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Evaluation of molecular and bacteriological detection methods performed on the formalin-fixed paraffin-embedded biopsy samples collected from endometrial and lymph node tuberculosis suspected patients

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Abstract

Background Endometrial Tuberculosis is one of the most common gynecological problems known to have serious implications for the quality of life like infertility. The commonly practiced histopathology solely relies on the suggestive feature of Tuberculosis (TB) with low specificity. Regarding the alternative bacteriological and molecular detection tools, little evidence was generated on their utility in the diagnosis of endometrial tuberculosis in Ethiopia. Therefore, we aim to investigate the detection rate of molecular and bacteriological detection methods on formalin-fixed paraffin-embedded biopsy samples for the diagnosis of endometrial and lymph node TB.

Methods A retrospective cross-sectional study was conducted on 90 formalin fixed paraffin embedded biopsy samples from patients with gynecologic and lymph problems collected between 2018 and 2022 at St. Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia. SPSS version 26 was used for statistical analysis. The diagnostic performance was calculated using the histopathology method as the reference standard. Cohen's Kappa value was used to measure the level of agreement. A test with a *P*-value of < 0.05 was considered statistically significant.

Results A total of 90 samples were analyzed in the current study. Auramine O, GeneXpert MTB/RIF assay, and Real-Time PCR tests have shown a detection rate of 32/90 (36%), 43/90 (47.8%), and 54/90 (60%) respectively ($P \leq 0.01$). The sensitivity and specificity of AO were 38.1% and 95% respectively. RT PCR showed superior sensitivity followed by

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GeneXpert MTB/RIF assay, 70% and 58.6%. AO and molecular methods have shown a similarly low level of agreement with histopathology (Kappa value = 0.2).

Conclusions In a resource-limited setting, the selection of diagnostic tools needs careful attention. Putting the patients on anti-TB treatments based solely on histopathological findings may lead to undesired and adverse complications. Therefore, applying molecular and bacteriological detection methods along with histopathology, could help minimize inappropriate antimicrobial use.

Keywords Endometrial TB, Auramine O, GeneXpert MTB/RIF assay, RT PCR

Introduction

Mycobacterium tuberculosis (MTB) often affects organs other than the lung resulting in the development of Extrapulmonary tuberculosis (EPTB) [1]. The prevalence of EPTB in Ethiopia was reported to be 43% [2]. The contribution of EPTB was also reported to be 15–20% of the total TB cases reported [3]. Tuberculous lymphadenitis (TBLN), an infection of the lymph node by MTB, was the most frequently reported form of EPTB. Endometrial TB (ETB), on the other hand, has a relatively lower contribution among EPTB cases, however, reports have shown a varying degree of prevalence [4]. The burden of ETB in females was reported to be underestimated as most of the patients are asymptomatic and usually diagnosed during evaluation for infertility [5]. The prevalence of ETB increases in countries with a high burden of Pulmonary Tuberculosis (PTB) [6].

The diagnosis of ETB & TBLN often is associated with challenges, due to the non-specific nature of the clinical manifestation of the condition and the absence of awareness of the clinicians [7]. In a resource-limited setting, histopathology is the most widely practiced diagnostic tool for ETB & TBLN. It is based on the demonstration of the typical caseous granulomatous lesion, which is highly suggestive of TB, however, many other conditions may result in granuloma formation. As the suggestive feature is less specific to TB, it requires differential diagnosis from other conditions that may result in granuloma formation [8]. Other diagnostic modalities such as Ziehl-Neelsen (ZN) staining and culture were often less practiced due to lack of sensitivity and time-consuming nature.

