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MULTIDRUG RESISTANCE PULMONARY TUBERCULOSIS PREVALENCE AND RISK FACTORS IDENTIFIED BY GENE XPERT RIFAMPICIN ASSAY IN THE RIVER NILE STATE-SUDAN

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ABSTRACT
The study carried out to find the prevalence of multidrug-resistance Mycobacterium tuberculosis (MDR-MTB) and associated risk factors associated with pulmonary tuberculosis (TB). This study conducted in a period from January 2018 to December 2019, in Atbara Diagnostic Central-Laboratory. Four hundred sputum specimens obtained from the patients referred from centers of different localities in the state using Gene Xpert rifampicin assay to identify the cases and to determine either sensitive or resistant TB case. Their ages ranged from 17.9 to 50.3 years, with a mean of 34.1±16.2 years. The male to female ratio was 3:1. The study revealed 25% (100/400) of the referred cases had TB, and MDR-MTB was encountered in 10% (10/100). The gender, age groups, social status, residence (rural or urban), and education were significant associated with MDR-MTB and pulmonary TB infection. Diabetes mellitus increased prevalence of MDR-TB ($P < 0.031$). The HIV co-infection was 1%. The study revealed the recurrence rate of TB was 59% (59/100), this rate was significantly high in relation to new TB cases (P. value = 0.000). The study revealed MDR-MTB was 10.9% (5/46) of new cases while 9.3% (5/54) had received previous treatment. The *MDR*- MTB and recurrence rates of pulmonary tuberculosis are high. This study highlights a significant relationship between MDR- MTB and some possible risk factors. The high recurrence rate was due to treatment failure, relapses or interrupted treatment. Drug resistance to tuberculosis and recurrence were serious public health problems in Sudan, and attention should direct to control and improve monitoring therapy for MDR-TB, especially in the new TB cases.

KEYWORDS: Gene Xpert RIF-assay, TB risk factor, MDR- MTB, Atbara.

INTRODUCTION
Tuberculosis care and treatment provided by the National Tuberculosis Control Program, which under the auspices of the Ministry of Health and by a number of Non-Governmental Organizations. The Tuberculosis Control Program in the River Nile State established in 1997. Treatment was also provided by the private sector.[1-2] The GeneXpert MTB/RIF assay was recommended by the WHO to detect pulmonary and extra pulmonary tuberculosis and resistance in adults and children.[3] In the first line of TB treatment rifampicin resistance occurs less frequently than resistance to isoniazid, and in many settings it was used as an indicator for the presence of MDR-MTB.[4] It is important to raise the issue of studying the prevalence of mono-resistance rifampicin in Sudan so to use RIF-resistance MTB assay as standardized accurate test for detection of MDR-MTB.[5] Resistance to both the key drugs rifampicin and isoniazid were termed multidrug-resistant tuberculosis. Patients with MDR-MTB were frequently fails to cure by the first line standard drug treatment and may remain infectious and a potential source of onward transmission.

Prevalence of MDR-TB in re-treatment patients in Ethiopia was (12%)[10], and for Kampala was (13%)[12], but reported 38% from Egypt.[11] However, the prevalence of MDR-TB, as reported by Sudan's National TB Program in 2012, was 19% in re-treated cases and 1.8% in new cases.[11] Hassan *et al.*, reported in north-eastern Sudan a MDR-TB prevalence of 6% among new TB cases.[13] Sharaf Eldin *et al.*, reported MDR-TB in Sudan was 5% in new cases and 24% in previously treated patients in (2011).[14] While Adam *et al.*, reported 6% for MDR-TB from North-eastern part of Sudan in new cases.[15] The human immunodeficiency virus (HIV) was a risk factor for TB infection. Sudan had a low HIV prevalence, which was reported to be 0.3% in (2015).[9] This Study aims to elaborate the prevalence of MDR-TB,

using RIF-resistant MTB assay, and the associate some risk factors to MDR MTB, which can be basic data for control and prevention measures of pulmonary TB.

MATERIALS AND METHODS

Study design and area

The study was a cross sectional laboratory-based, conducted in Atbara, Central Diagnostic Laboratory. Specimens collected from different TB program centers, distributed in different localities of the River Nile State. Moreover, the study design to determine TB infection and MDR-MTB, in addition to find the associations of some risk factors such as gender, age, social status, geographic residence, education level, occupation type, Diabetes mellitus and new TB or recurrence case in relation to TB and MDR- MTB.

