing morbidity and mortality worldwide. Despite the availability of various treatment options, it remains the world's ninth greatest cause of mortality (WHO, 2018; Balaky, *et al*., 2019). It is a chronic disease that mostly affects those of lower socioeconomic status and causes a variety of clinical infections (Khazaei *et al*., 2005). The central biology of the causative bacterium of the disease, *Mycobacterium tuberculosis*, makes it able to persist in the form of latent TB resulting in long-term asymptomatic infection (AL-Jebory *et al*., 2017).

The main cause of TB is *Mycobacterium tuberculosis* (MTB) , a small, aerobic, nonmotile bacillus (Bennett *et al*. , 2019). The high lipid content of this pathogen accounts for many of its unique clinical characteristics (Southwick, 2007). Mycobacteria have an outer membrane lipid bilayer (Niederweis *et al.,*2010). If a Gram stain is performed, MTB either stains very weakly «Gram-positive» or does not retain dye as a result of the high lipid and mycolic acid content of its cell wall (Madison, 2001).

The bacterium, Mycobacterium tuberculosis (MTB), is the main cause of TB. It is a small, slow growing, non-motile aerobic bacilli with peculiar cell wall structure that plays an essential role in virulence. It possesses an outer membrane functionally similar to what seen in gram negative bacteria, consisting in a asymmetric lipid bilayer made of long fatty acids (mycolic acids) and glycolipids and waxy components on the outer layer, that provides an exceptionally strong impermeable barrier to noxious compounds and drugs (Delogu G. *et al*., 2013).

Although TB is infectious, it doesn't spread easily. The infection occurs when few tubercle bacilli dispersed in the air from a patient with active pulmonary TB reach the alveoli of the host. It starts actively replicate, diffuse to nearby cells including epithelial and endothelial cells, reaching in few weeks of exponential growth a high bacterial burden.

Typical symptoms of active TB are chronic cough with blood-containing mucus, fever, night sweats,

and weight loss (World Health Organization, 2020) . TB infection begins when the mycobacteria reach the alveolar air sacs of the lungs, where they invade and replicate within endosomes of alveolar macrophages (Houben *et al*.,2006 ; Queval, *et al*.,2017). The primary site of infection in the lungs, known as the Ghon focus, is generally located in either the upper part of the lower lobe, or the lower part of the upper lobe(Kumar &Robbins ,2007). Tuberculosis of the lungs may also occur via infection from the blood stream. This is known as a Simon focus and is typically found in the top of the lung (Khan, 2011).

A definitive diagnosis of TB is made by identifying *M. tuberculosis* in a clinical sample (e.g., sputum, pus, or a tissue biopsy). However, the difficult culture process for this slow-growing organism can take two to six weeks for blood or sputum culture (Caulfield & Wengenack,2016).

Based on the facts indicated above, the current study conduct a retrospective analysis to evaluate TB cases discovered in Al-Rifai district between 1-1-2021 and 31-12-2021.

Methodology

A total of 300 TB patients, consisting of 149 males and 151 females, were involved in this study. Data for patients involved in the study, including name, age, gender, severity, and season, were collected from the Consulting Clinic for Chest Diseases in Al-Rifai Health Sector. The patients were subjected to Sputum examination by acid-fast staining. Acid-fast staining technique is the Ziehl–Neelsen stain which dye acid-fast bacilli a bright red that stands out against a blue background (Bayot et al. ,2019). Sputum examination by acid-fast staining is a diagnostic aid for tuberculosis. The test looks for the presence of AFB bacteria in sputum, which is a thick mucus coughed up from the lungs. In this test, the sample is "smeared" on a glass slide and looked at under a microscope. If an individual has pulmonary tuberculosis, and if the tubercles in the lungs are open, the bacteria (Mycobacterium tuberculosis) will be present in the sputum (Tansuphaisiri U, 2002). The procedure of the examination as follows:

1. Collecting the sputum sample in a sterile container.

2. Mixing the sputum with an equal amount of 2% sodium hydroxide (NaOH) solution.
3. Incubating the mixture at room temperature for 15 minutes.
4. Centrifuging the mixture at 3000 rpm for 15 minutes.
5. Discarding the supernatant and resuspend the sediment in 1 ml of phosphate buffer saline (PBS).
6. Transferring a drop of the sediment onto a clean glass slide and spread it evenly.
7. Air-drying the slide and heat-fix it by passing it through a flame three times.
8. Flooding the slide with carbol fuchsin stain and heat it gently until steam appears.
9. Rinsing the slide with water and decolorize it with acid-alcohol solution.
10. Counterstain with methylene blue for 30 seconds.
11. Rinsing the slide with water and blot it dry (Shen, F., & Sergi, C. , 2020).

