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ORIGINAL PAPER

Screening of Human Immunodeficiency Virus (HIV) among Newly Diagnosed Tuberculosis Patients in Eastern Sudan

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Abstract

Tuberculosis (TB) is a leading cause of death in patients infected with Human Immunodeficiency Virus (HIV), and HIV infection is the most potent risk factor for the development of active TB disease from a latent TB infection. This study aims to determine the seroprevalence of HIV among newly diagnosed TB patients in Kassala state eastern Sudan. This was a descriptive, hospital-based, cross-sectional study of 251 active and newly diagnosed TB patients, selected by simple random sampling. Blood samples and demographic data were collected from each patient. TB was diagnosed by direct ZN smear and molecular detection by Xpert MTB/RIF. The serum samples were tested for HIV using 4th generation enzyme-linked immunosorbent assay (ELISA). The prevalence of HIV was 13.9% (35/251), the infection rate among pulmonary TB was 17%, whereas that in extrapulmonary TB was 4.8%, the prevalence was (18.2%) in the males, and (7.2%) in the females. In conclusion: TB/HIV co-infection in the Eastern part of Sudan was high compared with the global prevalence, all TB patients should therefore be assessed for HIV risk factors and advised to undergo HIV testing.

K e y w o r d s: Tuberculosis, ELISA, HIV, ZN, Xpert MTB/RIF

Introduction

Tuberculosis (TB) and Human Immunodeficiency Virus/Acquired Immunodeficiency Syndrome (HIV/AIDS) are the major public health issue in many parts of the world particularly in resource-limited countries, TB remains an important cause of ill health, and the major cause of mortality from a single infectious agent, rated above (HIV/AIDS) in the top 10 diseases that cause high mortality rates (WHO 2019a).

HIV is one of the most significant threat to the global control of TB (Mukadi et al. 2001). By severely compromising the immune system, HIV facilitates TB dissemination and raises the mortality of co-infected individuals as opposed to TB patients who are HIV negative (Mukadi et al. 2001; Zumla et al. 2015). Both diseases are directly connected, and the number of co-infected patients continues to increase rapidly (Karim 2006).

HIV is a potential risk accountable for latent TB progressing to active TB (Davy-Mendez et al. 2019). People living with HIV are 19 (15–22) times more probable than people without HIV to develop active TB disease, which demonstrates the seriousness of this deadly combination. Worldwide about 251,000 people have died of HIV-associated TB in 2018, and an estimated 862,000 new cases of TB have been identified among HIV-positive individuals, 72% among whom live in Africa. With 95% of global TB deaths and more than 70% of the global HIV burden, sub-Saharan Africa bears the greatest burden of both diseases (Gwitira et al. 2018). Sudan one of the resources limited countries with major issues in health, in 2018 the total incidence of TB in Sudan was 30,000 (21,000–41,000), with 71 (49–98) rates per 100,000. The HIV-positive TB incidence was estimated to be 970 (300–2,000), with 2.3 (0.72–4.8) rate per 100,000 (WHO 2019b), and Eastern Sudan remain as an endemic area of TB (Abdallah et al. 2012).

While the HIV epidemic continues to fuel the global TB epidemic, the significance of HIV surveillance in TB patients is widely recognized (Manjareeka and Nanda

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2013). The main components of both HIV and TB programmers' are early diagnosis and treatment (Getahun et al. 2011). The 2012 updates issued by the World Health Organization on the Guidelines for TB/HIV Collaborative Activities in 2004 recommend HIV testing not only for diagnosed TB patient, but among patients with suspected TB also (WHO 2012). The optimal time to begin antiretroviral therapy (ART) has been carefully evaluated in patients with both TB and HIV infection (Han et al. 2014). Immune reconstitution inflammatory syndrome (IRIS), pharmacological interactions, and high pill burden have repeatedly claimed against concurrent therapy for both HIV and TB (Piscitelli and Gallicano 2001; Shelburne et al. 2002; Blanc et al. 2007; Kaplan et al. 2009). On the other hand, a delay in launching ART is correlated with disease progression and increased mortality, notably in severely immunosuppressed patients (Kwara et al. 2004; Breen et al. 2005).