Study population, duration and sampling

Suspected patients of pulmonary tuberculosis referred from different TB program centers of the localities in the River Nile State included in the study. Four hundred sputum specimens received for diagnosis of pulmonary TB, and MDR-TB. The specimens collected in the period from Jan to Dec 2018. Questionnaire was constructed for reporting patients information needed.

GeneXpert method principle

GeneXpert systems automate and integrate sample purification, nucleic acid amplification, and detection of the target sequence using Real-Time Reverse Transcriptase PCR (RT-PCR) and real-time PCR assay.

Each GeneXpert module processes one sample. The sample and applicable reagents inserted in to a GeneXpert cartridge and a test created on the Gene Xpert systems.[16]

Gene Xpert test steps

Sputum sample collected in container. (If delaying keep sample in refrigerator).

Add NaoH solvent in sputum sample in ratio of 2:1, and shake well. Sample incubated for 15 minutes, after that we took not less than 2 ml from the sample in cartridge then entered the cartridge in Gene Xpert machine. Move the sample and reagents into different chambers in the cartridge. For sample, preparation hydrates the reagent beads. Performs probe checks to ensure that the sample preparation is successful (only if the assay definition requires this step). Moves the sample and the reagent mixture which contains reverse transcription (if applicable) and real-time PCR specific components into the reaction tube. Starts the RT-PCR (if applicable), PCR cycles, and real-time detection. The GeneXpert Dx System uses the I-CORE module heating and fan cooling system to perform the real-time polymerase chain reaction used to exponentially amplify and detect the organism's DNA or cDNA sequence of interest.[17]

Ethical consideration

Written consents taken from patients and Ministry of Health in the River Nile State to conduct the study.

Data analysis

Data was analyzed using statistical package for Social Sciences Version 20 Software. Percentages and the Chi-square tests employed to compare the association of categorical variables. P-value of less than 0.05 considered as statistically significant.

RESULTS

Four hundred sputum samples obtained. The patient's ages ranged between17.9 to 50.3 years, and the mean age was 34.1± 16.2. GeneXPert MTB-RIF assay reveals100 out of 400 (25%) positive cases for B, and 10 out of 100 (10%) MDR-MTB. The TB recurrence in the study was 59 cases out of 100 patients. The reminder 41 patient's were new cases, who had not taken previous anti-tuberculosis therapy.

The recurrent TB patients in the study classified as interrupted treatment cases, which were 23 patients. Whose isolates tested positively having received less than one month of anti-tuberculosis chemotherapy, while 30 relapsed cases had previously completed a course of TB therapy, and six patients showed treatment failure due to irregular drug intake.

Table 1: Shows risk factors in relation to MDR and drug responder TB patients.

Risk factor	Drug responder (*N* = 90)	MDR (*N* = 10)	Total No - (%)	p. value
	No - (%)	No - (%)		
Sex				
Male	75 (100.0)	0 (0.0)	75 (100.0)	0.000
Female	15 (60.0)	10 (40.0)	25 (100.0)	
Total	**90 (90.0)**	**10 (10.0)**	**100 (100.0)**	
Age groups				
2-16 years	8 (100.0)	0 (0.0)	8 (100.0)	0.001
17-35 years	49 (100.0)	0 (0.0)	49 (100.0)	
> 35 years	33 (76.7)	10 (23.3)	43 (100.0)	
Total	**90 (90.0)**	**10 (10.0)**	**100 (100.0)**	
Social status				
Single	38 (100.0)	0 (0.0)	38 (100.0)	0.006
Married	52 (83.9)	10 (16.1)	62 (100.0)	
Total	**90 (90.0)**	**10 (10.0)**	**100 (100.0)**	
Residence				
Rural	67 (100.0)	0 (0.0)	67 (100.0)	0.000
Urban	23 (69.7)	10 (30.3)	33 (100.0)	
Total	**90 (90.0)**	**10 (10.0)**	**100 (100.0)**	

Table 2: Shows education status, occupation and diabetes mellitus in relation to MDR and drug responder TB patients.