The utility of Auramine O staining in the diagnosis of other forms of EPTB such as TBLN waswas shown to have considerable sensitivity, be user friendly, and less time-consuming [9, 10]. Regarding the diagnosis of ETB, a limited amount of evidence is available. GeneXpert MTB/RIF assay, the World Health Organization (WHO) endorsed automated molecular method, has been also considered a very useful tool in the diagnosis of tuberculosis forthwith [11]. Often, its application is limited to the diagnosis of pulmonary tuberculosis, but some recent studies reveal the very use of GeneXpert MTB/RIF assay in the analysis of several types of specimens and have proven to be effective in the detection of the presence of a very small number of bacilli. Another molecular method, RT PCR, was also shown to be effective in the diagnosis of different forms of TB [12, 13]. The molecular tests such as *G*eneXpert MTB RIF/assay and RT PCR are also reported to be rapid tests with higher sensitivity and are useful not only to deal with the paucity nature of the EPTB but also to detect the disease before it gets worse and damage the organs where the bacilli could be disseminated like the female genital organ where the consequences could be infertility [14]. Despite, the methods were proven to be sensitive, easy, and adaptable, there is a paucity of data in Ethiopia regarding their performance characteristic in the diagnosis of ETB.

Therefore, this study aimed to evaluate the diagnostic utility of bacteriological and molecular detection methods such as AO, ZN, and molecular methods on formalin-fixed paraffin embedded (FFPE) archived biopsy samples from ETB and TBLN suspected patients attending St. Paul Specialized Hospital, Addis Ababa, Ethiopia.

Materials and methods

Study settings

A retrospective cross-sectional study was conducted on 90 formalin-fixed embedded paraffin-embedded (FFPE) biopsy samples from patients with gynecologic and lymphadenitis problems collected between 2018 and 2022 at the Pathology Department of St. Paul's Hospital Millennium Medical College (SPHMMC), Addis Ababa, Ethiopia. The archived data was accessed on July 04, 2022. The data was accessed using a Medical Record Number (MRN) and authors didn't have access to other personal identifiers of the participants. The biopsy sample consists of 60 Endometrial and 30 TB lymphadenitis suspected patients. The blocks were stored at room temperature. The laboratory work was done at Armauer Hansen Research Institute (AHRI) from August 2022 – December 2022. Clinical information was extracted using a structured questionnaire from the database of IT unit at SPHMMC Pathology department. SPSS software package (Version 26) was used for statistical analysis. Due to the nature of the samples used in this study, FFPE biopsies, culture hasn't been conducted. Therefore, the sensitivity, specificity, and positive and negative predictive values

including their 95% confidence intervals (CI) were calculated by using histopathology results as the "reference standard". Samples with a negative outcome by histopathology were used as a negative control for specificity determination. Cohen's Kappa value was used to measure inter-rater variability and level of agreement. All statistical tests were considered significant if the P-value was <0.05. The study was conducted after obtaining ethical approval (Protocol number: PM23/797) from St. Paul's Hospital Millennium Medical College Ethical Review Board, Addis Ababa, Ethiopia. The need for informed consent was waived by the St. Paul's Hospital Millennium Medical College Ethical Review Board, Addis Ababa, Ethiopia.

Laboratory procedures

Tissue sectioning

Tissue sections of 4 µm thick were prepared using a rotary microtome (Leica RM2255, Germany) from each FFPE tissue after proper trimming. The trimmed underneath surface was exposed to the cold surface on the tissue embedding console to prevent tissue section from shrinking. Two slides were labeled with patient ID for ZN and AO. For GeneXpert and RT PCR analysis series of 20 slices of tissue sections of 4 µm thickness were transferred into each sterile cryovial tube for further processing.

Deparaffinization step

The slides containing the tissue section were placed in the laboratory oven for 1 h at 60°C. After removing the slide from the oven, it was then dewaxed in two changes of xylene for a duration of 5 min each, cleared with decreasing concentration of alcohol starting from two changes of absolute ethanol 5 min each, immersed into 95%, 80%, 70% alcohol concentration for 5 min each. Finally, the tissue section on the slide was rehydrated in distilled water.

