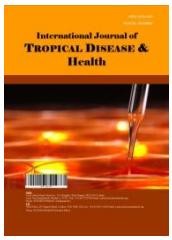

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Detection of Pulmonary Tuberculosis by Gene Xpert Method among HIV Patient in ART Center, Gadarif State, Sudan

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background: Tuberculosis (TB) is one of the most common and deadly infectious diseases in humans. Due to widespread poverty, inequity, and conflict, suboptimal health services in many countries, and the impact of the HIV/AIDS pandemic, TB/HIV co-infected patients frequently test negative for TB with direct microscopy, posing diagnostic challenges. Traditional HIV-associated TB diagnosis is complex, expensive, time-consuming, and technically demanding because it relies on conventional culture and drug susceptibility testing. The lengthy wait for results has disastrous consequences for patients who go undiagnosed or are diagnosed too late.
Objective: To detect the accuracy of gene X pert® MTB/RIF as a novel automated molecular tool to diagnose Mycobacterium tuberculosis in HIV patients.

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Methods: A cross sectional study was conducted on 70 HIV patients suspected to have *Mycobacterium tuberculosis* and who were referred to the ART and Tuberculosis Centers at Gadarif state, from June 2022 to December 2022. Sputum samples were tested by two methods involved the Gene X pert (Cepheid, California, United States of America) and direct smear microscopy (SM) stained with ZN stain for detection of acid fast bacilli. Out of 70 sputum samples analyzed for acid fast bacilli, 50(71%) cases were positive using GeneXpert whereas the DM was positive only for 28 (4o %) of the cases. GeneXpert detected another 22 AFB positive cases. Therefore GeneXpert is more accurate. Sensitivity of the GeneXpert was calculated as 93% and the specificity was 43%. The majority of the patients who contributed to (SM) were found to be co-infected with HIV.

Conclusion: GeneXpert demonstrated two-time case detection rate compared to the sputum smear microscopy so the direct smear showed poor performance tool for evaluation of TB-HIV co-infection. Also The Gene X pert's sensitivity was found to be high while the specificity was low. Although this was the case, the GeneXpert as compared to DM could greatly reduce false negatives and the wait on treatment initiation can be significantly shortened, minimising premature death and continued transmission.

Keywords: Pneumonia; mycobacterium tuberculosis; gene xpert; ZN stain; Sudan.

1. INTRODUCTION

"Tuberculosis (TB) is one of the most common and serious of all human infectious diseases. Regarding to widespread poverty, inequity and conflict, lack of health services in many countries and the impact of HIV/AIDS pandemic, the number of cases of TB today is greater than at any time in human history before. Estimated to 95% of all cases, and 98% of deaths due to TB, occur in tropical countries" [1]. "TB is the most frequent life-threatening opportunistic infection and a leading cause of death among PLHIV. HIV-positive people with latent TB infection have a 10% annual and 50% lifetime risk of developing active TB disease" [2]. "From the 1980s, infection by the human immunodeficiency virus (HIV) has lighted the concern with tuberculosis (TB)" [3]. "In 2015, according to the World Health Organization (WHO), 10.4 million people developed TB; 1.2 million corresponded to people with HIV/AIDS" [4]. "Before 35 years, HIV control strategies were established on passive detection of acid fast bacilli by investigating sputum smear microscopically in patients who were suffering from chronic cough. The clinical presentations of pulmonary TB in HIV patients differs in last stages with less frequent coughing and negative sputum smears" [5]. "Previously, Mycobacterium tuberculosis (MTB) culture has been the criterion standard for diagnosing TB, but this is a slow technique, it needs 2–6 weeks to grow, and is limited by the bacterial amount of the specimen" [6]. "In spite this microbiological diagnostic technique has advantages in terms of cheapness and simplicity, specificity and sensitivity are still considered precarious, especially among HIV patients" [7,8]. "In 2007, the WHO started to improve the diagnosis and management of smear-negative tuberculosis in HIV prevalent and resource constrained settings. The implementation required individuals with presumptive TB to be initially evaluated using two sputum microscopy examinations followed by clinical diagnosis such as chest X-rays in smear-negative individuals" [9] . "Since that time, there has also been a lot of advancement in technology to help with TB diagnosis such as the Gene X pert which uses Nucleic Acid Amplification Techniques (NAAT) to identify M. tuberculosis DNA and resistance to rifampicin. In December 2010, WHO endorsed the Gene X pert MTB/RIF for use in TB endemic countries and declared it a major milestone for global TB diagnosis" [10]. "However, during that period it was unclear how the assay performed as compared to the WHO 2007 algorithm in the diagnosis of smear negative pulmonary tuberculosis (SN-PTB)" [9]. "WHO's 2013 policy recommendations emphasized that after evaluating the GeneXpert MTB/RIF technology, it should be used rather than conventional microscopy and culture as the initial diagnostic test in adults suspected of multi drug resistant TB or HIV associated TB" [11] The high HIV prevalence rate in Gadarif makes effective TB diagnosis a priority in HIV positive patients to increase case detection and improve treatment outcomes. This study sought to evaluate the effectiveness of Gene Xpert technology against conventional diagnostic techniques used in Gadarif state Sudan [12].

