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ORIGINAL ARTICLE

Comparison of Ziehl-Neelsen Microscopy Technique and Genexpert in the Diagnosis of Tuberculosis in Sudanese Patients

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ABSTRACT

Aim: The quality of tuberculosis diagnosis service is indispensable for early infection control and preventing unnecessary anti-tubercular therapy use. TB diagnosis in Sudan mainly relies on detecting tubercule bacilli using the Ziehl-Neelsen (ZN) staining technique. Thus, the current study aimed to compare the ZN microscopy with GeneXpert in diagnosing pulmonary tuberculosis (PTB) in TB laboratories center in Khartoum state, Sudan.

Methods: A cross-sectional study was carried out at the TB laboratories diagnosis center in Khartoum state from April 2019 to January 2020. A total of 183 sputum samples were processed and examined by the Ziel-Neelsen (ZN) technique and GeneXpert assay for the detection of tubercle bacilli.

Results: Out of 183 sputum samples analyzed, 80.9% were AFB smear-positive, of which 65.6% were male patients. Most patients (57.4%) were within the age group of 20 – 39 years old. All smear-positive samples were detected positive for Mycobacterium tuberculosis by GeneXpert. However, one case of smear-positive PTB was given a negative result by GeneXpert. These results demonstrate that GeneXpert is crucial for confirming TB cases to control TB infection and avoid unnecessary anti-tubercular drug use.

Conclusion: ZN microscopy can result in false positivity, which may lead to the misuse of anti-tuberculosis drugs. A positive test should also be confirmed by the GeneXpert and culture techniques.

Keywords: Mycobacterium tuberculosis, Tuberculosis, GeneXpert, Ziel-Neelsen stain

INTRODUCTION

Tuberculosis (TB) is a chronic, highly infectious disease, and from ancient times till today, it has remained captain of death worldwide (1). In 2020, an estimated 10 million TB cases and roughly 1.5 million lives were lost from TB globally, including 214000 HIV-infected patients (2). Emerging a variety of drug-resistant Mycobacterium tuberculosis strains, such as mono, multidrug, pre-extensive, and extensive-drug-resistance phenotypes, threaten and weaken the global efforts to control and eradicate the disease (3). It has been reported that a third of the estimated burden in 2020 was enrolled in MDR treatment regimens, which spotlight the gap in early and accurate diagnosis of cases (3).

Sudan is a politically unstable African country that suffered from mismanagement of resources for several decades and is still excruciating from poverty (4) and limited healthcare infrastructure and disease prevalence data which is a vital necessity to inform better introduce interceptive measures (5). In Sudan, there were 28,000 new TB cases in 2020, and that includes 160 confirmed cases of mono- and multi-drug-resistant (6). Culture methods remain the gold standard for diagnosing TB and determining the susceptibility of isolated bacilli to anti-tubercular drugs (7). Mycobacterium tuberculosis is a slow-grower bacteria, and the culture process takes roughly 6 – 8 weeks for the final interpretation to be recorded (8), which can result in a delay in starting treatment regimens. In addition, culture needs well-equipped specialized laboratories and well-trained and skilled cadres. Ziehl-Neelsen's microscopic technique is commonly used as a standard method for the rapid diagnosis of TB, particularly in areas with limited resources. However, it has low sensitivity and cannot detect paucibacillary TB (8, 9). GeneXpert assay has been introduced recently for hasting and improving TB diagnosis and detecting drug-resistant strains within less than 120 minutes (10). Therefore, the current study aimed to compare the Ziel-Neelsen technique and GeneXpert test in diagnosing TB among Sudanese patients.

METHODS

A cross-sectional study was carried out at the TB laboratories diagnosis center in Khartoum state from April 2019 to January 2020. A total of 183 sputum specimens were collected from patients with specific signs and symptoms of pulmonary TB. A specimen without clinical information, improper samples such as saliva, or collected from a patient with lung cancer were excluded.

The sputum samples were processed and examined by Ziel-Neelsen (ZN) technique for acid-fast bacilli (AFB) (11). Next, positive and negative specimens were reanalyzed by GeneXpert methods according to WHO instructions [11] as follows: test cartilage containing master Mix plus DNA extraction reagent and fluorochrome probes was warmed up and labeled with sample ID. Next, 2ml of the specimen was pipetted into the cartilage and loaded into the GeneXpert machine, and the whole process till obtaining the result was fully automated.

The data gathered from the current study were statistically analyzed by SPSS version 26.0.