Hematoxylin and eosin (H&E) staining

The rehydrated tissue section containing slides was stained for 8 min in Harris's hematoxylin reagent, decolorized in 0.5% acid-alcohol for 3 s, and washed with tap water. It was then counterstained with 0.5% eosin for 1 min. Finally, the slides were properly mounted using a DPX mounting medium and examined under the microscope for the presence of suggestive features of TB by an experienced pathologist S1 Figure.

Auramine O staining

The deparaffinized and rehydrated slide was covered with 0.1% Auramine O solution for 20 min. The slide was washed using tap water and excess water was drained off the slide followed by decolorizing with 0.5% acid alcohol for 3 min. Finally, the slide was washed with tap water, counter-stained with 0.5% potassium permanganate for 1 min, properly mounted with DPX, and examined under 20x or 40X objectives using an LED fluorescence microscope (Primostar iLED, ZEISS, Germany) [9, 15] S2 Figure.

Ziehl–Neelsen staining (ZN)

Initially, the Carbol fuschin solution was filtered using filter paper. The rehydrated and air-dried slide was then fully covered with Carbol fuschin. Using Bunsen burner, heat was applied underneath the slide until vapor appeared from the slide. The slide was then left undisturbed for 5 min. Following 5 min incubation, it was washed with tap water, decolorized with 3% acid alcohol for 3 min, washed with water and stained with 1% of methylene blue for 1 min [10]. After mounting DPX and cover slide, it was examined for the presence of Acid-Fast Bacilli (AFB) using a 100X objective of bright field microscope (Primostar, ZEISS, Germany).

DNA extraction

For DNA extraction, 20 ribbons of 4 µm thick tissue sections were used from each block. After dewaxing, clearing, and rehydration steps, DNA extraction was performed using the QIAamp DNA FFPE Tissue Kit (Lot Number 169015087, QIAGEN GmbH, Germany) following the supplier's standard protocol. Tissue sections were suspended in 1 ml xylene to dissolve paraffin wax, vortexed, centrifuged, and the supernatant discarded; this step was repeated once. The pellet was resuspended in 1 ml absolute ethanol, vortexed, incubated at room temperature for 2 min, centrifuged, and the supernatant discarded. Then, 200 µl Lysis buffer and 20 µl proteinase K were added, vortexed, and incubated at 56 °C for 1 h, followed by incubation at 90 °C for 1 h to reverse formalin crosslinking. After vortexing, 200 µl buffer AL and 200 µl ethanol were added sequentially with thorough mixing. The solution was transferred to a QIAamp MinElute column and centrifuged at 6000 g for 1 min. After discarding the flow-through, 500 µl buffer AW1 was added, and centrifuged at 6000 g for 1 min, followed by 500 µl buffer AW2 with another centrifugation at 6000 g for 1 min. Residual contaminants were washed away in two wash steps, followed by a membrane drying step at 18,000 g for 3 min. Finally, 75 µl buffer ATE was applied to the membrane, incubated for 5 min at room temperature, and centrifuged at 18,000 g for 1 min, with the extract stored at -20 °C.

IS1081 RT PCR

The working solution of the primers and probes were prepared by diluting the stock solution 10X with molecular grade water to obtain a concentration of 100 µm. The following sequence of primers were used in this study.

10uM IS1081_Fw 5'-GATCCTTCGAAACGACCA-3' (18 bases).
10uM IS1081_Rev 5'- CGGTGTCGATAAGATGAGA-3' (19 bases).
10 µm IS1081_Probe [6FAM]-CGAAGGAAATGACGC AATGACCTC-[BHQ1] (24 bases).