2. METHODS

2.1 Study Design and Setting

This is a cross-sectional laboratory base study was conducted in the period from June2022 to December 2022 in Gadarif Tuberculosis and ART Centers. Most admitted patients came from the different localities in Gadarif State, Eastern of Sudan.

2.2 Study Area

This is a cross sectional laboratory base study, was conducted from june2022 to December 2022 at Gadarif ART and Tuberculosis Centers in Gadarif state, Eastern of Sudan neighboring Ethiopia and Eritrea countries

2.3 Study Population

The study population from different ages and sex enrolled in this research those who were attending to, Gadarif ART and Gadarif Tuberculosis centers suffering from HIV associated with Tuberculosis.

Inclusion criteria: Any HIV patients associated with tuberculosis symptoms.

Exclusion criteria: Any HIV patient who was not suffering from tuberculosis

2.4 Data Collection and Sampling

Socio-demographic characteristics of patients were collected using a questionnaire form through face-to-face interview with the patients. A total of 70 sputum sample were collected for detection of Acid fast bacilli by smear microscope method and gene X pert Technology.

2.5 Laboratory Procedure

2.5.1 Sampling

Participants were constructed to collect early morning sputum into a clean, sterile, leak proof, wide mouth containers. Collected specimens were sent immediately to the Medical Laboratory of Gadarif Tuberculosis Center, Ministry of Health, Gadarif State. Bio-safety considerations were followed for sputum processing including wearing of personal protective equipments and sodium hydroxide (NaOH) decontamination. From each sample; two ml were taken for gene Xpert analysis, then smear for ZN staining without concentration was prepared.

Sample size: Sample size determination by calculation of Yamane formula:

n=N1+Ne2
Where:
n=sample size.
N= population size=250.
e=error (0.1) reliability level 90% .
n=2501+250(0.1)2 = 71 or 70 participation

2.6 Smear Microscopy

The most common way for diagnosing TB worldwide is through sputum smear microscopy/DM using the fluorescence microscope (Auramine) or the Ziehl-Neelsen method (gold standard). However this method is susceptible to human error and other factors beyond control that can result in false negatives.

2.7 Procedure of ZN Stain

Smears were fixed over the glass slide by heating. Carbol fuchsine was poured over smear and heated gently until appearing of fumes. After standing for 5 minutes, water washing was done. Thin-staining was accomplished using 20% sulphuric acid. After water washing methylene blue was added for two minutes. Finally dried smears were examined under oil immersion lens. Degree of positivity was determined by 1 to 9 acid fast bacilli (AFB) per 100 high power field, 1 + (10 - 99 AFB/100 field), 2+ (1 - 10 AFB/ 50 field) and 3 + (more than 10 AFB/20 field) according to the WHO recommendations [13].