RESULTS

Of total sputum samples, 120 (65.6%) were collected from male patients, and 63 (34.4 %) were gathered from female patients with pulmonary TB (PTB) infection (Figure 1). The mean age of the patients enrolled was 40 ±20 years (Figure 2). The highest number of cases were 105 (57.4%) detected among those aged 20 – 39 years, while only 11 (6%) cases were observed in people older than 60.

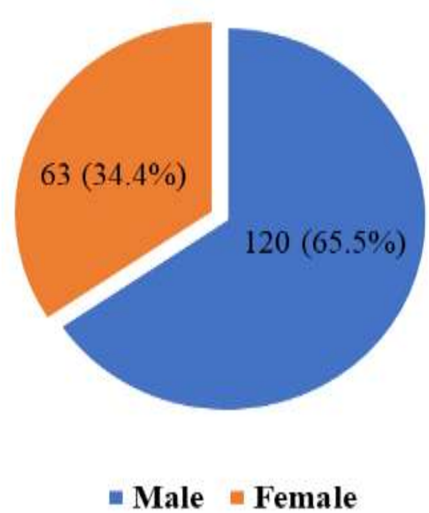

Figure 1: Sex distribution of cases with tuberculosis (n = 183).

There were 15 (8.2%) HIV-positive patients with PTB (Figure 3). Of the 183 sputum samples, 148 (80.9%) showed a positive smear for AFB using the ZN staining method (Figure 4). Further, gender-wise distribution presented that 102 (69.4%) were male and 46 (31.1%) were female patients (Figure 5). GeneXpert analysis of the 180 specimens showed confirmation of 147 (80.3%) cases (Figure 6). Out of 147 positive TB samples by GeneXpert, 102 (69.4%) were isolated from male patients, and 45 (30.6%) were female patients. Taken together, one sample detected positive for TB by ZN stain but showed a negative result on GeneXpert.

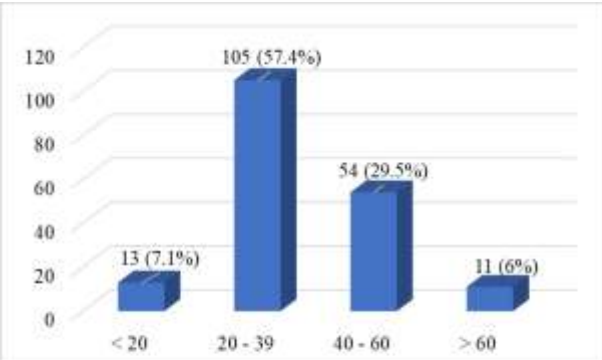

Figure 2: Age distribution of patients in years with tuberculosis (n = 183).

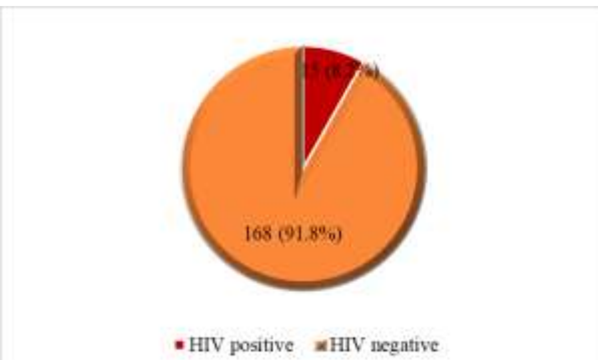

Figure 3: HIV- infected patients with tuberculosis.

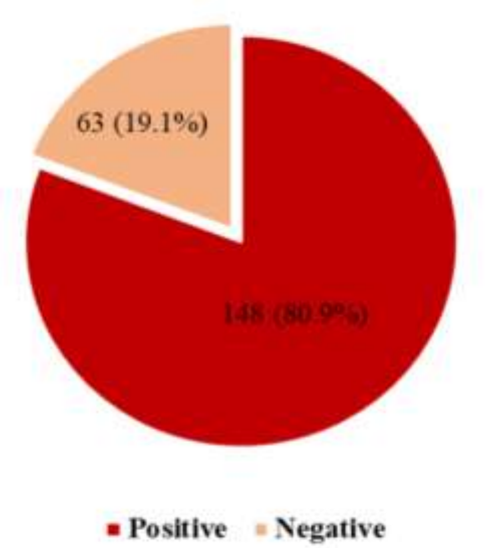

Figure 4: ZN stain positivity of tuberculosis cases (n = 183).

