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Accuracy of anti-mycobacterial protein 51 antibodies as a biomarker for latent TB infection in asymptomatic HIV positive individuals: a cross-sectional diagnostic study

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Abstract

Objective To evaluate the diagnostic accuracy of anti-Mycobacterial Protein 51 (MPT51) antibodies in latent Tuberculosis (TB) detection among asymptomatic Human Immune Virus (HIV) positive individuals using Interferon Gamma Release Assay (IGRA) (QuantiFERON-TB Gold Plus) as the gold standard and determine the factors associated with anti-MPT51 antibody positivity among asymptomatic HIV positive individuals.

Results Considering QuantiFERON-TB Gold Plus as the gold standard, antibody reactivity to MPT51 revealed sensitivity of 32.6% (95% CI 24.5–40.6), specificity of 56.1% (95% CI 47.6–64.6), positive predictive value of 61.7% (95% CI 53.4–70.1) and negative predictive value of 27.7% (95% CI 20-35.4). Among the factors tested, none was independently associated with an increased risk of antibody reactivity against MPT51.

Keywords Mycobacterial protein 51 (MPT51), IGRA, Latent TB, TB diagnosis

Introduction

Mycobacterium tuberculosis (*M. tb*) is an ancient bacterium that causes tuberculosis (TB), a disease that affects millions of people worldwide, accounting for over 4100 deaths in 2022 [1, 2]. Tuberculosis disease probability is heightened in people living with Human Immunedeficiency Virus (PLHIV), with approximately 214,000 deaths in 2022 [3, 4]. Despite the global introduction of a vaccine in 1953 and the discovery of an effective four-drug treatment regimen in the 1960s, *M. tb* continues to ravage the world [5–7]. The TB pandemic is fueled by several factors, including poverty and HIV, but also insufficient understanding of the spectrum of TB pathogenesis [8] that limits diagnosis and appropriate treatment [9–11].

Latent tuberculosis Infection (LTBI), defined as infection with viable *M. tb* for which progression to TB disease is not expected to occur soon without any significant immunological compromise, is increasingly described as a spectrum [12, 13]. Despite the progression from LTBI to active TB being higher in PLHIV [2], it is still diagnosed by proxy immune-based tests, the Tuberculin Skin Test (TST) and Interferon Gamma Release Assay (IGRA) [14–16], which have poor prediction of the progression

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and cannot differentiate between the phenotypes along the TB spectrum [17]. Singh et al. reported antibodies to the Mycobacterial Protein 51 (MPT51) antigen in 90% of asymptomatic patients with incipient and subclinical TB [18, 19], the most concerning phenotypes of the spectrum. Furthermore, studies have reported that these antibodies are elicited during the early stages of TB infection [20, 21] and can aid in early diagnosis and treatment.

However, evaluation against IGRA to map the use of these antibodies along the spectrum of TB pathogenesis for targeted diagnosis is still lacking. Information on factors associated with the positivity of these antibodies among asymptomatic HIV-positive individuals is also limited. This study, therefore, sought to evaluate the diagnostic accuracy of the anti-MPT51 antibodies in asymptomatic HIV-positive individuals against IGRA. Positive results would position anti-MPT51 antibodies as a biomarker to improve LTBI diagnosis [22, 23].

Methods

Ethical approval was obtained from Makerere University School of Biomedical Sciences Research and Ethics Committee. One hundred forty-six adult HIV-positive individuals asymptomatic for TB aged 18 years or more were enrolled in this cross-sectional study carried out between July 2023 and September 2023. Participants were recruited from the Infectious Diseases Institute adult HIV clinic and invited to give written consent to participate after being briefed about the study. Consenting participants gave social demographic information, including whether or not they share a house with a TB patient, experienced any TB signs and symptoms, were previously diagnosed with TB, were previously on any TB treatment, have been on any TB preventive therapy (TPT) and whether they have used any substances (alcohol/smoking). Also, information was collected on the last HIV viral load results, and weight and height were measured. Participants then donated 5 ml of blood, of which 4 ml were used for IGRA testing and 1 ml for MPT51 testing.

ELISAs. The ELISA for MPT51 testing was done according to Wanchu et al. [24]. Briefly, Wells of 96-well microtiter plates (Immulon 2HB; Dynax) were coated with MPT51 antigen at 2 µg/ml (100 µl/ well) and incubated for 16 h at 4 °C. After the incubation period, plates were brought to room temperature and then washed 6 times with 200µL of Phosphate Buffered Saline (PBS) in 0.05% tween 20. The wells were then blocked with 200µL of skimmed milk in 0.1% of tween 20 for one hour at 37 °C. After the incubation period, the plates were washed again 6 times with 200µL of PBS in 0.05% tween 20. 100µL of the sample was then added, and plates were incubated at 37 °C for one hour. After incubation, wells were washed again, and 100µL of antihuman IgA HRP (1:2000; sino biological) was added, after which incubation for an hour at 37 °C followed. 100µL of TMB substrate was then added and incubated for 30 min at 37 °C. 100µL of sulphuric acid was then added to stop the reaction. Optical Densities were read at 405 nm.

