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Burden of *Mycobacterium tuberculosis* and Associated Factors among Presumptive Extra pulmonary Tuberculosis Patients from Selected Health Facilities, Addis Ababa, Ethiopia



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ABSTRACT

Background: Extra pulmonary tuberculosis (EPTB) accounts for a significant proportion of tuberculosis (TB), a devastating disease of public health concern. The complexity of the cases, the involvement of many organs, resource constraints, and concerns regarding drug resistance make disease diagnosis and treatment difficult. This study aimed to determine the burden of tuberculosis and associated factors among presumptive EPTB patients in selected hospitals in Addis Ababa.

Material and methods: A cross-sectional study was conducted from February to August 2022 in selected public hospitals in Addis Ababa. Those who attended the hospitals and were presumptively diagnosed as EPTB patient were included in the study. Sociodemographic and clinical data were collected using a semistructured question-naire. The GeneXpert MTB/RIF assay, Mycobacterium Growth Indicator Tube (MGIT) culture, and solid culture using Löwenstein–Jensen (LJ) medium were used. The data were entered and analyzed using SPSS version 23, and a *p*-value ≤ 0.05 was considered as statistically significant.

Results: From a total of 308 participants enrolled in this study, the measured burdens of extrapulmonary tuberculosis using the Xpert MTB/RIF assay, liquid culture, and solid culture were 54 (17.5%), 45 (14.6%), and 39 (12.7%), respectively. In this study, sex, contact history with known TB cases, having a purulent type of aspirate, and being HIV positive had statistically significant associations with EPTB.

Conclusions: The burden of extrapulmonary tuberculosis among presumptive extrapulmonary tuberculosis cases was found to be significant. Sex, contact history with a known TB case, having apurulent type of aspirate, and being HIV positive were found to be associated with extrapulmonary tuberculosis infection. Strict adherence to the national tuberculosis diagnosis and treatment guidelines is important, while the true burden of the disease should be ascertained using standard diagnostic tests for better prevention and control interventions.

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Abbreviations: AFB, acid-fast bacilli; BHI, brain heart infusion; BSL-3, biosafety level 3; DM, diabetes mellitus; DNA, deoxyribonucleic acid; EPHI, Ethiopian Public Health Institute; EPTB, extrapulmonary tuberculosis; FNA, fine-needle aspiration; HIV, human immunodeficiency virus; LJ, Löwenstein–Jensen; *M. bovis, Mycobacterium tuberculosis*; MGIT, mycobacterium growth indicator tube; MTBC, mycobacterium tuberculosis complex; NAA, nucleic acid amplification; NALC, N-acetyl-L-cysteine; NaOH, sodium hydroxide; NTM, non-tuberculosis mycobacteria; NTRL, National Tuberculosis Reference Laboratory; PPE, personal protective equipment; PTB, pulmonary tuberculosis; SOP, standard operating procedure; SPHMMC, St Paul's Hospital Millennium Medical College; SPSS, Statistical Package for Social Sciences; TB, tuberculosis; TBL, tuberculosis lymphadenitis; WHO, World Health Organization; ZN, Ziehl–Neelsen.

Introduction

Tuberculosis (TB) is a major public health and economic concern caused by bacteria grouped in the genus *Mycobacterium*. It is believed that approximately 25% of the world's population is latently infected with tuberculosis. People who are latently infected with the bacterium have a 5–10% chance of contracting the disease throughout their lifetime. When the disease attacks the lungs, the condition is known as pulmonary tuberculosis [1]. Extra pulmonary tuberculosis is a form of tuberculosis that occurs outside the lung, usually resulting from hematogenous or lymphatic dissemination. Extra pulmonary tuberculosis is shought to account for 15–25% of all tuberculosis cases. Acid-fast bacilli staining, culture, and molecular-based methods are mostly used to diagnose the disease [2]. The diagnosis of EPTB is more difficult than for PTB, because the collection of tissue or fluid samples usually requires invasive manipulations of organs [3].

