Prevalence, associated factors and rifampicin resistance pattern of pulmonary tuberculosis among HIV-positive patients attending antiretroviral treatment clinic at East Gojjam Zone, Ethiopia: An institution-based cross-sectional study

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ABSTRACT
Background: Drug-resistant tuberculosis (TB) threatens global TB care and prevention, and it remains a major public health concern in many countries particularly in sub-Saharan countries. Pulmonary TB is the most common serious opportunistic infection on HIV-positive patients and it is the leading cause of death among HIV-positive patients in developing countries. Ethiopia is one of the high TB burden countries with high morbidity and mortality.
Objective: To determine the prevalence, associated factors and rifampicin resistance of pulmonary TB among HIV-positive attending antiretroviral treatment clinic at East Gojjam. Methods: Hospital-based cross-sessional study was conducted at Debre Markos Referral Hospital, from February to June 2019. A total of 112 HIV-positive TB suspected patients were included using convenient sampling techniques and a bacteriological confirmation test for tuberculosis was performed using Gene-Xpert MTB/RIF assay from a spot sputum sample. Viral load was determined by using a quantitative real-time polymerase chain reaction (RT-PCR) from the blood sample. Socio-demographic and clinical data were collected by face-to-face interview using a semi-structured questionnaire. The data were analyzed by using Statistical Package for Social Sciences (SPSS) software (version 24).
Result: Out of the 112 study participants, the prevalence of Pulmonary TB was 11.6 %. Among TB positives 23.1 % were rifampicin resistant. Rifampicin resistance was 100 % among female patients. Having family members treated for pulmonary TB (P = 0.003, [AOR = 4.5; 95 % CI = 3.59–58.8]), cigarette smoking (P = 0.039, [AOR = 2.18; 95 % CI = 1.17–40.5]), being on WHO HIV disease clinical stage II (P = 0.024, [AOR = 1.81; 95 % CI = 1.50–30.99]), and having viral load (1000–9999) RNA copies/ml (P = 0.031, [AOR = 1.54; 95 % CI = 1.32–31.41]) were found to be significantly associated with pulmonary TB.
Conclusion: The prevalence of pulmonary TB and rifampicin resistance was high among HIV patients. Having family members treated for Pulmonary TB, history of cigarette smoking, WHO HIV clinical stage, and high viral load were associated risk factors for TB. Therefore, strengthening awareness creation on TB transmission, drug resistance, and treatment adherence are essential. Moreover, early screening and treatment are vital for preventing the transmission and occurrence of drug-resistant TB among study populations.

1. Introduction
Pulmonary tuberculosis (TB) is a contagious air-borne bacterial infection caused by intracellular slow-growing acid-fast bacilli called Mycobacterium tuberculosis: that mainly infects the lungs. The disease is spread by close person-to-person contact through the inhalation of infectious aerosols [1,2]. According to the 2018 World Health Organization (WHO) report worldwide, TB is among one of the top 10 causes of
End TB Strategy. The End TB Strategy includes the targets of 80% associated factors among HIV-positive patients in Ethiopia, particularly in east Gojjam. Therefore, this study aimed to determine the prevalence, and WHO factors for TB [8–11].

Multidrug-resistant tuberculosis (MDR-TB) is TB resistant to two of the most important drugs Rifampin (RIF) and Isoniazid (INH) which are used to treat TB [5]. Drug-resistant TB continues to be a public health crisis. The best estimate is that, worldwide in 2018, about half a million people developed rifampicin resistance (RR), the most effective first-line drug, and of these, 78% had MDR-TB. Globally, about 3.5% of new TB cases and 18% of previously treated cases had MDR/RR-TB [3,7].

Different socio-economic and clinical factors contributed to the increased burden of TB among HIV-positive patients in sub-Saharan countries including Ethiopia. Among these HIV pandemics, depletion in CD4+ T cell, diabetes, history of previous TB, malnutrition, smoking, cigarette, alcoholism, poverty, homelessness, active TB contact, high viral load, and advanced WHO HIV disease clinical stages were significant factors for TB [8–11].

Global targets and milestones for reductions in the burden of TB disease have been set as part of the Sustainable Development Goals (includes a target to end the global TB epidemic by 2030) and WHO’s End TB Strategy. The End TB Strategy includes the targets of 80% reduction in the TB incidence rate and a 90% reduction in TB deaths between 2015 and 2030, with 2020 milestones of a 20% reduction in TB incidence rates and a 35% reduction in TB deaths. However, currently many high TB burden countries and most WHO regions were not on track to reach the End TB Strategy 2020 milestones [7].