This study aimed to estimate the seroprevalence of HIV infection among active pulmonary and extrapulmonary tuberculosis patients, and the level of knowledge in Kassala state, which is located in eastern Sudan near the Eritrean border, 600 kilometers from the Khartoum capital of Sudan with a great variety in culture, beliefs, language, and ethnicity.

Experimental

Materials and Methods

This was descriptive cross-sectional hospital-based study undertaken in Kassala State, Eastern of Sudan to investigate the prevalence of HIV among active pulmonary and extrapulmonary TB patients. A total of 251 TB patients attending Kassala Teaching Hospital during the period of the study were recruited by simple random sampling after consent was obtained. Information, such as age, gender, socioeconomic background, education level, and residence area, was collected by a structured questionnaire. All patients were tested for TB according to the recommendations of the national TB program by using direct ZN stain and Xpert MTB/RIF (Cepheid, Sunnyvale, CA, USA).

TB diagnosis. AFB smear microscopy. Specimens were processed using the N-acetyl-L-cysteine-NaOH (NALC-NaOH) method for digestion and decontamination. Specimens were concentrated by centrifugation at 3,200 × g for 20 min, and sediments were reconstituted with approximately 2 ml of 0.067 M sterile phosphate buffer (pH 6.8). Smear microscopy was performed on processed sediments using Ziehl-Neelsen (ZN) staining. Smear-positive specimens were graded from 1+ to 4+ according to CDC guidelines (American Thoracic Society/CDC 2000)

Xpert MTB/RIF assay. The Xpert MTB/RIF assay was run on the GeneXpert Dx instrument system according to the manufacturer's recommendations (Cepheid, Sunnyvale, CA, USA). Briefly, after digestion, decontamination and concentration, 0.5 ml of re-suspended sediment was transferred to a conical screw-capped tube, 1.5 ml of Xpert MTB/RIF sample reagent was added by sterile pipette, and the tube was recapped and shaken vigorously 10–20 times. The sample was incubated for a total of 15 minutes at 20–30°C, with manual agitation 10–20 times at one point between 5 and 10 minutes into the incubation period. The reagent-treated sample was then transferred by sterile pipette into the sample chamber of the Xpert MTB/RIF cartridge and loaded into the GeneXpert Dx instrument system for sample processing. In the event of "no result", "invalid" or "error" results, the test was repeated according to the manufacturer's recommendations using a new Xpert MTB/RIF cartridge.

Blood samples were collected from each patient, then serum samples were separated (Tognon et al. 2020) and investigated for HIV antibodies by using fourth-generation enzyme-linked immunosorbent assay (ELISA) according to the manufacturer's instructions.

Data was analyzed by IBM SPSS Statistics for Windows, Version 20 (Armonk, NY: IBM Corp) and iNZight (The University of Auckland New Zealand). *Chi*-square test was used to test the *p*-value, and it was deemed significant if it was less than 0.05. Ethical approval for this study was received from the Health Research Ethics Committee of the Ministry of Health in Kassala state. The patients consent was acquired from each participant prior to the sample collection.

Results

A number of 251 TB patients have consented to take part in this study. Of those patients, 188 (74.9%) were newly diagnosed with pulmonary TB, and the remaining 63 (25.1%) were complaining of extrapulmonary TB.

Gender, age, and residence. Out of 251 patients, 154 (61.35%) were male and 97 (38.65%) were female. Their age was ranged from 4 years to 80 years, and the mean was 41.7 ± 17.9 years. On the basis of the residence of the studied population, 145 (57.77%) were residing in urban areas in the city, whereas 106 (42.23%) resided in rural areas around the city.

