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Pulmonary Tuberculosis among Suspected Sudanese Patients in Wad Madani Tuberculosis Center

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Abstract

Background: Tuberculosis is a health problem in Sudan and may become a greater challenge in the future due to the weakness in infection prevention measures, increase in cases of drug-resistant and the difficulty of diagnosis. **Objective:** The aim of this study was to detect *Mycobacterium tuberculosis* (MTB) from sputum of clinically suspected patients using the three available techniques. **Methods:** Three hundred participants referred to Wad Madani Tuberculosis Center during 2017-2019 were included. Early morning fresh sputum samples were subjected to *Mycobacterium tuberculosis* examination by Ziehl-Neelsen (ZN) stain without concentration, ZN stain with centrifugation and geneXpert assay. **Results:** Of the 300 suspected cases; *Mycobacterium tuberculosis* detected in 17% (51/300) by ZN stain without concentration, 20% (59/300) by ZN stain with centrifugation and 34% (103/300) by geneXpert. The two techniques of ZN stains possessed 100% specificity and relative differences in sensitivity when compared to geneXpert assay. The significant association observed between ZN stains and geneXpert results indicated validity of ZN techniques for detection. **Conclusions:** The study confirmed that geneXpert is better for identification of *Mycobacterium tuberculosis* when compared to ZN techniques which are also important for diagnosis in poor places and where the geneXpert assay is not available.

Keywords

Mycobacterium Tuberculosis, GeneXpert, ZN Stain, Centrifugation, Sudan

1. Introduction

Tuberculosis (TB) is an infectious disease of lungs, abdomen and skin caused by *Mycobacterium tuberculosis*. Diagnosis of tuberculosis can be achieved using different approaches; direct smears from sputum for microscopy, culture-based testing, serology and nucleic acid amplification techniques (NAATs), in addition, chest radiography plays an important role in the diagnosis as well as disease prognosis and treatment follow-up [1] [2]. At last time, newly discovered diagnostic tools have been appeared, such as interferon-gamma release assays and geneXpert assay [3]. Successful elimination of TB needs selection of appropriate method for rabid and accurate detection, and this reduces mortality and transmission rates [4]. For several reasons, such as ease and low cost, ZN stain is used for diagnosis of tuberculosis in most developing countries where as culturing which has been considered as the golden standard method is not routinely used [1] [5] [6] [7] [8]. Major errors from ZN technique include false negativity and to a less degree false positivity [6]. In order to control the disease, it is important to use tests that increase the sensitivity of identification and help in the selection of treatment [9]. GeneXpert is a nucleic acid amplification technique that has been modified to specifically identify the species MTB and differentiate between the rpob resistance gene producers [4] [10] [11]. Globally, the test was endorsed by the WHO as the first choice for detection of drug-resistant tuberculosis [7] [12] [13], however, rpob resistance does not generalize to all types of resistance [14]. In Sudan TB considered as public health priority, through the national TB program, the Government has been striving to detect cases, give care to patients and involve other sectors in control efforts. GeneXpert assay has become an important part of tuberculosis diagnostic algorithms in many low- and middle-income countries [15] [16]. However, despite this effectiveness, smear for microscopy remains the primary diagnostic method for tuberculosis. In the context of negative tuberculosis detection, sensitivity and specificity of smear microscopy have been recorded to be 30% - 89% and 93% - 100% [17] [18], noted that, lower specificity has been observed in tuberculosis prevalence surveys. Cross positivity may be due presence of other acid fast bacilli like actinomycetes and nocardia [19]. In addition, laboratory errors such as analytic errors or sample mix-ups may lead to false positive smear microscopy results. Knowing that, smear microscopy enables to differentiate between *Mycobacterium tuberculosis* complex (MTBC) and non-tuberculous mycobacteria (NTM) [20]. This study was conducted to assess ZN stain, ZN stain with concentrated sputum and geneXpert assay for detection of MBT.

2. Methods

2.1. Study Design and Setting

A cross-sectional laboratory based was followed in the period from September 2017 to September 2018 in Wad Madani Tuberculosis Center. Most admitted patients came from Gezira State which is the biggest and well populated State in

Sudan.

2.2. Study Population

This study included all patients attending Wad Madani Tuberculosis Center during study period; suffer from chronic pulmonary symptoms and had tuberculosis suspected chest X-ray report. Patients under anti-tuberculous treatment, extra-pulmonary tuberculosis and known multi-drug resistance cases were excluded.