Tuberculosis disease is one of the top 10 causes of death and the leading infectious cause of death worldwide. In 2020, around 9.87 million people were diagnosed with TB, of whom around 1.5 million are expected to have died [4]. Both forms of TB are public health threats in Ethiopia. The country has the third-highest burden of EPTB cases worldwide, which is higher than PTB burdens in many other parts of the world [5]. According to WHO, Ethiopia is among the 30 countries with the highest overall TB burden. In 2020, the total TB incidence in the country was estimated to be around 151 000 cases, with a death rate of 21 500. The proportion of EPTB in the country during that year was 30% [4].

Extra pulmonary TB diagnosis is difficult because clinical samples are taken from inaccessible locations and may be paucibacillary, reducing the sensitivity of diagnostic testing. Every attempt should be made to obtain appropriate specimens for mycobacteriological and histopathological tests whenever possible [6]. A negative smear for acid-fast bacteria, the absence of granulomas on histology, and a failure to culture *M. tuberculosis* do not rule out the positivity of EPTB. New diagnostic methods, such as nucleic acid amplification, can be beneficial for a variety of EPTB types [7]. The proportion of EPTB is increasing, but there is an unexpected paucity of data in the medical literature on the prevalence and impact of the disease [8]. This may be because of challenges in diagnosing EPTB as a result of diverse manifestations of the disease [9]. Therefore, this study aimed to determine the burden of extrapulmonary tuberculosis and associated factors among presumptive extrapulmonary patients, using different diagnostic methods.

Materials and methods

Study design and study period

A cross-sectional study was conducted from February to August 2022, to determine the burden of tuberculosis and associated factors among presumptive EPTB patients in selected health facilities in Addis Ababa, Ethiopia. The facilities selected were St Paul's Hospital Millennium Medical College (SPHMMC), Ras Desta Damtew Memorial Hospital and Zewditu Memorial Hospital. The hospitals were selected specifically based on their accessibility and capability with regard to the proposed study design.

Study population

The study population consisted of all consecutive presumptive extrapulmonary tuberculosis patients who visited the selected public hospitals in Addis Ababa, Ethiopia.

Data collection

A pretested structured questionnaire was used to collect demographic and clinical data for all patients. Sociodemographic data were collected by trained health professionals. Clinical data were collected from clinicians, patients, and individual patient records.

Laboratory methods

Sample collection and transportation

Pathologists and physicians collected specimens from each presumptive EPTB patient under aseptic conditions. Fine needle aspirate, pleural fluid, peritoneal fluid, abscess, CSF, tissue biopsy, and genito-urinary samples were collected from each patient and transferred to a 50 ml Falcon tube. Half of the collected specimens were taken to the SPHMMC microbiology laboratory for the Xpert MTB/RIF assay and the remaining half were taken to the NTRL at EPHI via a cold chain. Samples to be processed within a week of collection were kept at 4°C. Samples to be processed within more than 1 week were stored at -20° C and brought to 4°C for 12 hours before the culture process.

GeneXpert MTB/RIF assay

The GeneXpert MTB/RIF assay (Cepheid, Sunnyvale, CA, USA) is a relatively rapid nucleic acid amplification (NAA) test. The test uses a cartridge that fits with the automated Xpert MTB/RIF assay instrument system, and can simultaneously detect the presence of MTBC and RIF resistance. The procedure was performed according to the manufacturer's instructions [10].

Mycobacterial culture (LJ and MGIT)

Both BACTEC MGIT 960 (BBL® MGITTM System) and LJ (egg-based) medium culture techniques were used to isolate bacteria. Samples were digested using freshly prepared N-acetyl-L-cysteine (NALC) and decontaminated using NaOH (1%). 50 ml of NaOH-Na citrate solution was added to 0.25 g of NALC powder to achieve a final concentration of 0.5%. Phosphate buffer (pH = 6.8) was added to neutralize the NaOH. Centrifugation was then used to concentrate the specimens (3000 RPM for 15 min). The supernatant was discarded, and the sediment was resuspended in 2 ml of sterile phosphate buffer. This suspension was then used to inoculate BACTEC MGIT 960 culture tubes (0.5 ml) and LJ slants (2–3 drops).