Social, behavioral, and HIV/TB knowledge data. In terms of social data (marital status, educational level, and occupation) and knowledge about TB and HIV, the study population was separated into two groups: children with less than 18 years old, and adults above 18 years old. Their distribution was 21 (8.37%) and

230 (91.63%) in the children and adult groups, respectively. These variables were analyzed separately.

As shown in Table I, the marital status of the adult group was divided into the following categories: married (167; 72.1%), single (61; 26.52%), and widowed (2; 0.87%). Their smoking behavior was as follows: 217 (94.35%) were nonsmokers, while 13 (5.65%) were smokers.

Table I
Frequency of social behavior, and knowledge about TB/HIV, of adults (n = 230).

Adults patients (Total 230)			
Marital Status	married	167	72.61%
	single	61	26.52%
	widow	2	0.87%
Educational level	illiterate	159	69.13%
	elementary	59	25.65%
	secondary	11	4.78%
	university	1	0.43%
Occupation	non	1	0.43%
	farmer	1	0.43%
	housewife	82	35.65%
	officer	2	0.87%
	worker	140	60.87%
	student	4	1.74%
Smoking	smoker	13	5.65%
	non smoker	217	94.35%
Alcohol	alcoholic	1	99.57%
	non alcoholic	229	0.43%
Knowledge about TB	good	5	2.17%
	poor	225	97.83%
Knowledge about HIV	good	24	10.43%
	poor	206	89.57%

The education level of 230 adults was as follows: illiterate (159; 69.13%), elementary education (59; 25.65%), secondary education (11; 04.78%), and university education (1; 0.43%).

Occupation of the adults' population was spread into unemployment, farmer, housewife, officer, free worker, and students, with the following frequencies: 1 (0.43%), 1 (0.43%), 82 (35.65%), 2 (0.87%), 140 (60.87%), and 4 (1.74%), respectively.

Alcohol drinking was one of the behavioral data collected, only one patient was alcoholic.

Knowledge about TB and HIV, transmission, and treatment were measured by asking different questions. Specifically, a patient who knew two-thirds or more was considered someone who had good knowledge, whereas a patient who knew one-third or less was considered someone with poor knowledge. Regarding TB knowledge, only 5 (2.17%) out of 230 adults showed good knowledge, while the remaining 225 (97.83%) showed poor knowledge.

In terms of HIV knowledge, 24 (10.43%) out of 230 adults showed good knowledge, while 206 (89.57%) showed poor knowledge.

The frequencies of the extracted children's data were as follows. Their mean age was 13.38 ± 2.94 years, 14 of them were male (66.67%), and 7 were female (33.33%). Thirteen of them were uneducated (61.90%), while 8 had an elementary education (38.10%). All of them had poor knowledge about TB, and only one had good knowledge about HIV (4.76%).

Overall, out of 251 patients tested for HIV, 35 showed positive results with a prevalence of 13.9%, while 216 (86.1%) showed negative results (Table II).

Table II
Frequency of gender, education, Knowledge about TB, knowledge about HIV, and occupation of children (n = 21).

Children patients (Total 21)			
Gender	male	14	66.67%
	female	7	33.33%
Education	elementary	8	38.10%
	uneducated	13	61.90%
Knowledge about TB	good	0	0.0%
	poor	21	100%
Knowledge about HIV	good	1	4.76%
	poor	20	95.24%

When the types of TB was compared with HIV infection, the infection rate among pulmonary TB was 17%, whereas that in extrapulmonary TB was 4.8%; this was statistically significant ($p = 0.03$; Table III).

Table III
Frequency of HIV in comparison to type of TB (n = 251).

	HIV positive	HIV negative	Total	p-value
Pulmonary TB	32 (17%)	156 (83%)	188	0.026
Extra pulmonary	3 (04.8%)	60 (95.2%)	63	
Total	35 (13.9%)	216 (86.1%)	251	

The mean age of HIV-positive patients with TB was 31.66 ± 12.80 years, while 43.35 ± 18.16 years in HIV-negative patients.