2.3. 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 Wad Madani Tuberculosis Center, Ministry of Health, Gezira State.

Sample size was calculated by the following formula:

$$n = \frac{Z^2 P(1-P)}{e^2}$$

n = sample size;

Z = confidence interval (=1.96);

P = prevalence of TB infection (=0.19);

e = marginal error (=0.05);

$$n = \frac{1.96^2 \times 0.19 \times (1-0.19)}{0.05^2} = 300 \text{ patients}$$

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 geneXpert analysis, then smear for ZN staining without concentration was prepared. The remnant of sputum subjected for smearing after concentration method.

2.4. Concentration of Sputum Samples

Suitable amount of sputum specimens were transferred into sterile centrifugal tubes with cotton stopper. Tubes were spun at 5000 round per minute (rpm) for 5 minutes. Then supernatant were discharged and sediments used for smear preparation.

2.5. Procedure of ZN Stain

Smears were fixed over the glass slide by heating. Carbol fuschin was poured over smear and heated gently until appearing of fumes. After standing for 5 minutes, water washing was one. De-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 [21].

2.6. GeneXpert Assay Procedure

To achieve liquefaction and deactivation of specimens; using screw-capped tube two ml of sample reagent were added to one ml of sputum. After shaking for 20 times tubes were incubated at room temperature for 15 min as the follows; 10 minutes firstly then additional 5 minutes. Two ml of liquefied sputum samples were loaded into MTB\RIF cartridges and inserted into the geneXpert chamber. Programmed machine was adjusted to finish after 1 hour and 52 minutes. Lastly the results were read and interpreted according the load of bacilli and rifampicin resistance gene detection [22].

2.7. Ethical Consideration

This study was approved by Ministry of Health, Gezira State and Faulty of Medical Laboratory Sciences, University of Gezira, Sudan.

2.8. Statistics

Data were analyzed by Statistical Package for Social Program (SPSS, V21.0. IBM Chicago). Chi-square used as significance test for the association at level of 0.05. Sensitivity and specificity were calculated according to geneXpert as standard diagnostic method:

$$\text{Sensitivity} = \frac{\text{True positive}}{\text{rue positive} + \text{False negative}} \times 100$$

$$\text{Specificity} = \frac{\text{True negative}}{\text{True negative} + \text{False positive}} \times 100$$

3. Results

A total of 300 sputum specimens from pulmonary tuberculosis suspected subjects were included, most of participants came from Gezira State. Socio-demographic and clinical characteristics of enrolled patients shown in (**Table 1**). Positive ZN staining without concentration for acid fast bacilli obtained from 17% (51/300) while centrifugation technique revealed positivity of 20% (59/300). Degree of ZN stain positivity for staining without concentration shown in (**Table 2**) and with concentration in (**Table 3**). Sensitivity of 57.28% and 49.51% recorded for ZN stains with and without centrifugation respectively (**Table 4**). *Mycobacterium tuberculosis* was detected in 34% (103/300) by geneXpert assay. The relationship between geneXpert result levels and ZN stain with and without centrifugation expressed a significant association (Chi square 0.000) at most levels while the very low level result recorded in 9 by geneXpert and at the same time showed negative by ZN stains, as shown in **Table 5**. The rpob gene was documented with frequency of 9.7% (10/103) (data not shown).

Table 1. Socio-demographics and clinical characteristics of study subject (No 300).

		Frequency	Percent
Sex	Male	183	61
	Female	117	39
Age groups/years	Less than 20	35	11.7
	21 - 40	130	43.3
	41 - 60	87	29
	More than 60	48	16
Residence	Rural	179	59.7
	Urban	121	40.3
	Total		
Occupation	House wife	69	23
	Farmers	60	20
	Workers	84	28
	Students	38	12.7
	Others	49	16.3
Fever	Yes	110	36.7
	No	190	63.3
Cough	Yes	300	100
	No	0	0
Loss of weight	Yes	200	66.6
	No	100	33.4
Night sweat	Yes	75	25
	No	225	75

Table 2. Results of sputum examination using ZN stain without centrifugation among tuberculosis suspected subject (N = 300).

ZN smear (without centrifugation)	N	%
AFB positive	51	17
1-9/100 field	0	0
1+	28	9.3
2+	14	4.7
3+	9	3.0
AFB negative	249	83.0

Table 3. Results of sputum examination using ZN stain with centrifugation among tuberculosis suspected subject (N = 300).