Using DNase-free Eppendorf tube, Hot Start Master mix was mixed with water and primers/probe using filter tips when pipetting. The reaction mixture was prepared by adding 1×20ul of the master mix into a reaction tube. The DNA template was then added with a volume of 5ul, and the same volume of positive and negative control was added to the corresponding wells on the plate. The PCR was run on Bio-Rad (Bio-Rad, Singapore). The thermal conditions consisted of initial denaturation at 95°C for 15 s. followed by 40 cycles of 95 °C for 15 s, 58 °C for 1 min (16). Samples having a Ct value of above 36 were considered negative.

Table 1 Site of the lymph node and clinical presentation of endometrial and lymph node TB patients of the study participants (90)

Variables			Frequency (Percentage)
Site of lymph node	Cervical		13 (43.3)
	Abdominal		3 (10)
	Axial		4 (13.3)
	Inguinal		3 (10)
	Pancreatic		2 (6.67)
	Mesenteric		3 (10)
	Ovary		1 (3.3)
	Unknown		1 (3.3)
Patient group			
ETB	Symptoms	Lower abdominal pain	9 (15)
		Pelvic mass	2 (3.3)
		Vaginal discharge (Leukorrhea)	10 (16.7_
		Dysmenorrhea	2 (3.3)
		Pelvic pain	7 (11.7)
		Infertility	2 (3.3)
		Post menopausal bleeding	4 (6.7)
		Pyrexia	2 (3.3)
		Menstrual disorder	2 (3.3)
		Unknown	20 (33.3)
Total			60
TBLN	Symptoms	Fever	7 (23.3)
		Fatigue	11 (36.7)
		Weight loss	4 (13.3)
		cough	2 (6.7)
		Enlarged lymph node only	6 (20)
Total			30

GeneXpert MTB/RIF assay

The volume of the sample for GeneXpert analysis was made up to 0.5 ml by adding buffer ATE to 35 µl of DNA extracted. 1.5 ml of the digestion solution provided with the kit was mixed with 0.5 ml sample. Following digestion of the sample with digestion solution, 2 ml of the reaction mixture was transferred into the cartridge. The cartridge was then loaded in the GeneXpert machine (Cephied, USA) for reading.

Results

Site of lymph nodes and clinical presentation of the endometrial and lymph node TB patients

A total of 90 FFPE biopsy block samples from ETB (60/90) and TBLN patients (30/90) collected and archived from 2018 to 2022 were analyzed in the current study. Most of the lymph node was collected from the cervical region followed by the abdominal, pancreatic, and mesenteric regions. Most of the ETB patients have been presented with vaginal discharge followed by Lower abdominal pain and pelvic pain, 10/60 (16.7%), 9/60 (15%), and 7/60 (11.7%) respectively. The TBLN patients mostly presented with fatigue and fever, 11/30 (36.7%) and 7/30 (23.3%) respectively. Table 1.

Detection rate and comparison of AO, ZN, GeneXpert MTB/RIF assay, and RT PCR against histopathology

Histopathology

Among the FFPE blocks with a suggestive feature of TB, granulomatous inflammation was seen in 32/70 (45.7%), necrosis in 35/70 (50%), and caseous necrosis in 3/70 (4.3%). Among the 32 cases that showed granulomatous inflammation, AO, ZN, and GeneXpert were positive in 16/32 (50%), 12/32 (37.5%), and 19/32 (59.4%) respectively. Among the 3 cases showing ceasating granulomatous inflammation, 2/3 (66.67%) also had a positive outcome by AO and ZN, while GeneXpert was positive in all the 3 cases. Table 2.

AFB staining methods

Among all 90 FFPE biopsy blocks, AO staining has shown a positive outcome in 32/90 (35.5%). Of this figure, 21/32 (65.6%) and 10/32 (31.2) were from ETB and TBLN groups respectively. The remaining positive case by AO was missed by histopathology and reported as negative. ZN on the other hand has shown a positive outcome in 25/90 (27.8%) of all the participants, where 16/25 (64%) were from endometrial TB and 8/25 (32%) were from TBLN group. The remaining one positive case shown by ZN was reported as negative by histopathology. Table 3.