Regarding gender, the infection of HIV among males was 18.2%, while that among females was 7.2%; the difference was statistically significant ($p = 0.02$; Table IV).

No statistical significance was detected when comparing the residence of patients with HIV infection frequency ($p = 0.9$). The frequency was 14.5% in patients residing in the urban area, while that was 13.2% in patients residing in a rural area (Table IV).

Table IV
Frequency of HIV in comparison to gender, education, Knowledge about TB, knowledge about HIV, and occupation of children (n = 251).

		Positive	Negative	Total	*p*-value
Gender	male	28 (18.2%)	126 (81.8%)	154	0.02
	female	7 (7.2%)	90 (92.8%)	97	
Residence	urban	21 (14.5%)	124 (85.5%)	145	0.92
	rural	14 (13.2%)	92 (86.8%)	106	
Knowledge about TB	good	1 (20%)	4 (80%)	5	0.69
	poor	34 (13.8%)	212 (86.2%)	246	
Knowledge about HIV	good	6 (24%)	19 (76%)	25	0.13
	poor	29 (12.8%)	197 (87.2%)	226	
Total		4 (19.05%)	17 (80.95%	21	

Table V
Frequency of HIV in comparison to gender, education, and occupation of adults (n = 230).

		Positive	Negative	Total	*p*-value
Education	illiterate	23 (14.5%)	136 (85.5%)	159	0.89
	elementary	7 (11.9%)	52 (88.1%)	59	
	secondary	1 (9.1%)	10 (90.9%)	11	
	university	0 (0.0%)	1 (100%)	1	
Marital status	married	11 (6.6%)	156 (93.4%)	167	0.6
	single	20 (32.8%)	41 (67.2%)	61	
	widow	0 (0.0%)	2 (100%)	2	
Occupation	unemployed	0 (0.0%)	1 (100%)	1	0.03
	housewife	5 (6.1%)	77 (93.9%)	82	
	freeworker	24 (17.1%)	116 (82.9%)	140	
	officer	0 (0.0%)	2 (100%)	2	
	farmer	1 (100%)	0 (0.0%)	1	
	student	1 (25%)	3 (75%)	4	
Smoking	smoker	8 (61.5%)	5 (38.5%)	13	0.7
	non smoker	23 (10.6%)	194 (89.4%)	217	
Alcohol	yes	0 (0.0%)	1 (100%)	1	0.69
	no	31 (13.5%)	198 (86.5%)	229	
Total		31 (13.5%)	199 (86.5%)	230	

Regarding the patients' knowledge about TB in comparison with HIV infection, out of all 251 patients, 20% of patients with good knowledge were found to be HIV positive, while 13.8% of patients with poor knowledge were HIV positive. The *p*-value was 0.69, it was not statistically significant (Table IV). Considering patients' knowledge about HIV and the frequency of infection, 24% of patients with good knowledge were positive for HIV, while 12.8% HIV-positive patients had poor knowledge, with no statistical significance between the two groups ($p = 0.13$; Table IV). Table V demonstrates the social and behavioral characteristics of adult patients (above 18 years old) with TB compared with HIV infection. First, regarding the education of HIV-positive patients, 14.5% were illiterate, 11.9% had an elementary education, 9.1% had secondary education, and 0.0% were university studied patients; this was not statistically significant ($p = 0.89$).

Second, marital status and HIV results were compared. The frequency of HIV-positive patients was 6.6% married, 32.8% single, and 0.0% widowed; the difference was not statistical significant ($p = 1.6$).

Third, the occupation of patients with TB was compared with the HIV results. No positive HIV results were found among the unemployed and officers, and 6.1% were found to be HIV positive in housewives. Approximately 17.1% of HIV-positive samples were free workers, only one farmer was HIV positive (100%), and 25% of students were found to be HIV positive.