In liquid medium, a mycobacterial culture supplement was mixed with a collection of antimicrobials called PANTA, which is used to support the growth of mycobacteria and inhibit other microorganisms. The tubes for the solid culture were incubated at 37°C in a slant position to ensure an even distribution of inoculums for 1 week, and thereafter in a straight position for another 7 weeks. The tubes were checked once every week for mycobacterial growth. The MGIT tubes were inoculated using an automated MGIT 960 culture system, in which the machine detects the growth of oxygen-consuming bacteria every hour, indicating the presence of growth through UV fluorescence. The MGIT culture was followed for 42 days, beyond which the system automatically considered growth as negative. The machine shows only the presence of growth and does not identify bacterial isolates. Print-outs for both positive and negative culture results were accessed by scanning bar codes on the tubes [11].

Identification of MTBC

Tubes with no growth were considered as negative after 8 weeks of incubation on LJ medium, while the machine itself registered a negative result for tubes after 42 days in MGIT culture. Positive liquid cultures were subcultured on brain heart infusion agar plates and incubated for 48 hours at 37°C to identify potential contaminants. All positive tubes were confirmed by ZN staining and using an MPT64 protein-specific immunochromatographic test (SD Bioline Kit, Standard Diagnostics, Inc., Korea). Those isolates that were AFB positive, BHI growth negative, and SD Bioline positive were confirmed as MTBC positive. Isolates that were AFB positive, BHI growth negative, and SD Bioline negative were confirmed as non-tuberculosis mycobacteria (NTM) [11].

Table 1

Sociodemographic characteristics and burden of tuberculosis among presumptive extrapulmonary tuberculosis patients in selected health facilities in Addis Ababa, Ethiopia, 2022

		n	Gene Xpert result		Culture result/MGIT	
Variables		(%)	Negative n (%)	Positive n (%)	Negative n (%)	Positive n (%)
Age category						
	≤ 5	13 (4.2)	10 (76.9)	3 (23.1)	12 (92.3)	1 (7.7)
	6–20	44 (14.3)	34 (77.3)	10 (22.7)	37 (84.1)	7 (15.9)
	21-40	147 (47.7)	122 (83.0)	25 (17.0)	123 (83.7)	24 (16.3)
	41-60	73 (23.7)	59 (80.8)	14 (19.2)	62 (84.9)	11 (15.1)
	≥ 61	31 (10.1)	29 (93.5)	2 (6.5)	29 (93.5)	2 (6.5)
Sex						
	Male	148 (48.1)	120 (81.1)	28 (18.9)	119 (80.4)	29 (19.6)
	Female	160 (51.9)	134 (83.8)	26 (16.2)	144 (90.0)	16 (10.0)
Marital status						
	Single	119 (38.6)	96 (80.7)	23 (19.3)	103 (86.6)	16 (13.4)
	Married	167 (54.2)	140 (83.8)	27 (16.2)	141 (84.4)	26 (15.6)
	Divorced	11 (3.6)	8 (72.7)	3 (27.3)	9 (81.8)	2 (18.1)
	Widowed	11 (3.6)	10 (90.9)	1 (9.1)	10 (90.9)	1 (9.1)
Educational status						
	Illiterate	89 (28.9)	76 (85.4)	13 (14.6)	80 (89.9)	9 (10.1)
	Primary school	73 (23.7)	58 (79.5)	15 (20.5)	55 (75.3)	18 (24.7)
	Secondary school	69 (22.4)	51 (73.9)	18 (26.1)	57 (82.6)	12 (17.4)
	College diploma	57 (18.5)	51 (89.5)	6 (10.5)	52 (91.2)	5 (8.8)
	\geq Degree	20 (6.5)	18 (90.0)	2 (10.0)	19 (95.0)	1 (5.0)
Residency						
	Urban	207 (67.2)	169 (81.6)	38 (18.4)	175 (84.5)	32 (15.5)
	Rural	101 (32.8)	85 (84.2)	16 (15.8)	88 (87.1)	13 (12.9)
Family size						
	≤ 3	49 (15.9)	38 (77.6)	11 (22.4)	40 (81.6)	9 (18.4)
	4–6	219 (71.1)	184 (84.1)	35 (16.0)	192 (87.7)	27 (12.3)
	≥ 7	40 (13.0)	32 (80.0)	8 (20.0)	30 (75.0)	10 (25.0)

MGIT = mycobacterial growth indicator tube

Data analysis

Data for each study participant were collected at each stage and finally analyzed using SPSS version 23 software (IBM Corporation, NY, USA). Descriptive statistics were used to show quantitative data. Binary and multinomial logistic regression analyses were conducted to determine the association of independent variables with the dependent variable. Values of $p \le 0.05$ were considered to be statistically significant.