Molecular methods

GeneXpert was positive in 47/90 (52.2%) of the total cases, where 26/43 (60.5%) were from ETB and 18/43

Table 2 Distribution of the detection rate of various methods as compared to histopathology

Methods		Histopathology			Total
		Granulomatous inflammation	Necrotizing granulomatous inflammation	Ceasating granulomatous inflammation	
AO	**Positive**	16	13	2	31
	Negative	16	22	1	39
ZN	**Positive**	12	10	2	24
	Negative	20	25	1	46
GeneXpert	**Positive**	19	19	3	41
	Negative	13	16	0	29
RT PCR	**Positive**	24	22	3	49
	Negative	8	13	0	21

Table 3 Detection rate for AO, ZN, GeneXpert and RT PCR across disease groups

		Histopathology Findings			Total	*P* value	Kappa
		ETB	TBLN	Negative			
AO	Positive	22	9	1	32	0.2	0.23
	Negative	18	21	19	58		
Total		40	30	20	90		
ZN	Positive	17	7	1	25	0.3	0.2
	Negative	23	23	19	65		
Total		40	30	20	90		
GeneXpert	Positive	27	14	2	43	0.04	0.33
	Negative	13	16	18	47		
Total		40	30	20	90		
RT PCR	Positive	30	19	5	54	0.02	0.4
	Negative	10	11	15	36		
Total		40	30	20	90		

(41.9%) were from the TBLN group. GeneXpert detected 2 positive cases which have shown no suggestive feature of TB by histopathology. Among the ETB patients, RIF-resistant cases were identified in 1/40 (2.5%) of the participants. The RT PCR test detected 54/90 (60%) positive cases among the total participants. Among histopathology-negative cases, 5/20 (40%) of them were shown to have positive outcomes with RT PCR test. The two AFB staining methods have shown poor agreement with Histopathology (Kappa of 0.2) Table 3. GeneXpert and RT PCR positive case detection were 43/90 and 54/90 respectively, both methods agreed on 38 cases from the total positive cases by both methods. Figure 1.

Diagnostic performance of AFB staining and molecular methods against histopathology as a reference standard

The AFB staining methods have shown similar specificity of 95%, while the sensitivity of AO, 38.1%, was higher when compared with that of ZN, 34%. The sensitivity of GeneXpert, 58.6%, was shown to be lower about that of RT PCR which has shown a sensitivity of 70%. However, GeneXpert has shown relatively increased specificity, 90%, when compared with RT PCR which has shown a value of 75%. The specificity of GeneXpert was comparable with that of the AFB staining methods while its sensitivity was way higher than the two staining methods Table 4.

Comparison of semiquantitative results as shown by GeneXpert and the combined use of molecular methods with AFB staining method

Both AO and ZN missed the considerable number of positive cases having a very low bacterial load by GeneXpert, constituting 11/31 (34.5%) and 15/31 (48.4%) of the total cases respectively (P value < 0.0001). ZN has detected one RR case as negative. In most ZN negative cases, 34/65 (52.3%) have shown a positive outcome by the combined molecular method S1 Table.

Comparative analysis of the combined use of various bacteriological and molecular detection methods with histopathology and their diagnostic performances

GeneXpert when combined with AO has shown a detection rate of 45/90 (50%) and its level of agreement with Histopathology was good (Kappa = 0.4). The detection rate increased to 72/90 (80%) when GeneXpert was combined with Histopathology showing an excellent kappa value of 0.9. The combined use of AFB staining methods with histopathology has shown an increased specificity of 95% when compared with other methods (P value < 0.0001). The combined use of histopathology with

