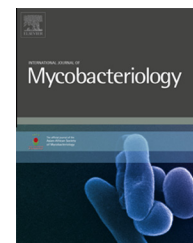


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Active tuberculosis case finding and detection of drug resistance among HIV-infected patients: A cross-sectional study in a TB endemic area, Gondar, Northwest Ethiopia

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ABSTRACT

Background: Tuberculosis (TB) patients co-infected with human immunodeficiency virus (HIV) often lack the classic symptoms of pulmonary tuberculosis, making the diagnosis difficult. Current practices in resource-limited settings often indicate that these co-infected patients are diagnosed when they clinically manifest disease symptoms, resulting in a delayed diagnosis and despite continued transmission. The aim of this study is to determine the prevalence of undiagnosed pulmonary tuberculosis cases through active case finding and including multidrug-resistant TB (MDR-TB) among HIV-infected patients.

Materials and methods: A total of 250 HIV-infected patients, aged 18 years and above were evaluated in a cross-sectional design between February 2012 and November 2012. Socio-demographic and clinical data were collected using a structured questionnaire. Sputum samples were collected from all participants for acid fast bacilli (AFB) direct smear microscopy and *Mycobacteria* culture. A PCR-based RD₉ deletion and genus typing, as well as first-line anti-TB drug susceptibility testing, was performed for all culture-positive isolates.

Results: Following active TB case finding, a total of 15/250 (6%) cases were diagnosed as TB cases, of whom 9/250 (3.6%) were detected by both smear microscopy and culture and the remaining 6/250 (2.4%) only by culture. All the 15 isolates were typed through RD₉ typing of which 10 were *Mycobacterium tuberculosis* species; 1 belonged to *Mycobacterium* genus and 4 isolates were non-tuberculous mycobacteria. The prevalence of undiagnosed pulmonary TB disease among the study participants was 4.4%, which implies the possibility of identifying even more undiagnosed cases through active case finding. A multivariate logistic regression showed a statistically significant association between the presence of pneumonia infection and the occurrence of TB (OR = 4.81, 95% CI (1.08–21.43), $p = 0.04$). In addition,

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all the isolates were sensitive to all first-line anti-TB drugs, except for streptomycin, seen in only one newly diagnosed TB patient, and MDR-TB was not detected.

Conclusion: The prevalence of undiagnosed pulmonary TB infection among HIV-infected patients in Gondar was 4.4%. Additionally, the possibility of these undiagnosed TB cases in the community could also pose a risk for the transmission of the disease, particularly among family members. Active screening of known HIV-infected individuals, with at least one TB symptom is recommended, even in persons with opportunistic infections.

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Introduction

Tuberculosis (TB) is one of the leading opportunistic infections that affect people living with human immunodeficiency virus (HIV) leading to significant morbidity and mortality [1]. People living with HIV are about 21–34 times more likely to develop TB disease compared with those who are HIV-negative [2]. TB in such patients is often difficult to diagnose because of nonspecific symptoms, atypical or normal chest radiograph findings, negative results of sputum smear microscopy and lack of culture facilities in resource-limited settings [3].

Active TB screening can prompt earlier treatment initiation, reducing the burden of disease in an individual and transmission within the community [4,5]. In 2004, the World Health Organization (WHO) has recommended routine screening for active TB disease in all patients living with HIV, with treatment provided for active TB disease or isoniazid preventive therapy (IPT) for latent TB to reduce morbidity and mortality [6]. However, since this recommendation, only 2.3 million individuals have been screened for TB, of whom 178,000 were offered IPT [2]. Africa accounted for 82% of new TB cases among people living with HIV [2]. According to the 2011 WHO tuberculosis report, Ethiopia ranked seventh among the world's 22 high-burden countries with an estimated incidence of 333 per 100,000 populations per year [2,7].