Results

Sociodemographic characteristics of participants

Of a total of 308 study participants, more than half (51.9%) were females. The mean age of the participants was 35.74 years, with a standard deviation of \pm 17.73 years. The majority (167; 54.2%) were married, 89 (28.9%) were illiterate, and 207 (67.2%) came from urban settings. The sociodemographic characteristics of the participants are summarized in Table 1.

Clinical characteristics of participants

Of the 308 study participants, 276 (89.6%) were new presumptive patients, while 32 (10.4%) had a disease relapse. Only 21 (6.8%) had known contact with TB cases. Of all study participants, 29 (9.4%) were HIV positive and 39 (12.7%) had known COVID-19 history. The clinical characteristics of the patients are summarized in Table 2.

The majority of the study participants were recruited from OPD, pathology, and emergency wards, accounting for 113 (36.7%), 95 (30.8%), and 33 (10.7%) respectively. The ward distribution of study participants is shown in Figure 1.

The patients' samples included pleural fluid, FNA, peritoneal fluid, tissue biopsy, genito-urinary samples, CSF, abscess, and bone tissue. Pleural fluid was the predominant sample type, accounting for 99 of the cases (32.1%), followed by FNA and peritoneal fluid, accounting for

91 (29.5%) and 44 (14.3%) of cases, respectively. Figure 2 shows the frequency distribution of sample types.

Burden of Mycobacterium tuberculosis

Xpert MTB/RIF assay results

The burden of MTBC was found to be 54 (17.5%) from a total of 308 samples evaluated using the Xpert MTB/RIF assay. Only one sample tested positive for rifampicin resistance. Of the 54 Xpert MTB/RIFpositive cases, 34 (63%) were culture positive and 20 (37%) were culture negative, while 11 (4.3%) of the Xpert MTB/RIF-negative cases were culture positive. Despite the higher number of female participants, males outnumbered females in the Xpert MTB/RIF-positive cases by 51.9% to 48.1%. The majority 24 (53.3%) of the positive cases were between the ages of 21 and 40. Tuberculosis lymphadenitis (TBL) accounted for 34 (63%) of the 54 Xpert MTB/RIF-positive patients, with pleural fluid and abscess samples accounting for seven (13%) and five (9.3%) of the positive cases, respectively. Nine (16.7%) of all positive cases had a known contact with a TB patient. Of the 54 positive cases, 37 (68.5%) were new cases, whereas 17 (31.5%) were relapses. Purulent aspirate samples exhibited a high positivity rate, accounting for 81.5% of all positive cases. Ten (18.5%) and nine (16.7%) of the positive cases had known HIV and COVID-19 positivity history, respectively.

Culture results

The burden of culture-positive EPTB among 308 presumptive EPTB patients using MGIT was 45 (14.6%). One isolate was NTM, while the remaining 44 isolates were MTBC. When employing LJ medium, the burden of culture-positive results was 39 (12.7%). As for the the Xpert MTB/RIF results, the majority of culture-positive cases (53.3%) were between the ages of 21 and 40. Most culture-positive samples (64.4%) were from male subjects. The sociodemographic characteristics and the burden of tuberculosis among the study participants are summarized in Table 3. Out of 45 MGIT culture-positive cases, seven (15.6%) had known contact history with TB patients and 40 (88.9%) were new cases.

Table 2

Clinical characteristics and burden of tuberculosis among presumptive extrapulmonary patients in selected health facilities in Addis Ababa, Ethiopia, 2022