Fourth, smoking behavior in adult patients was compared with HIV results. The frequency of HIV-positive results was 61.5% among smokers, and 10.6%

among nonsmokers; the difference was not statistically significant ($p = 1.7$).

Lastly, regarding adult data, alcohol drinking was compared with HIV results. Approximately 13.5% non-alcoholics were positive for HIV, while only one patient who drank alcohol was HIV negative.

Discussion

The prevalence of HIV in patients with TB is a responsive predictor of the spread of HIV to the general population in many regions. In order to respond to a growing commitment to providing comprehensive HIV/AIDS treatment and support, including anti-retroviral therapy (ART), for HIV-positive patients with TB, information on HIV prevalence in patients with TB is important. Currently, while TB cases are increasingly being found in most countries, most cases of HIV are not.

The current study revealed that the prevalence of HIV in TB patients was 13.9%. These findings were lower than a similar study conducted in Kassala in 2012, where the frequency was reported as 18.3% (Abdallah et al. 2012).

This frequency was also lower than those reported by studies carried out in Nigeria, Ghana, Ethiopia and Zambia (Yassin et al. 2004; Erhabor et al. 2010; Pennap et al. 2010; Chanda-Kapata et al. 2017; Osei et al. 2017) but higher than those in studies in India, China, Pakistan, and Vietnam (Thanh et al. 2010; Wang et al. 2010; Hasnain et al. 2012; Manjareeka and Nanda 2013). The large variation in TB/HIV co-infection rates worldwide is partly due to the following reasons: under-reporting, diagnostic procedures used, disparity in TB diagnosis, TB epidemiology in different countries, and methods used in the study.

The present study showed a high prevalence of HIV in males than in females, which was in line with a study in southern Ethiopia, wherein the HIV prevalence was 18% for females and 21% for males (Yassin et al. 2004), in Eastern India including 42 (10.3%) males and 8 (02%) females (Manjareeka and Nanda 2013), and in Pakistan (Hasnain et al. 2012). However, the present work was contradicted by studies in Nigeria in which the prevalence of co-infection was found to be higher among females (44.82%) than among males (38.30%) (Pennap et al. 2010), and in a study conducted in Ghana, wherein the percentage was 15.1% in males and 24.1% in females (Osei et al. 2017).

The current study also showed slightly higher frequency in patients residing in an urban area (14.5%), while only 13.2% was noted in patients residing in a rural area. This outcome was in line with a related study carried out in the southern region of Ethiopia (Yassin et al. 2004).

Statistical significance was detected when comparing HIV co-infection in pulmonary TB and extrapulmonary TB; the frequencies of pulmonary TB and extrapulmonary TB were 17% and 4.8%, respectively. Similar findings have been found in studies carried out in southern Ethiopia, which reported 19% of pulmonary TB, and 11% of the patients with extrapulmonary TB were HIV positive (Yassin et al. 2004). Nevertheless, these findings were not in agreement with studies in India and Pakistan (Hasnain et al. 2012; Manjareeka and Nanda 2013), because of a limited number of HIV cases detected in their reports. The seroprevalence of HIV infection among TB-infected patients was identified in this study in Kassala State, Eastern Sudan, and showed a high burden of HIV infection among active TB patients.

In strict compliance with the WHO, the CDC recommends that all patients with newly diagnosed TB be screened for HIV after consultation. TB reactivation can be minimized by TB preventive therapy and universal access to ART for people living with HIV.

Ethics approval and consent to participate

Approval for this study was issued by the Health Research Ethics Committee of the Ministry of Health in Kassala state. Consent was acquired from each participant before sample and data collection.

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Authors' contributions

Gada MA Mustafa, collection of sample and conduction of all practical tests. Mustafa E Yassin, study planning, performed data analysis and wrote the manuscript. Samah AbduRahim revised and helped to draft the manuscript. Ashwag Shami AbduRahim revised the paper and helped to draft it. The manuscript was read and accepted by all the authors.

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Conflict of interest

The authors do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

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