In addition, the emergence of multidrug resistance (MDR) is also one of the threatening factors for many TB control programs globally [8]. Drug-resistant TB is expected to exist in countries like Ethiopia where there is high risk of infection with HIV, as drug resistance has been significantly associated with HIV infection [9]. According to the WHO estimate, the prevalence of MDR-TB in Ethiopia has been estimated to be 1.6% among newly diagnosed cases and 12% among retreatment cases [2]. MDR-TB has progressed to extensively-drug resistant status (XDR-TB) [10] and the latter have evolved in some parts of the world to totally-drug resistant (TDR-TB) infections [11]. The presence of XDR-TB has not been thoroughly investigated in Ethiopia. Recently, two XDR-TB strains were reported from a total of 45 MDR-TB cases in a study conducted at St. Peter's TB Specialized Hospital and Ethiopian Health and Nutrition Research Institute [12]. TDR-TB – a yet-to-be-defined infection – is believed to be resistant to all forms of therapy, and mortality is almost certain [11].

Although many factors such as the degree of immunosuppression, high-risk behavior, lifestyle and TB contact history contribute to the development of active TB in HIV-positive

patients [13–15], delays in early diagnosis of TB can also play an important role in morbidity and mortality among HIV patients. To the knowledge of this study, this is the first report done among HIV-infected patients to assess the prevalence of undiagnosed pulmonary TB, anti-TB drug susceptibility patterns and associated risk factors, who attend anti-retroviral (ARV) clinics in the Gondar area, Ethiopia, through active case findings.

Materials and methods

Study design and participants

A cross-sectional study was conducted between February 2012 and November 2012 among HIV patients attending the ARV clinic at the Gondar University Hospital, a tertiary-level teaching and referral hospital in Northwest Ethiopia. A total of 250 consecutive HIV patients, aged 18 years and above and who at least had one of the TB symptoms (cough, night sweats, fever or weight loss), but undiagnosed during their visit at the clinic were enrolled.

In addition, patients with a history of previous TB and those on anti-retroviral treatment (ART) or pre-ART were also enrolled. Those patients with severe illness and unable to provide sputum and who already started anti-TB treatment or IPT were excluded. Socio-demographic features (Table 1) and clinical data (Table 2), including chest X-ray findings, were collected using structured questionnaires.

The clinical presentation of HIV positive cases were non-specific, such as an acute mononucleosis-like illness accompanied by fevers, sweats, malaise, lethargy, anorexia, nausea, myalgia, arthralgia, headache, sore throat, diarrhea, lymphadenopathy and rash [16].

Sputum collection and processing

Three sputum samples (spot-morning-spot samples) were collected from all eligible participants, pooled and a direct acid fast bacilli (AFB) smear microscopy was performed using the conventional Ziehl-Neelsen staining technique at the Gondar Microbiology Laboratory as described earlier [17]. The remaining aliquots of sputa were transported in a cold chain from Gondar Microbiology Laboratory to the P₃ TB laboratory facility at Armauer Hansen Research Institute (AHRI), Addis Ababa, for *Mycobacterium* culture, RD typing and drug susceptibility testing.

Table 1 – Socio-demographic characteristics of the study participants (n = 250) from antiretroviral Clinic at Gondar University Hospital, Northwest Ethiopia, 2012.

Socio-demographic variables	Frequency (n = 250)	Percentage (%)
Sex		
Male	92	36.8
Female	158	63.2
Age, mean (SD)	35.72 (9.42)	
Residence area		
Urban	210	84.0
Rural	40	16.0
Marital status		
Single	47	18.8
Married	111	44.4
Divorced	52	20.8
Widowed	40	16.0
Educational background		
Illiterate	98	39.2
Read & write	135	54.0
Higher education	17	6.8
Occupational status		
Employed	38	15.2
Unemployed	136	54.4
Retired	4	1.6
Housewife	45	18.0
Daily labor	20	8.0
Farmer	7	2.8

Abbreviation: SD = standard deviation.

Mycobacterial culture

Sputum samples were decontaminated and homogenized by the modified Petroff's method as described previously [18]. Briefly, about 1 ml of the sediment was inoculated into the conventional Lowenstein-Jensen (LJ) egg slant medium containing 0.6% sodium pyruvate and glycerol for primary isolation. After inoculation, LJ slants were held for 8 weeks at 37 °C and visually inspected for growth every day for the first week and twice per week thereafter for the total of 8 weeks for the presence of mycobacterial colonies. Microscopic examinations of the colonies were performed using Ziehl-Neelsen staining method so as to select AFB positive isolates.