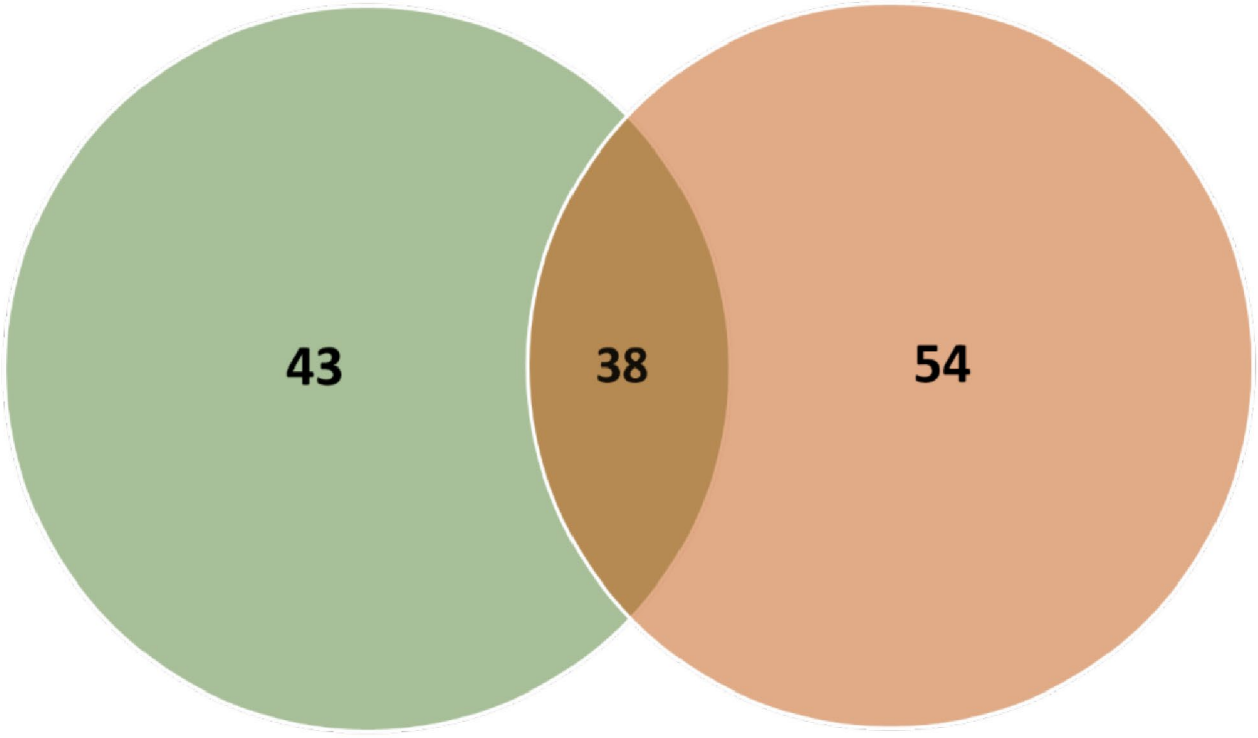

Fig. 1 Venn diagram showing the positive case detection of GeneXpert (Left) and RT PCR (Right)

Table 4 Diagnostic performance of the 4 methods against the histopathological method as a reference standard

Methods	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95%CI)	NPV (95%CI)	Positive Likely hood ratio (95% CI)	Negative Likely hood ratio (95% CI)	P value
ZN	34.30% (23–45)	95% (85–100)	96% (88–100)	29.20% (18–40)	6.8 (0.99-47)	0.69 (0.57–0.84)	0.01
AO	38.10% (26–50)	95% (85–100)	96% (88–100)	32.80% (20–45)	8.8 (1.3–60.9)	0.58 (0.46–0.74)	0.001
GeneXpert	58.60% (47–70)	90% (77–100)	95.30% (89–100)	38.30% (24–52)	5.86 (1.5–22)	0.46 (0.33–0.63)	< 0.001
RT PCR	70% (59-80.7)	75% (56-93.9)	90.70% (83–98)	41.60% (25–57)	2.8 (1.3-6)	0.4 (0.26–0.62)	< 0.001

Due to the nature of the sample used in this study, FFPE biopsy, culture hasn't been conducted and the reference standard used was Histopathology

GeneXpert has also shown a better diagnostic yield, sensitivity, and specificity of 100% and 90% respectively (*P* value < 0.0001) S2 Table.