ZN smear (with centrifugation)	N	%
AFB positive	**59**	**19.7**
1-9/100 field	**0**	**0**
1+	20	6.7
2+	25	8.3
3+	14	4.7
AFB negative	**241**	**80.3**

Table 4. Sensitivity and specificity of ZN stains with and without centrifugation according to geneXpert among study subjects (No. 300).

	ZN Smear	
	Without centrifugation	**With centrifugation**
Sensitivity	49.51	57.28
Specificity	100	100

Table 5. The association of the levels of geneXpert results with ZN stains (with and without centrifugation) (No. 300).

	GeneXpert						Chi square
	Very Low	**Low**	**Medium**	**High**	**Negative**	**Total**	
ZN smear (without centrifugation)							
1+	0	2	20	6	0	28	0.000
	0.0%	7.2%	71.4%	21.4%	0.0%	100	
2+	0	1	10	3	0	14	
	0.0%	7.2%	71.4%	21.4%	0.0%	100	
3+	0	0	4	5	0	9	
	0.0%	0.0%	44.4%	55.6%	0.0%	100	
Negative	9	20	19	4	197	249	
	3.6%	8.0%	7.6%	1.6%	79.1%	100	
Total	9	23	53	18	197	300	
	3.0%	7.7%	17.7%	6%	65.6%	100	
ZN smear (with centrifugation)							
1+	0	2	15	3	0	20	0.000
	0.0%	10.0%	75.0%	15.0%	0.0%	100	
2+	0	1	15	9	0	25	
	0.0%	4.0%	60.0%	36.0%	0.0%	100	
3+	0	0	8	6	0	14	
	0.0%	0.0%	57.1%	42.9%	0.0%	100	
Negative	9	20	15	0	197	241	
	3.7%	8.3%	6.3%	0.0%	81.7%	100	
Total	9	23	53	18	197	300	
	3.0%	7.7%	17.7%	6%	65.6%		

4. Discussion

Low level of income in Sudan, increase in poverty and insecurity especially in the places of boarder disputes led to an increase in the spread of diseases such as tuberculosis, in addition to the poor quality and continuation of health care services provided to citizens. Despite the endemicity of tuberculosis in Sudan, information like the actual prevalence, diagnostic data and monitoring of drug-resistance situations are limited. Early diagnosis of tuberculosis cases achieves cure and reduces the areas of spread of drug resistant cases and thus, enables control [23].

In view of results of participants whose were positive in the tests used in the present study, the small proportions (17%, 20% and 34%) that showed positive results necessitates a re-evaluation of the criteria for expectation of pulmonary tuberculosis. From the results obtained ZN stain without concentration revealed lowest sensitivity for detecting MTB from sputum; false negative results may occur due to sputum contamination with saliva diluents, personnel competence and or poor quality of equipment and reagents used. Thus, in the current study 49.5% (51/103) of geneXpert positive cases were negatively detected by ZN stain without concentration.

The findings revealed sensitivity and specificity of ZN stain without centrifugation of 49% and 100% for detecting MBT, near results were concluded in France [24] and Pakistan [25]. ZN stain with centrifugation gave better sensitivity when compared to that without, thus false negative results were 42.7% (44/103). By looking to the given time required to complete centrifugation method, which is approximately 15 minutes, and simplicity of this technique, negative results of ZN satin without concentration should be repeated using a concentration method especially in case of no geneXpert availability. In line, other study recommended concentration approaches for examine pre-bronchoscope sputum in order to enhance sensitivity [26]. Since ZN stain without concentration can detect MTB amount of 5000 - 10,000 CFU/ sputum ml [27], centrifugation method actually detect less than 5000 CFU/ml.

So far, the geneXpert assay has the best results for rabid pulmonary tuberculosis diagnosis but, beside possibility of contamination during sample collection and processing the high infrastructures requirements remain major factors that affect diagnosis by this method [21]. Approximately two thirds of tuberculosis suspected participants gave negative results for sputum test, which proves the hypothesis of re-examination and follow-up, moreover, those patients could be checked to exclude other causes of non-infectious and infectious chronic pulmonary diseases such as aspergillosis, actinomycosis and nocardiosis [28] [29] [30].

5. Conclusion

As conclusion, sputum concentration for suspected tuberculosis cases is strongly recommended to increase detection sensitivity when direct smears negatively interpret.

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Limitation of Study

The study did not include follow-up of patients who showed negative results for TB by the three used tests, such as taking additional investigations; culture and lung biopsy, as well as investigations for other chronic respiratory infections.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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