2.8 Gene X pert Assay

The Gene X pert (X pert MTB/RIF) is a cartridge based automated diagnostic test that can identify Mycobacterium tuberculosis (MTB) DNA and resistance to rifampicin (RIF) by nucleic acid amplification technique (NAAT). The X pert MTB/RIF assay consists of two main components: 1) the X pert MTB/RIF plastic cartridge, which contains liquid sample processing and PCR buffers and lyophilized real-time reagents 2) the Gene X pert instruments which controls intra-cartridge fluids and performs real-time PCR analysis.

2.9 Gene X pert Assay Procedure

“A sample reagent was added to the sputum specimen in a 2:1 ratio. The mixture was incubated at room temperature for 10 minutes and was manually agitated. A total of 1 ml of

sample was introduced into cartridge, which was then loaded into the Gene X pert instrument, where the subsequent steps of sample lysis, nucleic acid extraction, and amplification occurred automatically with results in1 hour and 52 minutes .5 Lastly the results were read and interpreted according the load of bacilli and rifampicin resistance gene detection" [14].

2.10 Data analysis

The information related to patients and research results were first entered in Microsoft Excel and later analyzed using SPSS version 20 computer program. The significance of the association between variables was measured by Chi-square test, and a *p value* of less than 0.05 was considered significant.

3. RESULTS

A total of 70 sputum specimens from HIV patient suspected with tuberculosis in ART and pulmonary tuberculosis centers subjects were included, most of patients came from Gadarif State. Socio-demographic characteristics of enrolled patients according to their gender were males more infected than females, 36/70 (51%) and females 34/70 (49%) respectively as indicated in Table 1. In Table 2 showed their age, 13% less than 20 years, 40% were from 20 to 29 years, 20% were from 30 to39 years, 16% were from 40 to 49 years and 11% were more than 50 years. The detection of positive acid fast bacilli by direct microscope smear (ZN) stains yielded 28/70 (40%). Out of 70 sputum samples analyzed for TB, 50(71%) cases were positive using GeneXpert whereas the DM was positive only for 28 (4o %) of the cases as pointed in Table 3. The relationship between gene X pert result levels and direct microscope smear (ZN) stains without centrifugation showed a significant different between them (*P.v.* = 0.05) as indicated in Table 4. The sensitivity of 93%, specificity 43%. Positive predictive value (50%), Negative predictive value (90%) indicated in Table 5, Gene X pert demonstrated twice case detection rate versus the sputum smear microscopy.

Table 1. Distribution of participation according to their gender (n=70)

Gender	Frequencies	Percentage %
Male	36	51
Female	34	49
Total	**70**	**100**

Table 2. Distribution of participation according to their age group (n=70)

Age range	Frequencies	Percentage %
Less than 20 years	9	13
20-29	28	40
30-39	14	20
40-49	11	16
More than 50 years	8	11
Total	**70**	**100**

Table 3. Comparison of positive TB case between direct smear microscope and gene X pert

	TB/ Gene X pert		TB/SM	
	Frequencies	Percentage %	Frequencies	Percentage %
Positive	50	71	28	40
Negative	20	29	42	60

Table 4. The comparison of the Gene X pert and SM microscopy

		SM		Total	p-value
		+Ve	-Ve		
Gene X pert	+Ve	26	24	50	
	-Ve	2	18	20	0.001*
Total		**28**	**42**	**70**	

Table 5. Comparison of gene X pert to Direct smears method

Sensitivity = (26/28)*100 = 93%	**Specificity** = (18/42)*100 = 43%
Positive predictive value = (26/50)*100 = 50%	**Negative predictive value** = (18/20)*100 = 90%

4. DISCUSSION

"The HIV epidemic has led to great increase in the frequency of smear negative pulmonary tuberculosis which has negative prognosis and increased early mortality versus to smear positive disease. HIV-positive individuals have a greater rate of smear-negative disease because they are less likely to have cavity lesions due to the impairment of granuloma formation. Sensitivity of sputum microscopy in HIV ranges from 43% to 51%" [15]. "About 24% to 61% of HIV and TB co-infected patients their smears were negative. 30-60% of people with HIV infection may die with tuberculosis often undiagnosed" [16].