Discussion

The routine diagnosis of ETB and TBLN in resource-limited settings is based on histopathological findings suggestive of TB. Due to this, histopathological diagnosis is often associated with limitations such as lack of specificity, an event that leads to a false positive outcome. In testimony of its non-specific nature, our study has shown its exaggerated positive case detection rate, 78%, from a total FFPE biopsy sample. This is due to its core principle of finding TB suggestive features to diagnose TB which often may be caused by conditions other than TB and result in a false positive outcome. This may lead to unnecessary use of antibiotics, delaying the early diagnosis of other severe diseases.

Alternative diagnostic tools such as AO, though not commonly practiced, are thought to alleviate the consequences associated with low specificity of histopathology. In our study, the detection rate of AO was 36%, with a sensitivity and specificity of 38.10% and 95% respectively. An inconsistent finding was shown by Girma S et *al* which has shown a sensitivity of 65.5% and specificity of 100% [17]. The discordance might be caused due to the type of sample used in both studies. Our finding was also inconsistent with the report Atnafu et *al* which has shown a sensitivity of 75% and specificity of 98.3%. The difference in using different reference standards in the

previous studies. Culture as the gold standard in previous studies can increase the sensitivity of AO since culture has a low detection rate when compared to using histopathology. Sample type usage might have also caused the discrepancy, Atnafu A et *al* have used fresh FNA samples [16].

Ziehl Neelson staining (ZN) is easy, cheap, and the most frequently used method for the detection of AFB especially in resource-limited areas. However, it is often reported with varying sensitivity, ranging from 18 to 71.4% [18, 19]. The sensitivity of ZN in the current study, 34.30%, was lower than that of AO's. In a similar pattern, a higher sensitivity of AO was shown by Laifangbam et al. who reported the sensitivity of ZN to be 41.2% and AO's to be 71.6% [20].

GeneXpert MTB/RIF assay in our study has shown sensitivity and specificity of 58.60% and 90% respectively. This finding is consistent with Jia Qing et *al* who used an FFPE biopsy sample and reported sensitivity and specificity of 57.81% and 100% respectively [21]. A slightly lower sensitivity of GeneXpert was reported by Njau et *al* which has shown a sensitivity of 53.2% [22]. A higher and inconsistent finding was reported by Romdhane et *al* which has shown a sensitivity of 74% [23]. The lack of concordance might have been caused by the difference in the cartridge used in both studies, where the later study used the GeneXpert Ultra cartridge which claims to have higher sensitivity than the GeneXpert MTB/RIF assay.

In an unusual instance, in this study, among GeneXpert-positive cases, 1/47 (2.1%) of the endometrial sample was found to be Rifampicin resistant while the rest were sensitive to rifampicin. The finding of RR case among endometrial TB is not common yet a very alarming event that needs careful attention. The burden of EPTB itself is one of the main challenges in TB elimination programs, let al.one the emergence of drug-resistant cases. A similar finding to our study was the case report of a 20-year-old female who was found to be rifampicin mono-resistant TB of the endometrium [24].

In the diagnosis of EPTB such as endometrial TB where there is the paucity of bacilli it is important to employ a highly sensitive diagnostic tool such as RT PCR to help the health care provider and the patients access more reliable results. In this study, the diagnostic yield of RT PCR targeting IS1081 primer was superior to the rest of other methods employed. The sensitivity and specificity of RT PCR in our study were 70% and 75% respectively. The sensitivity reported by Meenu et *al* was 75% which showed a slight difference with our study [25]. Another study conducted on endocervical swabs to diagnose endometrial TB has shown a sensitivity of 100% and 92% specificity by RT PCR [26]. The inconsistency might be caused by the sample type used in both studies.