Molecular typing

Heat-killed cells were prepared from AFB positive isolates by mixing two loops-full of colonies in 200 µl of distilled water and by heating at 80°C for one hour. Polymerase Chain Reaction (PCR)-based deletion typing was performed to check for the presence or absence of regions of difference-9 (RD₉) so as to identify *Mycobacterium tuberculosis* (*M. tuberculosis*) from other species of *Mycobacteria* [19]. The method was applied to heat-killed mycobacterial suspensions. A multiplex PCR was designed to amplify the non-deleted RD₉ region. Two external primers (RD₉_FlankFW: 5'-AACACGGTCACGTTGTCGTG-3' and RD₉_FlankRev: 5'-CAAACCAGCAGCTGTCGTTG-3') and one internal reverse primer (RD₉_InternalR: 5'-TTGCTTCCCCGGTTCGTCTG-3') were used per locus.

Table 2 – Baseline clinical data of the study participants (n = 250) from antiretroviral Clinic at Gondar University Hospital, Northwest Ethiopia, 2012.

Characteristics	Total n = 250 (%)	Tuberculosis negative n = 239 (%)	Tuberculosis positive n = 11 (%)	p Value
Previous contact with TB	26 (10)	25 (11)	1 (9)	0.88
History of TB treatment	73 (29)	69 (29)	4 (36)	0.59
Alcohol drinking	29 (12)	27 (11)	2 (18)	0.49
Tobacco use	4 (2)	4 (2)	0	0.66
Shisha use	8 (3)	7 (3)	1 (9)	0.26
Receiving HAART	167 (67)	161 (67)	6 (55)	0.37
Mean household members (IQR)	3.8(2-5)	3.7	5.4	0.52
WHO clinical stage				0.63
I	126(50)	122(51)	4(37)	
II	39(16)	36(15)	3(27)	
III	80(32)	76(32)	4(36)	
IV	5(2)	5(2)	0	
Current OIs				
Oral Thrush	37 (15)	36 (15)	1 (9)	0.59
Pneumonia	24 (10)	21 (9)	3 (27)	0.04
Herpes Zoster	62 (25)	60 (25)	2 (18)	0.60
Chest X-ray findings	61 (24)	57 (24)	4 (36)	0.35
Infiltrate	14 (6)	13 (5)	1 (9)	0.61
Effusion	7 (3)	7 (3)	0	0.56
Cavity	4 (2)	4 (2)	0	0.67
Miliary	2 (1)	2 (1)	0	0.76

TB – tuberculosis; HAART – highly active antiretroviral therapy; IQR – interquartile range; OIs – opportunistic infections; WHO – World Health Organization.

* TB status was categorized based on the results of a combination of AFB smear microscopy, culture, RD9 and genus typing.

After identification by RD₉ typing, *Mycobacterium* genus typing was performed from non-deleted RD₉ region to further differentiate species of the *M. tuberculosis* complex from other *Mycobacterium* species. A reference strain of *M. tuberculosis*, H37Rv (ATCC 27249) and water were also run simultaneously with samples in every reaction as positive and negative controls, respectively.

Drug susceptibility testing (DST)

Indirect drug susceptibility testing was performed for first-line anti-TB drugs (isoniazid, rifampicin, ethambutol and streptomycin), based on proportion method on 24-well tissue culture plates (Becton Dickinson, USA) using Middlebrook 7H10 medium supplemented with glycerol and 10% oleic acid albumin dextrose catalase (OADC; Becton Dickinson, Sparks, MD). Primary isolates grown within 2–3 weeks were used for preparing the suspension, then the DST plates were inoculated by adding 10 µl of the suspension which was adjusted with McFarland standard. The plates were securely sealed with Parafilm and incubated at 35 °C in a 5% CO₂ incubator with a water reservoir. Drug susceptibility was determined by visually comparing the drug containing media (1:1 bacterial suspensions) with the drug-free control on which 1:100 bacterial suspensions was inoculated. The drug sensitivity of the bacterial isolate was later determined using the critical concentration level as recommended by the WHO 2009 [20].