Statistical analysis

Two software programs were used: The Statistical Package for Social Sciences (IBM® SPSS® V. 20) and Microsoft Office Excel 2010. The discrete nominal variables were expressed as number and percentage. Statistical analyses and reporting o obtained data were carried out by using the computerized database structure. One way analysis of variance (ANOVA) was used for more than three or more groups and $P < 0.05$ was the accepted statistical significance.

RESULTS

During the study period, a total of 300 clinically suspected patients were subject to sputum exams at Consulting Clinic for Chest Diseases in Al-Rifai Health Sector. The number of females and males was approximately equal, which recorded 151(50.33 %) and 149 (49.66%), respectively (Table 1).

The results of the acid-fast staining of the sputum showed that 14 patients were positive to pulmonary tuberculosis. The prevalence rate of the disease was equal between males and females, that recorded 7 for each. There is no significant difference between males and females. Age-wise analysis of the data shows a high number of cases (5) were recorded in the age group (20-29) and group (50-59) years. The age

group (40-49) shows (3) cases, whereas the lowest cases were observed in people aged (60-69) years old was (1) case. As for the age (30-39), there were no infections (Figure 1).

The severity of tuberculosis distributed between severe (+++) was present in one male case, whereas common (++) AFB smear results were seen in two females and one male. Finally, 5 males and 5 females had less severity TB (+).

Table (2) demonstrates the pattern of prevalence of pulmonary TB across one study year. Moreover, the prevalence of pulmonary TB was observed among the different year seasons. Winter had the highest prevalence (five cases) whereas the lowest in Autumn (two case) compared to different year seasons.

Table 1: Descriptive statistics of study population

The total number of cases (300)		P- value
Male (%)	Female (%)	0.002
149 (49.66%)	151 (50.33%)	0.002

Significant differences at $p \leq 0.05$ between male and female

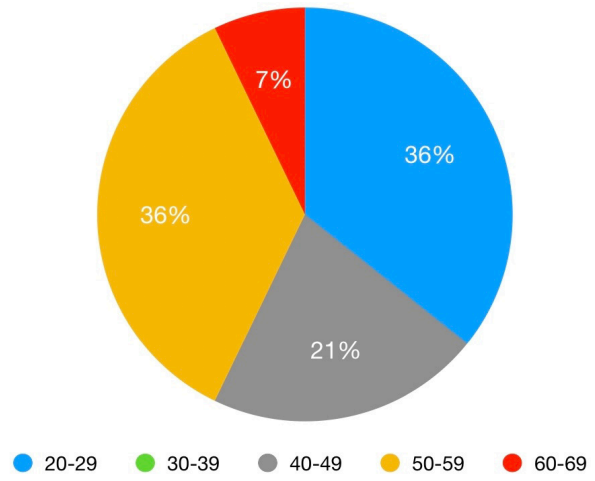

Figure 1: Distribution of Positive samples according to age

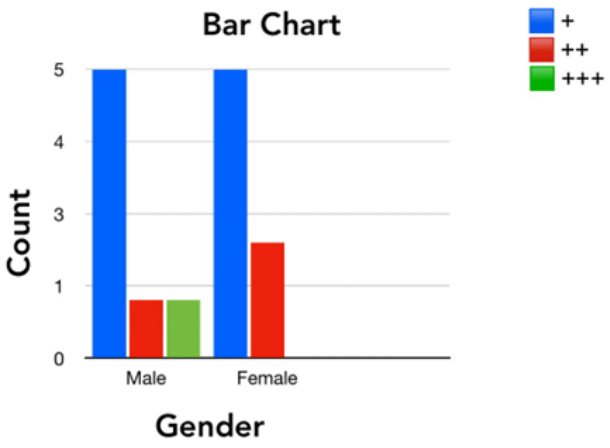

Figure 2: Distribution of cases according to the severity of the infection and sex.