Strength and limitation of the study

Strength

The strength of this study lies in its demonstration that molecular and bacteriological detection methods, particularly GeneXpert, offer superior specificity and significantly increased sensitivity compared to traditional histopathological methods. GeneXpert, in particular, not only provides more reliable results than RT PCR but also enables simultaneous assessment of rifampicin resistance, highlighting its utility in early TB detection and drug resistance assessment. This study underscores the practical benefits for healthcare providers and patients, emphasizing the efficiency and economic advantages of these diagnostic tools, and raises awareness of the critical need to address rifampicin-resistant endometrial cases.

Limitations

One primary limitation of our study is the use of formalin-fixed paraffin-embedded (FFPE) biopsy samples, which, while widely accepted for long-term tissue preservation, present challenges that impact the integrity and quality of extracted DNA. Formalin cross-linking in FFPE leads to DNA fragmentation and chemical modifications, hindering downstream molecular analyses such as PCR amplification and sequencing due to the production of fragmented and degraded DNA.

Conclusions

From our findings, we have observed that the utility of molecular and bacteriological detection methods provides a result with a more specific outcome than the routinely employed histopathological method. On the other hand, molecular tools such as GeneXpert generate an outcome with significantly increased sensitivity when compared with other methods such as AO and ZN. Particularly, the utility of GeneXpert provides more reliable results with better specificity than RT PCR. On top of this, it also provides an opportunity to assess the RIF resistance pattern simultaneously. In this study, one RR endometrial case was reported. Therefore, the application of bacteriological and molecular detection methods, particularly GeneXpert, along with histopathology, could help achieve early detection of TB cases with more reliable outcomes in addition to assessing the drug resistance patterns. The health care provider and patients in this regard will benefit from the utility of such diagnostic tools in terms of time and economy. On another note, due emphasis should be given to the emergence of RR among endometrial cases as it leads to undesirable consequences if left unaddressed.

Recommendations

We recommend the development of diagnostic algorithm for extrapulmonary TB including endometrial TB

by combining bacteriological and molecular detection methods such AO with histology to increase the specificity of diagnosis. We also recommend the utilization of GeneXpert MTB/RIF assay for endometrial and lymph node TB diagnosis specially to identify whether the TB is drug resistance or not. Since FFPE sample has effect especially on molecular tests, we recommend the use of better DNA extraction method or the use of fresh endometrial sample side by side for molecular analysis during sample collection for FFPE procedure.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12879-024-09908-7.

Supplementary Material 1

Supplementary Material 2

Supplementary Material 3

Supplementary Material 4

Supplementary Material 5

Supplementary Material 6: Peer reviews

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Peer reviews
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Author contributions
NB, AA, SG, AG, and MC – Conceptualization, Data curation, formal analysis, investigation, methodology, supervision, writing original manuscript, reviewing the manuscript YB, SY, BG, SA – Investigation, methodologyKB, ZC – Investigation, methodology, Reviewing manuscript.
We hereby declare that the manuscript titled "Evaluation of molecular and bacteriological detection methods performed on the formalin-fixed paraffin-embedded biopsy samples collected from endometrial and lymph node Tuberculosis suspected patients" is our original work and has not been submitted or published elsewhere in any form. All authors have significantly contributed to the research and preparation of this manuscript, and we have approved the final version.

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Data availability
Data is provided within the manuscript or supplementary information files (S1 File).

Declarations

Ethics approval and consent to participate
The study was conducted after obtaining ethical approval (Protocol number: PM23/797) from St. Paul's Hospital Millennium Medical College Ethical Review Board, Addis Ababa, Ethiopia. The need for informed consent was waived by the St. Paul's Hospital Millennium Medical College Ethical Review Board, Addis Ababa, Ethiopia.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Clinical trial number
Not applicable.

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