Statistical analysis

Data analysis was performed using SPSS software packages Version 20.0 (SPSS Inc., Chicago, 2011, USA). Descriptive statistics was used to report patient socio-demographics and clinical data, prevalence of undiagnosed pulmonary TB and rates of MDR-TB. Logistic regression and odds ratio were used to measure the degree of association between the different variables. A *p*-value of <0.05 was considered statistically significant.

Ethical consideration

The study received ethical approval from the ethics board of the University of Gondar, School of Biomedical and Laboratory Sciences, and AHRI/ALERT Ethics Review Committee and all participants gave written informed consent.

Results

Of the 250 participants who were actively screened for TB, 15 (6%) cases were identified as having TB, of whom 9 were newly detected TB cases and positive by both smear microscopy and culture, and the remaining were previously treated TB cases (patients who had a previous history of TB and completed TB treatment at least 3 months previously and not identified as TB suspect during the study period) and detected only using culture. RD₉ typing also showed that ten of the isolates were *M. tuberculosis* species, one belonged to *Mycobacterium* genus, which could not be specified due to inaccessibility of the test kits and the remaining four isolates

were non-tuberculous mycobacteria, further indicating the prevalence of undiagnosed pulmonary TB disease in the study cohort to be 4.4% (11/250).

The overall CD₄ T-cell count was relatively higher among non-TB cases compared with TB cases (Table 3), although there was no statistically significant association. Logistic regression analyses showed no significant association between different variables and risk factors for pulmonary TB disease, except for the presence of pneumonia (OR = 4.81, 95% CI [1.08–21.43], *p* = 0.04) (Table 4).

On the other hand, analyses of drug susceptibility testing showed that all of the 11 *Mycobacterium* isolates became sensitive to all first-line anti-TB drugs, except for one isolate, which was obtained from a newly diagnosed TB case that was resistant to streptomycin. No MDR-TB was detected in the study cohort in both newly diagnosed cases and previously treated TB cases.

Discussion

The current study identified about 4.4% of undiagnosed pulmonary TB among a cohort of HIV-infected individuals visiting ART clinics in the Gondar area, where the majority were also smear positive/culture positive (3.6%) implying the possibility of identifying even more undiagnosed cases through active case finding. In addition, the possibility of these undiagnosed TB cases in the community could also pose a risk for the transmission of the disease, particularly among family members. This observation is relatively higher than what has been reported from South Africa among HIV-positive gold miners, where the point prevalence of undiagnosed TB and the rate of smear-positive TB were 3.8% and 0.4%, respectively [21]. This difference could also be attributed to the use of IPT among the South African gold miners that could reduce the occurrence of symptomatic disease due to TB.

A case-control study conducted in Northwest Ethiopia by Animut et al. showed that out of 282 TB patients, 54.6%, 23.8% and 21.6% had smear negative, smear positive and extra pulmonary TB, respectively, and identified several predictors of HIV counseling and testing among TB patients which is in contrast to this study [22]. The difference is in this study all participants were known HIV patients screened for TB but not TB patients.

On the other hand, a study conducted in one of the research clinics in Gambia reported a significantly higher proportion of TB (43.2%) among HIV patients, of whom 66% had pulmonary TB confirmed by microscopy and/or culture [23]. In a similar trend, a higher prevalence of pulmonary TB was reported among HIV-positive patients in Cambodia [24]. Part of these differences in the two reports was mainly due to differences in inclusion and exclusion criteria, where in this case all patients who were already diagnosed with TB and started anti-TB treatments were excluded.

The finding from the RD₉ typing also demonstrates that the dominant causative agent for TB in the study population was *M. tuberculosis*, as more than 60% of the isolates were confirmed to be *M. tuberculosis*. This has been also evident from earlier studies conducted in other regions of Ethiopia [25,26]. This study also evaluated the association of TB

Table 3 – Immunological status of the study participants (n = 250), as depicted by CD₄⁺ T-cell count, Gondar University Hospital, Northwest Ethiopia, 2012.

Tuberculosis status				
CD ₄ ⁺ T-cell count	Total n = 250 (%)	Tuberculosis negative n = 239 (%)	Tuberculosis positive n = 11 (%)	p value
<200	58 (23)	53 (22)	5 (46)	0.07
≥200	192 (77)	186 (78)	6 (56)	
Mean CD ₄ ⁺ T cell (IQR)	363 (211–475)	368	245	0.09

CD – cluster of differentiation; IQR – interquartile range.