Additionally, according to a WHO report, there are also big gaps in the detection and treatment of HIV-associated TB and MDR/RR-TB [3]. Ethiopia is one of the sub-Saharan countries with a high burden of TB and HIV infections. Assessment of associated factors, early detection using rapid diagnostic method and treatment is still vital solution to prevent the distribution of drug-resistant TB among the most susceptible and risk groups like HIV infected patients in resource-limited countries like Ethiopia. There is also limited data on the burden of TB and its associated factors among HIV-positive patients in Ethiopia, particularly in east Gojjam. Therefore, this study aimed to determine the prevalence, associated factors, and rifampicin pattern resistance of pulmonary tuberculosis among HIV-positive patients attending ART clinic at Debre Markos Referral Hospital, East Gojjam Zone, Ethiopia using the Gene-Xpert MTB/RIF assay method.

2. Materials and methods

2.1. Study area, design and period

The study was conducted at Debre Markos referral hospital located in Debre Markos town which is 299 km far away from the capital Addis Ababa and 256 Kms from Bahar Dar, the capital city of Amhara Region. Currently, the hospital serves people in East Gojjam Zone and neighboring towns. In addition to other services, the hospital has been providing anti-retroviral treatment follow-up care services from the time when 2005. An institution-based cross-sectional study was conducted from February to June 2019.

2.2. Study population and eligibility criteria

All HIV-positive patients attending ART clinic and clinically suspected of pulmonary TB having productive cough with or without blood for ≥ 2 weeks, night sweat, chest pain, weight loss and fatigue were the target population for this study. All HIV-positive patients who were suspected of TB and sent with sputum examination request paper to the hospital laboratory for sputum examination during the study period were included and those HIV positive unable to produce sputum and on TB treatment follow-up were excluded from the study.

2.3. Sample size determination and sampling technique

The sample size was determined using a single population proportion formula (N = by considering previous prevalence (P) of TB among HIV/AIDS patient 7.5% [12], 5% none response rate, 95% confidence level and 5% margin of error (d)). Therefore, by considering 5% non-response, the final calculated sample size was 112. A convenient sampling technique was used to recruit all PTB suspected known HIV positive patients attending the ART clinic in the hospital. The aims of the study and the benefits of participation were clearly explained to the participants before data collection and written informed consent was obtained. For the children <18 years informed consent was obtained from their parents/legal guardians and assent was obtained from children. Participation in the study was voluntary and each participant was informed that withdrawal from the study at any time during the data collection is their right.

2.4. Data collection and processing

2.4.1. Socio-demographic and clinical data collection

The data collection tool was prepared in English and translated to Amharic which is the local language of the area. The data on socio-demographic characteristics (age, sex, residence, marital status, family size, and occupation), clinical and behavioral information (contact history with PTB positives, family history of TB, smoking, feeding status per day, alcoholism, duration of HIV infection, diabetes mellitus, other chronic illness, viral load, WHO HIV clinical-stage, substance abuse, and CD4+ cell count) were collected by trained health professionals by face to face interview and referring patients medical records using a pre-tested structured questionnaire.

2.5. Laboratory data collection methods

2.5.1. Sputum specimen collection method and processing

After giving adequate instruction on how to collect appropriate purulent sputum specimens for voluntary study participants, a single spot sputum specimen was collected aseptically on a dry, leak-proof, labeled sputum cup. The collected sputum specimen was immediately submitted to TB diagnosing laboratory unit for investigations. Additionally, the sputum specimen was transported and processed according to the standard operating procedure of the Debre Markos Referral hospital TB diagnosing laboratory unit and general instructions of the manufacturer’s leaflet. After the arrival of the specimen to the laboratory, the sputum was inspected for physical appearance and those sputum specimens (saliva and very low volume) which do not fulfill the required specimen quality were discarded and new specimens re-collected.

2.5.2. Xpert MTB/RIF assay testing

The collected sputum specimen was processed and tested according to the WHO implementation manual for Gene-Xpert MTB/RIF assay (Cepheid, Inc.) with molecular technique, i.e. collected 2 ml sputum specimen was mixed with (8 ml) commercially prepared sodium chloride reagent. Then two to three ml of the prepared mixture was
transferred to pre-labeled cartilage and scanned with a barcode reader. Finally prepared specimen was loaded into Gene-Xpert MTB/RIF assay for testing [13,14]. Then the result of the Gene-Xpert assay was read and interpreted as RIF resistance detected, RIF resistance not detected and RIF resistance indeterminate according to the protocol [13,15].