		Frequency	GeneXpert result		Culture result/MGIT	
Variable		(%)	Negative n (%)	Positive n (%)	Negative n (%)	Positive n (%)
Contact history with TB patient						
	No	153 (49.7)	121 (79.1)	32 (20.9)	123 (80.4)	30 (19.6)
	Yes	21 (6.8)	12 (57.1)	9 (42.9)	14 (66.7)	7 (33.3)
	Unknown	134 (43.5)	121 (15.7)	13 (22.3)	126 (94.0)	8 (6.0)
Fever						
	No	138 (44.8)	123 (89.1)	15 (10.9)	125 (90.6)	13 (9.4)
	Yes	170 (55.2)	131 (77.1)	39 (22.9)	138 (81.2)	32 (18.8)
Weight loss						
	No	78 (25.3)	64 (82.1)	14 (17.9)	66 (84.6)	12 (15.4)
	Yes	230 (74.7)	190 (82.6)	40 (17.4)	197 (85.7)	33 (14.3)
Cough						
	No	169 (54.9)	132 (78.1)	37 (21.9)	139 (82.2)	30 (17.8)
	Yes	139 (45.1)	122 (87.8)	17 (12.2)	124 (89.2)	15 (10.8)
Case type						
	New	276 (89.6)	239 (86.6)	37 (13.4)	236 (85.5)	40 (14.5)
	Relapse	32 (10.4)	15 (4.7)	17 (5.3)	27 (84.4)	5 (15.6)
Site of infection						
	TBL	91 (29.5)	57 (62.6)	34 (37.4)	59 (64.8)	32 (35.2)
	CSF	13 (4.2)	13 (100.0)	0 (0.0)	13 (100.0)	0 (0.0)
	Pleura	99 (32.1)	92 (92.9)	7 (7.1)	93 (93.9)	6 (6.1)
	Bone	3 (1.0)	2 (66.7)	1 (33.3)	3 (100.0)	0 (0.0)
	Peritoneum	44 (14.3)	43 (97.7)	1 (2.3)	41 (93.2)	3 (6.8)
	Genito-urinary	22 (7.1)	20 (90.9)	2 (9.1)	21 (95.5)	1 (4.5)
	Biopsy	26 (8.4)	22 (84.6)	4 (15.4)	24 (92.3)	2 (7.7)
	Abscess	10 (3.2)	5	5 (9.3)	9 (90.0)	1 (10.0)
Duration of illness						
	\leq 6 month	218 (70.8)	177	41 (75.9)	182 (83.5)	36 (16.5)
	6 months to 1 year	62 (20.1)	53	9 (16.7)	56 (90.3)	6 (9.7)
	1–2 years	17 (5.5)	15	2 (3.7)	16 (94.1)	1 (5.9)
	≥ 2 years	11 (3.6)	9	2 (3.7)	9 (81.8)	2 (18.2)
Type of aspirate						
	Hemorrhagic	40 (13.0)	37	3 (5.6)	35 (87.5)	5 (12.5)
	Purulent	88 (28.6)	44	44 (81.5)	57 (64.8)	31 (35.2)
	Caseous	17 (5.5)	15	2 (3.7)	15 (88.2)	2 (11.8)
	Normal	163 (52.9)	158	5 (9.3)	156 (95.7)	7 (4.3)
HIV status						
	Negative	171 (55.5)	143	28 (51.9)	151 (88.3)	20 (11.7)
	Positive	29 (9.4)	19	10 (18.5)	22 (75.9)	7 (24.1)
	Unknown	108 (35.1)	92	16 (29.6)	90 (83.3)	18 (16.7)
History of COVID-19						
	No	269 (87.3)	224	45 (83.3)	231 (85.9)	38 (14.1)
	Yes	39 (12.7)	30	9 (16.7)	32 (82.1)	7 (17.9)

MGIT = mycobacterium growth indicator tube, HIV = human immunodeficieny virus, COVID-19 = coronavirus disease 19, TBL = tuberculosis lymphadenitis, CSF = cerebrospinal fluid



Figure 1. Distribution of study participants among wards in selected health facilities, Addis Ababa, Ethiopia, 2022



Figure 2. Sites of infection and types of sample collected from presumptive extrapulmonary tuberculosis patients in selected health facilities Addis Ababa, Ethiopia, 2022.