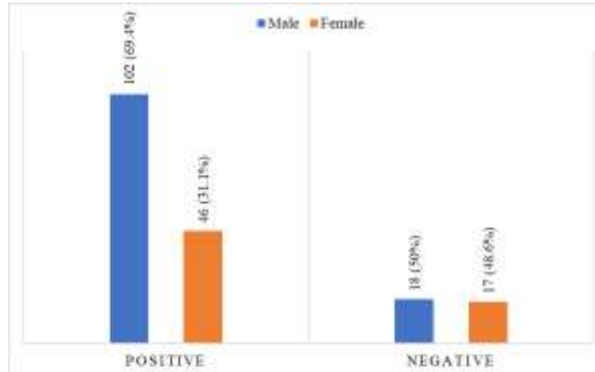

Figure 5: Gender-wise ZN stain positivity of tuberculosis cases (n = 183).

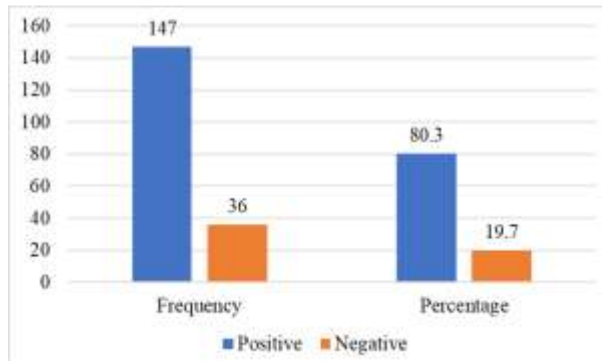

Figure 6: GeneXpert positivity of tuberculosis cases (n = 183).

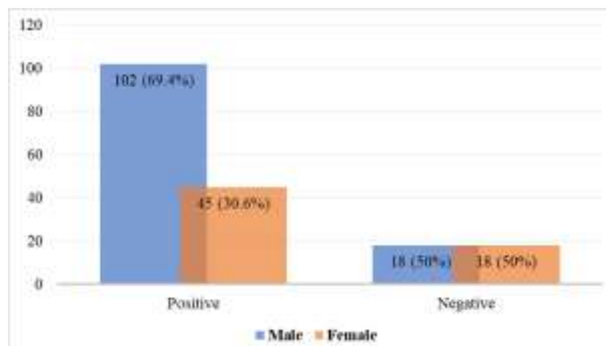

Figure 7: Gender-wise GeneXpert positivity in tuberculosis cases (n = 183).

DISCUSSION

TB remains the leading cause of morbidity and mortality globally, with approximately 10 million TB cases reported annually (12). The high incidence of TB occurs in low-income and below-poverty-line countries as TB is considered a disease of poverty (13). In addition, TB has serious socioeconomic effects, prevalent in males more than females (14) and among the age group 20 – 40 years old (15, 16). Similar findings were observed in the current study, as 65.6% of patients were male, and 57.4% of cases were among the age group between 20 – 39 years old.

Human immunodeficiency virus infection (HIV) and related AIDs complications remain public health obstacles to eradicating TB (17, 18). HIV can cause abrogation of immune responses, leading to increased incidence of active TB and increased possibility of reactivation of latent TB or reinfection (19-21). The present study found that the incidence of TB cases among HIV patients was 8.2%, in accordance with the global prevalence of TB among HIV patients (22).

Case identification and accurate diagnosis of TB are key strategies for ending the spread of TB disease through early enrolment of patients in treatment regimens (23). An acid-fast bacilli (AFB) "ZN technique" sputum smear microscopy remains the most commonly utilized technique in low-income countries for the rapid diagnosis of PTB, particularly in peripheral areas (24). Even though WHO recommended Xpert MTB/RIF assay as the initial test for accurate and rapid diagnosis of TB (25). In the current study, ZN smear microscopy detected AFB in 148 patients, whereas, GeneXpert detected 147 positive sputum samples. One sample detected positive for the AFB by ZN smear, but it was shown negative by GeneXpert. It has been reported that ZN smear microscopy has less accuracy and provides false results compared with GeneXpert and culture (26, 27).

CONCLUSION

ZN smear microscopy is the cornerstone for diagnosing tuberculosis in low-income countries, including Sudan; however, test results could contain many false negative and positive findings. One of the smear-positive samples was detected negative for Mycobacterium tuberculosis by GeneXpert. Therefore, misdiagnosis of TB may have serious consequences, such as unnecessary administration, which may cause harmful side effects or delay of anti-tubercular drugs that may lead to extended TB transmission and increasing mortality rates.

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