2.5.3. Viral load testing
To determine the viral load of the study participant about 3–5 ml of venous blood was collected according to the standard operating procedure for venous blood collection for viral load. Then the collected blood specimen was transported to ART molecular laboratory for further processing. Finally, a viral load test was done by using a quantitative real-time polymerase chain reaction (QRT-PCR) HIV-1 assay with the COBAS® instrument (Roche, Homburg, Germany) following standard Biosafety precautions and protocol [16,17].

2.6. Data quality assurance
To ensure the quality of data; the data collectors were trained and the instrument was also pre-tested on at least 5 % of the respondents and quality control test was done before each step in laboratory tests and the necessary correction was made based on the findings. The Gene-Xpert Diagnostic System automatically performs internal quality control for each sample. The principal investigators frequently supervised the data collection process by checking the completeness of the required type of data to correct faults if any on the site of data collection and transportation of specimens following standard operational procedure. The data collection forms (questionnaires) were checked for completeness and consistency before data entry by the principal investigators.

2.7. Data analysis
Data was entered and analyzed using Statistical Package for Social Sciences (SPSS) software version 24. Summary findings were presented by tables. Descriptive statistics were used to determine the demographic characteristics and the prevalence. The potential predictor variables were tested in bivariate analyses separately for their association with prevalence. The variables which are significant in bivariate analysis at a cut point of P-value of <0.25 were a candidate for multiple logistic regression analysis. Finally a p-value <0.05 was used as the cut-off point for the presence of statistical significance.

3. Results
3.1. Socio-demographic characteristics of the study participants
A total of 112 HIV-positive patients participated in the study with a 100 % response rate. The mean age of the study participant was 37.17 (S.D. ± 13.75) years. Among the total study participants included in our study 68 (60.7 %) were males and the majority of the study participants 51 (45.5 %) were in the age group 30–44 years. Regarding occupation, the majority 29 (25.9 %) were farmers and around 56 (50 %) was living in a rural area (Table 1).

3.2. Clinical characteristics of study participants
The majority of the participants, 85 (75.9 %) and 97 (86.6 %) had no family members treated for TB and contact active TB cases respectively. The majority, 104 (92.9 %) of the study participants had no history of cigarette smoking. Regarding duration since tested positive for HIV, the majority of the study participants 55 (49.1 %) had been diagnosed with HIV over 11 years. According to WHO HIV disease clinical staging, 24 (21.4 %) of our study participants were in clinical stages three (Table 2).

### Table 1

| Variables         | Frequency percent (%) |
|-------------------|-----------------------|
| Age               |                       |
| <15               | 7                     | 6.3                  |
| 15–29             | 23                    | 20.5                 |
| 30–44             | 51                    | 45.5                 |
| ≥45               | 31                    | 27.7                 |
| Sex               |                       |
| Male              | 68                    | 60.7                 |
| Female            | 44                    | 39.3                 |
| Residence         |                       |
| Urban             | 56                    | 50                   |
| Rural             | 56                    | 50                   |
| Marital status    |                       |
| Married           | 89                    | 79.5                 |
| Single            | 17                    | 15.2                 |
| Divorced          | 6                     | 5.4                  |
| Educational status|                       |
| College and above | 32                    | 28.6                 |
| High school       | 9                     | 8                    |
| Elementary school | 15                    | 13.4                 |
| Read and write    | 30                    | 26.8                 |
| Cannot read and write | 26      | 23.2                 |
| Family size       |                       |
| <3                | 17                    | 15.2                 |
| 3–4               | 70                    | 62.5                 |
| ≥5                | 25                    | 22.3                 |

3.3. Prevalence of pulmonary tuberculosis
In our study, the overall prevalence of pulmonary TB among HIV-positive patients was 13 (11.6 %). The highest proportion of pulmonary TB was observed among the age group 15–26 years (21.7 %) followed by 45–59 years (16.1 %) (Fig. 1). The prevalence of TB among the female participant was 7 (15.9 %) which is almost twice more likely to acquire pulmonary TB than males (8.8 %), but there is no statistically significant difference between participant’s sex and TB (P > 0.05). The prevalence of pulmonary TB was high (20.0 %) among participants with family size ≥ 5 followed by 3–5 which was 8 (11.4 %). The highest prevalence 7 (29.1 %) was observed among study participants in WHO disease clinical stage III followed by clinical stage II 4 (9.7 %) with statistically significant difference (P < 0.05). None of the study participants were found in WHO disease clinical stage IV (Table 3).