Table 3

Burden of tuberculosis, and bivariate and multivariate analysis of associated factors among presumptive extrapulmonary tuberculosis patients in selected health facilities, Addis Ababa, Ethiopia, 2022

Variables		Frequency, n	Test result/	MGIT	Bivariate analysis		Multivariate analysis	
		(%)	Pos (%)	Neg (%)	COR (95% CI)	<i>p</i> -value	AOR (95% CI)	p-value
Sex	Male	148 (48.1)	29 (64.4)	119 (45.2)	2.193 (1.137, 4.230)	0.019	0.238 (0.097, 0.583)	0.002
	Female	160 (51.9)	16 (35.6)	144 (54.8)	_	-	-	-
Educational status	Illiterate	89 (28.9)	9 (20.0)	80 (30.4)	2.137 (0.255, 17.909)	0.484	0.164 (0.013, 2.105)	0.165
	Primary	73 (23.7)	18 (40.0)	55 (20.9)	6.218 (0.777, 49.779)	0.085	0.120 (0.011, 1.367)	0.088
	Secondary	69 (22.4)	12 (26.7)	57 (21.7)	4.000 (0.487, 32.829)	0.197	0.089 (0.007, 1.095)	0.059
	Diploma	57 (18.5)	5 (11.1)	52 (19.8)	1.827 (0.200, 16.660)	0.593	0.163 (0.012, 2.133)	0.167
	\geq Degree	20 (6.5)	1 (2.2)	19 (7.2)			-	-
Contact history	Yes	21 (6.8)	30 (66.7)	123 (46.8)	3.841 (1.694, 8.709)	0.001	0.150 (0.034, 0.667)	0.013
	No	153 (49.7)	7 (15.6)	14 (5.3)	-			
	Unknown	134 (43.5)	8 (17.8)	126 (47.9)	7.875 (2.481, 24.993)	0.000	0.244 (0.085, 0.705)	0.091
Fever	No	138 (44.8)	13 (28.9)	125 (47.5)	_	-		
	Yes	170 (55.2)	32 (71.1)	138 (52.5)	0.449 (0.225, 0.893)	0.022	3.751 (1.435, 9.807)	0.007
Cough	No	169 (54.9)	30 (66.7)	139 (52.9)	_	-	-	-
	Yes	139 (45.1)	15 (33.3)	124 (47.1)	1.784 (0.917, 3.471)	0.088	0.890 (0.293, 2.707)	0.838
Site of infection	FNA	91 (29.5)	32 (10.4)	59 (19.2)	4.881 (0.592, 40.275)	0.141	0.192 (0.018, 1.995)	0.167
	CSF	13 (4.2)	0 (0.0)	13 (4.2)	0.000 (0.000, 0.000)	0.999		0.998
	Pleura	99 (32.1)	6 (1.9)	93 (30.2)	0.581 (0.063, 5.372)	0.632	0.439 (0.032, 6.069)	0.539
	Bone	3 (1.0)	0 (0.0)	3 (1.0)	0.000 (0.000, 0.000)	0.999	-	-
	Peritoneum	44 (14.3)	3 (1.0)	41 (13.3)	0.659 (0.061, 7.083)	0.730	0.336 (0.019, 5.963)	0.457
	Genito-urinary	22 (7.1)	1 (0.3)	21 (6.8)	0.429 (0.024, 7.632)	0.564	0.575 (0.023, 14.612)	0.737
	Biopsy	26 (8.4)	2 (0.6)	24 (7.8)	0.750 (0.060, 9.319)	0.823	1.733 (0.102, 29.482)	0.704
	Abscess	10 (3.2)	1 (0.3)	9 (2.9)				
Aspirate type	Hemorrhagic	40 (13.0)	5 (11.1)	35 (13.3)	3.184 (0.954, 10.621)	0.060	0.755 (0.163, 3.492)	0.719
	Purulent	88 (28.6)	31 (68.9)	57 (21.7)	12.120 (5.055, 29.060)	0.000	0.139 (0.036, 0.535)	0.004
	Caseous	17 (5.5)	2 (4.4)	15 (5.7)	2.971 (0.566, 15.602)	0.196	0.367 (0.036, 3.704)	0.395
	Normal	163 (52.9)	7 (15.6)	156 (59.3)	-	-	-	-
HIV status	Negative	171 (55.5)	20 (44.4)	151 (57.4)		0.171	-	-
	Positive	29 (9.4)	7 (15.6)	22 (8.4)	0.662 (0.333, 1.318)	0.241	3.399 (1.309, 8.829)	0.012
	Unknown	108 (35.1)	18 (40)	90 (34.2)	1.591 (0.591, 4.280)	0.358	0.573 (0.138, 2.379)	0.444

COR = crude odds ratio, AOR = adjusted odds ratio, CI = confidence interval, yr = year

The majority 32/45 (71.1%) of the culture-positive cases were TBL. Purulent aspirates exhibited a high positivity rate, accounting for (68.9%) of all positive cases. Seven (15.6%) of the positive cases had known positivity status for HIV and COVID-19 history. The clinical characteristics and burden of tuberculosis among presumptive extra pulmonary patients are summarized in Table 2.