Our study focused on the importance of Gene X pert in TB patients notification under the program of WHO intensified TB case finding among PLHIV. "Gene X per is a relatively a modern diagnostic fashion in the battle to combat TB as a world public health problem, and is emphasized that it is to promote bacteriology confirmed TB cases with shorter turnaround time as shown in past researches" [17,18].

In our result finding, Out of 70 sputum samples analyzed for TB, 50(71%) cases were positive using GeneXpert whereas the DM was positive only for 28 (4o %) of the cases as pointed in Table 3. The difference in TB diagnosis between the two methods was statistically significant.

"All patients who turned positive for smear AFB were also positive for GeneXpert, confirming that it can substitute the conventional smear AFB microscopy in the clinical care of TB" [19-21]. GeneXpert detected another 22 AFB positive cases. And this is typical to the finding of the study from north-western part of Ethiopia and other multicenter studies [17,18,22,21]. Also congruent with study by Abebe et al. [23]. However, the study finding reported by Habte et al indicated "additional TB case detection of GeneXpert among smear AFB negative cases was 64.3%" [13], which was higher than our finding while the report from Cochrane review and meta-analysis by Steingart and his collogues, and studies from other areas were in the range of quarter which were lower than our results [19,14,24–28].

Table 5 shows that the GeneXpert had a sensitivity of 93% and the specificity of 43%, respectively when compared to the Ziehl-Neelson method. Other studies have reported high specificity and sensitivity of the GeneXpert method as in comparison to the results of this current study. A study conducted by WHO (2011) indicated "the GeneXpert sensitivity of 80% and the specificity of more than 80% in patients with smear negative PTB" [29]. "When the GeneXpert was compared to the TB culture gold standard the sensitivity was 67% while the specificity was 98%" [30]. "In a similar study the GeneXpert sensitivity was 95% and the specificity 33%" [31]. Since we employed the Ziehl-Neelsen method as the fundamental standard instead of another study's use of culture, our findings may not be comparable to those of other studies.

This study found that men had more HIV/TB Confections 36 (51.0%) than women 34(49%) as shown in Table 4. This is in corresponding with other studies involving a study by WHO [32,33]. These findings, however, are based solely on global statistics from developed countries. There is currently no definitive evidence of a gender disparity in the occurrence of TB and HIV co-infection in developing countries.

As shown in Table 2, the most affected age category with HIV/TB Co-infections was 20 to 29 years 28 (40.0%), followed by 30 to 39 years 14 (20%). The economically productive age groups are primarily insulted and thus impacting the society in terms of loss of economic productivity due to absenteeism, loss of potential tax revenue, lack of trained human experts, and expensive treatment costs. This is similar with findings of other studies [34,33]. "The age preponderance to HIV/TB Confections could also be due to the fact that these groups are sexually active, therefore encountering sexual partners in whom both TB and HIV are both prevalent" [33].

5. CONCLUSION

The direct smear showed poor performance method for detecting of TB-HIV co-infection. Even though this was the case, the Gene X pert

as compared to the direct microscopy could significantly decreases false negatives and the delay on treatment initiation can be significantly shortened, Also The Gene X pert's sensitivity was found to be high while the specificity was low; Gene X pert is likely to substantially promote the diagnostic confirmation of the Mycobacterium bacilli.

CONSENT

It is not applicable.

ETHICAL APPROVAL

This study was approved by the Ministry of Health, Gadarif State and by the IRB in Faculty of Medical Laboratory Sciences, University of Gezira, Sudan. A written informed consent was obtained from each participant before being enrolled in this study.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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