3.4. Prevalence of rifampicin resistance
Among the total pulmonary TB-positive participants, the prevalence of rifampicin resistance was 3/13 (23.1 %). All rifampicin resistance was observed among female study participants without statistically significant difference (P > 0.05) and all of them have no previous history of TB (are primary MDR). Regarding the proportion of rifampicin resistance pattern among the age group, the highest resistance pattern 33.3 % was observed in the age group 30–44 (Fig. 1).

3.5. Factors associated with pulmonary tuberculosis
From variables tested for the presence of association with pulmonary TB among study participants, a multivariate logistic regression test showed that there was no statistically significant difference between the prevalence of pulmonary TB and socio-demographic characteristics such
as age, sex, residence, marital status, and family size (p-value > 0.05). However, having family members treated for pulmonary TB (P = 0.003, [AOR = 4.5; 95 % CI = 3.59–58.8]), cigarette smoking (P = 0.039, [AOR = 2.18; 95 %CI = 1.17–40.5]), being on WHO HIV disease clinical stage II (P = 0.024, [AOR = 1.81; 95 %CI = 1.50–30.99]), and having viral load 1000–9999 copies of viral RNA/ml of blood (P = 0.031, [AOR = 1.54; 95 %CI = 1.32–31.41]) were significantly associated with prevalence of pulmonary TB as shown in (Table 3).

4. Discussion

The majority of the people who are co-infected with TB/HIV live in sub-Saharan Africa. People living with HIV are most vulnerable to contracting active tuberculosis due to the weak body’s immune response [18]. In Ethiopia, TB remains the third major cause of hospital admissions and one of the leading causes of mortality [19].

In our study, the overall prevalence of pulmonary TB among HIV-positive patients attending antiretroviral treatment clinic in the study area was 11.6 %. This finding is comparable to the study findings reported from Ghana 12.7 % [20], Oyo state, Nigeria 12.6 % [21], Northwestern Tanzania 11.0 % [22], and Eastern India 12.3 % [23], but lower than the study findings reported from Guangxi, China 15.7 % [24], South Africa 32.7 % [25], Republic of Congo 29 % [26], Nigeria 22.9 % [27], South Central Ethiopia 20.3 % [28], South Ethiopia 15.5 % [29], and Amhara Region in Ethiopia 27.7 % [9]. However, our finding is higher than studies reports 9 % from South Sudan [30], 2.7 %–5.6 % from Nigeria [31,32], 2.08 % from Tanzania [33], 7.5 % from Arba Minch [8] and 7.2 % from North Gondar [12]. The possible explanation for the variation in prevalence among studies might be due to difference in geographical location, study design, sampling technique, study populations, improvement in awareness creation and diagnostic techniques used.

In this study, a higher prevalence of pulmonary TB was observed among female participants with no statistically significant difference. This finding is supported by another study conducted in South Central Ethiopia [28] and the Amhara region [9]. In contrast, a study report from Tanzania showed a high prevalence of TB among male patients [22]. The highest proportion of pulmonary TB was observed among the age group 15–26 years (21.7 %) in our study. Our study report is strengthened by a study report from another part of the country in which high prevalence was observed among ages >15 years [28]. These age groups are sexually active and involve actively in socio-economic and recreational activities that predispose them for Tb.

Infection by multi-drug resistant tuberculosis is a serious public health problem and result in high mortality among these populations due to Immunosuppression [34]. The prevalence of rifampicin-resistant Tuberculosis (RR-TB) in our study is 23.1 %. This finding is higher than study finding from HIV patients in Oyo state, Nigeria 12 % [21] and non-HIV patients in Espírito Santo, Brazil 5 % [35], Botswana 5.4 % [36], Yenagoa, Nigeria 14.7 % [37], and Addis Ababa 9.9 % [38]. The vulnerability of the HIV patient to infection by multi-drug resistant strains M. tuberculosis increases in the advanced HIV clinical stage in

| Clinical variables | Frequency | Percent (%) |
|--------------------|-----------|-------------|
| Having family members treated for TB | Yes | 27 | 24.1 |
| | No | 85 | 75.9 |
| Contact with family members with active TB | Yes | 15 | 13.4 |
| | No | 97 | 86.6 |
| Cigarette smoking | Yes | 8 | 7.1 |
| | No | 104 | 92.9 |
| Chronic disease* | Yes | 38 | 33.9 |
| | No | 74 | 66.1 |
| Duration of being HIV positive | <5 | 32 | 28.6 |
| | 5–10 | 25 | 22.3 |
| | ≥11 | 55 | 49.1 |
| Alcohol drinking | Yes | 45 | 40.2 |
| | No | 67 | 59.8 |
| WHO HIV clinical stage | Stage 1 | 47 | 42.0 |
| | Stage 2 | 41 | 36.6 |
| | Stage 3 | 24 | 21.4 |
| | Stage 4 | 0 | 0.0 |
| VIRAL load | <20 | 6 | 5.4 |
| | 20–999 | 59 | 52.7 |
| | 1000–9999 | 25 | 31.3 |
| | ≥9999 | 12 | 10.7 |
| Substance abuse | Yes | 3 | 2.7 |
| | No | 109 | 97.3 |