Factors associated with M. tuberculosis

Based on the binary logistic regression, sex (COR = 2.193, 95% CI 1.137–4.230; p = 0.019), contact history with a known TB case (COR = 3.814, 95% CI 1.694–8.709; p = 0.001), site of infection (COR = 0.630, 95% CI 0.508–0.782; p = 0.000), fever (COR = 0.449, 95% 0.225–0.893; p = 0.022), and purulent aspirate (COR = 12.120, 95% 5.055–29.060; p = 0.000) were found to be statistically significant for culture results, with a 95% confidence interval. Multinomial

Discussion

A total of 308 study subjects participated in this investigation, and the burden of confirmed tuberculosis among the EPTB presumptive patients was 45 (14.6%), using MGIT culture. The Xpert MTB/RIF assay showed the frequency of MTBC to be 54 (17.5%). Sex, contact history with known TB cases, having a purulent type of aspirate, and being HIV positive had statistically significant associations with EPTB infection.

logistic regression tests performed for variables with $p \le 0.25$ and sex (AOR = 0.238, 95% CI 0.097–0.583; p = 0.002), known contact

history (AOR = 0.150, CI 0.034–0.667; *p* = 0.013), purulent aspirate

(AOR = 0.139, CI 0.036-0.535; p = 0.004), and being HIV positive

(AOR = 3.399, CI 1.309–8.829; p = 0.012) were found to be statisti-

cally significant for culture results, with a 95% confidence interval.

The frequency of EPTB (MTBC) according to the Xpert MTB/RIF assay was consistent with studies conducted in Morocco (20.6%), and findings from Goba, southeast Ethiopia (15.3%) [12,13]. Our findings showed that the prevalence of MTBC was lower than in studies conducted in Dessie (23.4%), Gondar (26.2%), and Addis Ababa (49.3%) [14–16]. These differences could be the result of a lower proportion of TBL samples in the Dessie study, and a lower proportion of TBL samples and relatively fewer HIV-positive patients included in the Gondar study. In the Addis Ababa study, not only was the number of TBL participants relatively low, but also the positivity rate for TBL was much higher than for other sites of infection.

Our study showed a higher culture prevalence of tuberculosis among presumptive EPTB patients than the study conducted in Dessie (8.8%) [17], which could be due to sociodemographic differences among the study participants. The culture prevalence was also higher than in a study conducted in Bangladesh (10.5%) [18]. One possible reason for this difference was the solid culture technique used in the latter study, which has a relatively lower sensitivity compared with liquid culture. Our study found a lower frequency than studies conducted in Gondar (29.8%), northern Ethiopia (51.7%), and Addis Ababa (44.7% and 29.5%) [15,16,19,20]. The studies in northern Ethiopia and Addis Ababa were conducted entirely on TBL patients, who show a higher positivity rate than those with other sites of infection. Likewise, other studies cited have included a high proportion of TBL patients and relatively high HIV positivity rates, which could explain the variation in results.

There was no significant patient or bacterial strain factor identified as being responsible for the high occurrence of TBL, and there was no link to HIV infection. Since the 1990s, the prevalence of EPTB, the majority of which is TBL, has increased progressively among TB patients in Ethiopia, reaching an average of roughly 33% across the country. The idea that TBL in Ethiopia was caused by zoonotic transmission of *M. bovis* from cattle was recently examined, but molecular identification of the causal organisms revealed that *M. bovis* contributed to less than 1% of human TB [21].