Table 4: Distribution of cases according to seasons.

The part	Frequency	Male	Female	p-value
Winter	5	2	3	0.000
Spring	4	2	2	0.000
Summer	3	2	1	0.000
Autumn	2	1	1	Non-significance

The Significance for the relationship Gender and Season at p-value < 0.05

DISCUSSION

The number of female TB patients was slightly larger than the male patients. There is no significant difference between male and females in TB infection. These results are greatly watching with Beaky and his colleagues, who studied TB in Al-AL Sulaimaniyah and found no significant difference between male and females (Balaky et al.,2019). While This finding is inconsistent with the global sex distribution of TB reported by WHO which showed, a male/female ratio of 1.8 male cases of TB disease for every female case (World Health Organization, 2008). In the study of Durib and Blinova, (2020) that conducted in Baghdad, they concluded that the number of female patients is slightly higher than that of males. According to WHO study about TB in 7 countries of the eastern Mediterranean region, a little predominance of female patients was reported from Pakistan and Iran (World Health Organization,2006). The role of sex hormones such as testosterone and estrogen in immune protection and the pattern of high male TB mortality in patients over 40 years old remained unexplained (Blondiaux *et al.,*2015). Concerning the age distribution of the patients, the current study showed that most of the age groups (20-29 and 50-59) were susceptible to infection with TB, but the most predominant age group was more than 40 years. These results are matching with what was shown by other studies, which showed that TB as a disease of older people, or of the immunocompromised people (WHO, 2006; Butcher et al.,2001; CDC,2015). Balaky *et al*. (2019) showed that (73.6 %) of Erbil TB patients are between

the ages of 18 and 64 years Kareem, & Kadhim (2017) showed that 40% of AL-Diwaniyah TB patients are from the age groups of 55 years.

The most predominant age group was more than 40 years which was matched with that reported by other studies that showed TB as a disease of older people, or of the immunocompromised people (WHO, 2006; Butcher et al.,2001). However, in countries where TB has gone from high to low incidence, such as the United States, TB is mainly a disease of older people, or of the immunocompromised (Kumar *et al*.,2007). The aging has a negative effect on human immunity, resulting in increased susceptibility to bacterial infection (Butcher et al.,2001)

Tuberculosis is a seasonal disease, and its peak occur in spring and summer (Douglas *et al*., 1996; Martineau *et al*.,2011; Parrinello *et al*.,2012; Korthals *et al*., 2012). Kuddus *et al.* (2019) observed a relationship between weather fluctuations and tuberculosis infection. Low temperature, low humidity and low rainfall increase tuberculosis rates.

In winter, the crowding and lack the exposure to sunlight that reduce the level of vitamin D, may lead to increase transmission of TB infections. There is a substantial association between vitamin D deficiency and TB prevalence (Nnoaham & Clarke, 2008). It is required for gamma interferon-mediated macrophage responses, which play a critical role in the host response to M. tuberculosis infection (Fabri et al,2011), and it effect on the synthesis of antimicrobial peptides such as cathelicidin and defensins (Liuet et al., 2008; Wang, et al.,2014).

During the previous two decades, Iraq has experienced dramatic changes, after 13 years of tough economic sanctions that left their imprint on the health system (Daponte& Garfield, 2000; Ahmed, 2013), followed by 10 years marked by many efforts to recover from years of war and sanctions. During those two decades, TB detection rates fluctuated between growing and falling. In 2011, a total of 9,248 cases of TB were recorded, placing Iraq the eighth among Eastern Mediterranean Region Organization (EMRO) nations, with an incidence of 45/100,000 people and a prevalence of 74/100,000 population (WHO /EMRO / NTP Iraq , 2011), however, looking at the TB lev-

els during the last two decades, one can note that, during the years under penalties, the reported detection rates were ‘‘unprecedented’’, with high detection rates for several times above the estimated incidence in many years (United Nations,2011; Ahmed,2013).

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