Table 4 – Analyses of risk factors for pulmonary TB among study participants (n = 250), Gondar University Hospital, Northwest Ethiopia, 2012.

Characteristic	Univariate analysis		Multivariate analysis	
	OR (95% CI)	p value	OR (95% CI)	p value
Age (year)	1.02 (0.96–1.08)	0.60		
Female	1.58 (0.41–6.20)	0.51		
Married	0.27 (0.06–1.25)	0.09	0.31 (0.06–1.52)	0.15
Unemployed	0.69 (0.20–2.31)	0.54		
Illiterate	0.57 (0.15–2.20)	0.41		
Any alcohol use	1.75 (0.36–8.50)	0.49		
Previous contact with TB	1.17 (0.14–9.51)	0.89		
Household members >4	1.58 (0.47–5.34)	0.46		
HIV				
WHO stage III/IV vs. I/II	1.12 (0.32–3.92)	0.87		
HAART use	0.58 (0.17–1.96)	0.38		
CD ₄ ⁺ T cell count <200	2.93 (0.86–9.96)	0.09	2.54 (0.68–9.56)	0.17
History of TB treatment	1.41 (0.40–4.96)	0.59		
Current OIs				
Thrush	0.56 (0.07–4.54)	0.59		
Pneumonia	3.89 (0.96–15.79)	0.06	4.81 (1.08–21.43)*	0.04*
Herpes Zoster	0.66 (0.24–3.15)	0.61		
Symptoms				
Fever	0.83 (0.24–2.93)	0.77		
Cough	2.31 (0.49–10.92)	0.29		
Weight loss	2.62 (0.78–8.87)	0.12	2.38 (0.67–8.43)	0.10
Night sweats	1.76 (0.46–6.80)	0.41		
CXR infiltrate	0.58 (0.07–4.84)	0.61		

TB – tuberculosis; OR – odds ratio; CI – confidence interval; HAART – highly active antiretroviral therapy; CD – cluster of differentiation; OIs – opportunistic infections; CXR – chest X-ray.
* Statistically significant.

occurrence with different risk factors, such as age, sex, residence area, occupation, education, marital status, alcohol intake, tobacco or shisha use, previous TB contact history, stages of HIV or ART use and CD4 count; however, none of these factors significantly correlated with the occurrence of TB ($p > 0.05$). Similar observations were also reported earlier [27].

All the mycobacterial isolates tested in this study showed no resistance to all first-line anti-TB drugs, except for one isolate which was resistant to streptomycin. This is in contrast to previous MDR-TB reports conducted in the country, which is about 1.6% among new cases and 12% in re-treatment cases [9,28] and elsewhere [29–32]. It should be noted from the current study that only a few HIV positive cases that had at least one TB symptom but were undiagnosed at the attending clinic (newly diagnosed) or those who completed TB treatment at least 3 months prior to the study (previously treated

cases, but not retreatment cases) were considered, and hence a few TB-positive cases were identified, which may underestimate the overall prevalence of drug resistance among the HIV-infected population. On the other hand, a study from Cambodia showed a decreasing trend of anti-TB drug resistance (48% in 1999 to 7.9% in 2004) since the introduction of ART drugs [33]. These observations may possibly imply the fact that the introduction of ART may play a role in limiting the emergence of anti-TB drug resistance. Despite this, the observed prevalence in the current study could not be overlooked, as these identified cases could still be major sources of infection and transmission in the community.

In addition, TB is the most common opportunistic infection and is the leading cause of mortality in HIV-infected persons. TB disease has been shown to accelerate the natural course of HIV disease, and, similarly, HIV infection results in

a more rapid progression from latent TB infection to TB disease. However, diagnosing TB in HIV-infected persons is a major public health challenge.

In general, considering the observed prevalence of undiagnosed TB among HIV-positive patients, health professionals, particularly those working in ART facilities, should critically evaluate HIV patients clinically regardless of their ART use and actively screen for TB at least through microscopic examination of TB to detect cases early and initiate anti-TB or isoniazid preventive treatments. Further community-based surveys are warranted to confirm the current findings.

Conflict of interest

The authors declare that they have no competing interests.

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