Phylogenetic analysis has revealed that dissemination from the lungs to extrapulmonary areas is just as common as dissemination between lung sites, lending credence to the hypothesis of similar migration paths within and between organs, at least in HIV patients. As a result, genomic diversity provides a record of disease diversification and repeated spread throughout the body [22]. In the case of TBL, mycobacteria are actively phagocytosed by macrophages after invasion. These then collect in regional lymph nodes, resulting in clinical symptoms of lymph node TB. *Mycobacterium tuberculosis* complex spread to different tissues is usually limited in immune-competent hosts. However, in immunocompromised patients, tubercle bacilli can spread to other regions of the human body.

In this study, male participants accounted for the highest proportion of culture-positive EPTB cases (64.4%). This finding was in agreement with the studies carried out in Gondar and Dessie [14,15], and could be due to underreporting, because of social and cultural factors. The finding differed from the previous study conducted in Bangladesh [18], which could be due to a relatively lower sample size and various social factors. The presence of contact history with known TB patients increased the chance of becoming diseased to four times more than for those who had no contact history. This finding was supported by studies conducted in Goba and Gondar [13,15]. In our study, TBL was the most common confirmed type of EPTB (71.1%). This finding was supported by reports from Dessie (48.1%) and Gondar (78.4%) [14,15]. Purulent aspirate was the common type of aspirate among the positive cases (68.9%). This was supported by findings in Addis Ababa [16]. The reason for this could be due to the high bacterial burden in purulent aspirates from patients at an advanced stage of the disease.

Comparing the diagnostic performances of different diagnostic procedures for different types of EPTB is problematic. FNA cytology, a less intrusive method than excision biopsy, has become more significant in the diagnosis of TBL. Unfortunately, the amount of material recovered in FNA is often so tiny that it is insufficient for using in AFB smear and culture examinations [23]. Identifying tubercle bacilli in pleural fluid and pleural biopsy specimens, or revealing granulomas in pleural tissue, are insensitive and time-consuming methods of diagnosing pleural TB. In TB meningitis, the volume of CSF sample is critical for achieving acceptable PCR findings, and therefore difficulties in obtaining an appropriate volume of CSF sample make diagnosis of TB meningitis a formidable problem [24].

The findings of this study clearly indicated that the WHOrecommended diagnostic approaches showed relatively lower frequencies of confirmed EPTB cases than expected due to variations in diagnostic capability among the different techniques. This demonstrates the need to increase the availability and capability of standardized tuberculosis diagnosis laboratories in health facilities in order to diagnose and treat the disease more effectively.

Limitations of the study

The hospitals were selected based on their accessibility rather than their representativeness in the city, which means the results may not show the actual burden of the disease. The sample of study participants was relatively small. Other tests, such as drug susceptibility testing and molecular characterization of the isolates, were not performed.

Conclusion

Our study revealed a significant burden of EPTB in the study area.

Tuberculosis lymphadenitis was the predominant presentation among cases. Sex, contact history with known TB cases, having a purulent type of aspirate, and being HIV positive were found to have statistically significant associations with EPTB infection. As a result, clinicians, public health experts, and health policy decision makers should give more attention to EPTB case detection, treatment, prevention, and control by adhering to national and WHO guidelines.

Declarations

Consent for publication

Not applicable. This study did not include any individual or personal data.

Availability of data and materials

All data relevant to this study are available within the manuscript.

Authors' contributions

Elias Alehegn: conceptualization, methodology, investigation, software, and manuscript – drafting. Addisu Gize: conceptualization, methodology, and manuscript – editing of final draft. Alganesh Gebreyohannes: conceptualization and methodology. Bereket Berhane: conceptualization and methodology. Jayne Wright: conceptualization and manuscript – writing, reviewing, and editing. Mengistu Fantahun: validation. Michael Hailu: validation and resources. Bedo Buta: validation and resources. Melkayehu Kassa: validation. Tsedale Woldu: validation. Natnael Dejene: validation. Nuhamin Zena: validation. Tewodros Tesfaye: validation. Azeb Gezahegn: validation. Tilahun Getinet: formal analysis.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

Ethical statement

Ethical clearance was obtained from the institutional review board (IRB) of SPHMMC and the Addis Ababa Public Health Research and Emergency Management Directorate. Informed consent was obtained from each study participant. Participants who tested positive for EPTB had their results forwarded to clinicians for treatment. Any information gathered during the course of the study remained confidential. Furthermore, the clinical specimens collected during the study period were used solely for the stated objectives.

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