Risk factor	Drug-responder (*N* = 90)	MDR (*N* = 10)	Total No - (%)	P. value
	No - (%)	No - (%)		
Education				
Illiterate	27 (100.0)	0 (0.0)	27 (100.0)	0.000
Under graduate	63 (100.0)	0 (0.0)	63 (100.0)	
Graduated	0 (0.0)	10 (100.0)	10 (100.0)	
Total	**90 (90.0)**	**10 (10.0)**	**100 (100.0)**	
Occupation				
Unemployed	30 (100.0)	0 (0.0)	30 (100.0)	0.000
Employed	16 (100.0)	0 (0.0)	16 (100.0)	
Free business	44 (95.7)	2 (4.3)	46 (100.0)	
Student	0 (0.0)	8 (100.0)	8 (100.0)	
Total	**90 (90.0)**	**10 (10.0)**	**100 (100.0)**	

Diabetes mellitus				
Yes	28 (100.0)	0 (0.0)	28 (100.0)	0.031
No	62 (86.1)	10 (13.9)	72 (100.0)	
Total	**90 (90.0)**	**10 (10.0)**	**100 (100.0)**	

Table 3: Shows distribution of TB patients according to new or recurrent status.

Patient	No	Percentage
New	41	41
Recurrence	59	59
Total	**100**	**100**

Table 4: Shows distribution of recurrence cases of TB in relation to the cause.

Cause	No	Percentage
Interrupted treatment	23	39
Relapse	30	50.8
Treatment failure	6	10.5
Total	**59**	**100**

Table 5: Shows MDR and drug responder cases distribution among patients.

TB Patient	No	Percentage
RIF-responder	90	90
MDR	10	10
Total	**100**	**100**

Table 6: Shows relation between previous treatment and TB patients.

TB Patient	Previous treatment		Total No - (%)
	Yes (recurrence) No-(%)	No (new) No -(%)	
MDR	5 (5)	5 (5)	10 (10)
RIF-responder	49 (49)	41 (41)	90 (90)
Total	**54 (54)**	**46 (46)**	**100 (100)**

P value= (0.000)

DISCUSSION

The patients referred to central laboratory either suspected cases of pulmonary tuberculosis or multidrug resistance patients.

Patient's ages ranged from17.9 to 50.3years and the mean age was 34.1±16.2. The study reveals 25% (100/400) positive cases for MTB from the referred suspected patients, identified by GeneXpert- RIF assay for MTB.

Tuberculosis was higher in males75%, with ratio of M/F 3:1, which was not consisted with national reported ratio of M/F 1:7. But near to Elmadhoun *et al*,. study who reported males was 65.9%.[18-1] The frequency of TB was more in married individual (54%) than in single. Patients from rural area represent 67%; this high frequency may be due to low socioeconomic status compared with urbanized individual. TB patients not routinely screened for HIV, 97% of patients was unscreened. While 1% shows sero-positivity. HIV sero-prevalence among TB cases was (5.8%) reported in a study in The River Nile State by El Elmadhoun *et al*.[1] Therefore, mandatory TB patients should be screened for HIV. The study reveals significant association between diabetes mellitus and MDR-TB, which consisted with the study done by Tegegne *et al*.[18] The study reveals the recurrent rate of infection is 59% (59/100) due to interrupted treatment, relapse or treatment failure which was high compared with Sharaf Eldin *et al*., Who reported recurrence rate of 30%.[14] This study shows MDR-MTB is 10% (10/100), which is not far from previous studies done in Ethiopia and Kampala, reported 12% and 13% respectively, but a study in Egypt reported high rate of 38% for MDR-TB.[10-12-11] The study reveals a MDR- MTB is 10%, higher in comparison with study conducted by Mekonnen, in districts of North West of Ethiopia, who reported 5.7% MDR-MTB.[10] The study reveals lower prevalence of MDR- MTB than study conducted by Ikuabe and Ebuenyi, in Yenagoa, Nigeria whom reported 22.9% MTB in the sputum specimens, and MDR- MTB was14.7%.[19] The study reveals a significant difference between new and previously treated cases of TB patients, for sensitive and MDR-MTB cases (P. value=0.000). However the study reveals frequencies of MDR-MTB as

5% in re-treated cases and 5% in new cases. The MDR-MTB either new cases or retreated patients disagree with a study done by Sudan's National TB Program in, which reported 20% for retreated cases and 1.9% for new cases.[11] While Hassan *et al*., Sharaf Eldin *et al.*, and Adam et al., done studies in different regions of Sudan, reported similar results to the study, whom stated that prevalence of MDR-MTB in new cases, were 6%, 5% and 6% respectively.[13-14-15]

CONCLUSION

The MDR-MTB and recurrence rates of pulmonary tuberculosis are high. This study highlights a significant relationship between MDR- MTB and some possible risk factors. The high recurrence rate was due to treatment failure, relapses or interrupted treatment. Drug resistance to tuberculosis and recurrence were serious public health problems in Sudan, and attention should direct to control and improve monitoring therapy.

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