*Diabetes, Heart disease, Cancer, Kidney disease, Asthma.

![Fig. 1. Distribution of pulmonary TB and rifampicin resistance in different age groups detected from sputum samples of HIV-positive patients DMRH, Ethiopia, 2019.](image-url)
patients with immunosuppression. Therefore, variation in RR-TB among studies might be due to differences in the study population, sample size, distribution of resistant strains in different geographical locations, inadequate treatment or adherence to treatment, and use of rifampicin for treatment.

In the present study, having family members treated for pulmonary TB was significantly associated with pulmonary TB (P = 0.003, [AOR = 4.5; 95 %CI = 3.59–58.8]). This could be due to living with or close contact with pulmonary TB positive family will lead to latent TB and later which will reactivate during immune suppression among HIV patients compared with the general population [39,40]. In this study, cigarette smokers are twice more likely to have pulmonary TB as compared to non-cigarette smokers (P = 0.039, [AOR = 2.18; 95 %CI = 1.17–40.5]). It is a confirmed truth that cigarette smoking increases the risk of TB by dropping the body immune response in humans [41]. This study finding is supported by previous studies conducted in China [24], Arbaminch [8], and Gondar [11] reported smoking was significantly associated with TB among HIV patients.

In this study, being on WHO clinical disease stage II was statistically significantly associated with pulmonary TB among HIV-positive patients at antiretroviral therapy (P = 0.024, [AOR = 1.81; 95 %CI = 1.50–30.99]). This study finding is also supported by study reports from different parts of Ethiopia [10,12,28,29,42] and Tanzania [22] in which the advanced WHO clinical stage was significantly associated with TB (P = 0.031, [AOR = 1.54; 95 %CI = 1.32–31.4]). Lastly, having an increased HIV viral load level (1000–9999 copies of RNA/ml) in blood was significantly associated with TB. This is due to patients having a high viral load in their blood leading them to decline in their cell-mediated immune response, which predisposes them to opportunistic infections including TB [12,40,43].

5. Conclusions

The prevalence of TB and rifampicin resistance among HIV patients in the study area was high. Having family members treated for Pulmonary TB, history of cigarette smoking, WHO HIV disease clinical stage, high viral load were significantly associated with TB among HIV patients. Strengthening awareness creation on TB transmission, drug resistance and treatment adherence are very essential. Moreover, early screening and treatment are vital for preventing the occurrence and distribution of multi-drug resistant pulmonary TB among study populations and its morbidity and mortality in the region, particularly in the study area.
6. Strength and limitation of the study

The strength of the current study was the application of Gene-Xpert MTB/RIF assay as a diagnostic method that makes the current study differs from previous studies in the area. CD4 + T cell count of the patient was not determined.

Data sharing statement

Research data can be presented upon a reasonable request.

Ethical approval and consent to participate

The proposal was ethically reviewed and approved by Debre Markos University Health Science College, Department of Medical Laboratory Science. Following the endorsement by the research review committee, the selected Hospital was informed about the objective of the study through a support letter from the Department of Medical Laboratory Science. And written permission was secured from the hospital. The written informed consent was obtained from each participant’s parents/guardians before inclusion in the study (as per the Declaration of Helsinki 1975, as revised in 2013). The results of each study participants were communicated to their physicians. To preserve confidentiality, no personal identifiers were used on the data collection form.

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CRediT authorship contribution statement

Milkiyas Toru: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Resources, Data curation, Writing – original draft, Writing – review & editing, Visualization, Supervision, Project administration. Amanuel Baye: Conceptualization, Methodology, Software, Formal analysis, Investigation, Resources, Writing – original draft, Writing – review & editing. Zemenu Gebyeheu: Conceptualization, Methodology, Software, Formal analysis, Investigation, Resources, Writing – original draft, Writing – review & editing. Alemayehu Reta: Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Resources, Data curation, Writing – original draft, Writing – review & editing, Visualization, Supervision, Project administration.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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