Blood for IGRA was collected directly into the IGRA tubes. The IGRA assay, QuantiFERON-TB Gold-plus (Qiagen, Hilden, Germany), was performed according to the manufacturer's instructions. The results were calculated using QFT-Plus analysis software version 2.71.2.

Statistical analysis

A 2 by-2 table was used to calculate the diagnostic accuracy (sensitivity, specificity, positive and negative predictive values) of anti-MPT51 antibodies against QuantiFERON-TB Gold-plus at 95% CI. In inferential analysis, a random effect logistic regression analysis was used to determine factors associated with antibody positivity against MPT51. Bivariate logistic regression models were used to obtain unadjusted odds ratios for all characteristics potentially associated with antibody positivity against MPT51. Multivariate logistic regression analysis with forward stepwise logit function was done with variables included as a priority based on biological plausibility with antibody positivity against MPT51. Adjusted odds ratios were reported with 95% confidence intervals (95% CI). Data was analyzed using Stata/IC 15.0, Stata Corp LLC Texas USA. For all comparisons, a two-tailed P-value < 0.05 was considered significant.

Results

Demographic characteristics of the study participants

130/146 of the participants enrolled in the study were included in the analysis [25]. Sixteen [16] participants had indeterminate IGRA results; their -anti-MPT51 ELISA was not done.

Of the 130 participants, 82/130 (63.1%) were females, 68/130 (52.3%) were 33 years and below, 85/130 (65.4%) were of normal weight, 18/130 (13.85%) shared a house with a TB patient, 16/130 (12.31%) experienced signs and symptoms of TB, 41/130 (31.54%) were previously diagnosed with TB, 44/130 (33.85%) were previously on TB treatment, 84/130 (64.62) had been on TPT before while 37/130 (28.68%) had used substances (smoking or taking alcohol) before, and 127/130 (97.69%) had suppressed viral load, (Table 1).

Diagnostic accuracy of antibody reactivity against MPT51

Considering IGRA (QuantiFERON-TB Gold Plus) as the gold standard, antibody reactivity to MPT51 revealed a sensitivity of 32.6% (95% CI 24.5–40.6), specificity of 56.1% (95% CI 47.6–64.6), positive predictive value of 61.7% (95% CI 53.4–70.1) and negative predictive value of 27.7% (95% CI 20-35.4) (Table 2).

Table 1 Socio-demographics and clinical characteristics of the study participants

Variable	Category	Overall *n* (%)
Sex	Female	82 (63.1)
	Male	48 (36.9)
Age	33 and below	68 (52.3)
	Above 33	62 (47.7)
BMI	underweight	8 (6.2)
	Normal	85 (65.4)
	Overweight	26 (20.0)
	Obese	11 (8.5)
Shared a house with a TB patient	No	112(86.15)
	Yes	18(13.85)
Experienced any TB signs and symptoms	No	114(87.69)
	Yes	16(12.31)
Previously diagnosed with TB	No	89(68.46)
	Yes	41(31.54)
Previously on TB treatment	No	86(66.15)
	Yes	44(33.85)
Had been on any TB prophylaxis	No	46(35.38)
	Yes	84(64.62)
Used substances	No	92(71.32)
	Yes	37(28.68)
Viral Load status	Suppressed	127(97.69)
	Non-suppressed	3(2.31)

Table 2 A 2 by-2 table and antibody reactivity against MPT51 in comparison with QuantiFERON TB Gold Plus as the gold standard*

		IGRA (QuantiFERON-TB Gold plus)		
		Positive	Negative	Total
Anti-MPT51 antibodies	**Positive**	29	18	47
	Negative	60	23	83
	Total	89	41	130

Characteristics	Anti-MPT51 antibody reactivity
Sensitivity (95% CI)	32.6% (24.5–40.6)
Specificity (95% CI)	56.1% (47.6–64.6)
Positive predictive value (95% CI)	61.7% (53.4–70.1)
Negative predictive value (95% CI)	27.7% (20-35.4)

*Sensitivity = [29/ (29 + 60)) x100] = 32.6%; Specificity = [23/ (23 + 18)) x 100] = 56.1%; Positive Predictive value (PPV) = [29/ (29 + 18)) x 100] = 61.7%; Negative predictive value (NPV) = [23/ (60 + 23)] x 100 = 27.7%

Factors associated with antibody reactivity against MPT51

At bivariate analysis, among the factors tested, only sex was associated with an increased risk of antibody reactivity against MPT51 with a (Prevalence Ratio (PR) 0.52 95% CI 0.24–1.13). However, at multivariate analysis, none of the factors was independently associated with an increased risk of antibody reactivity against MPT51: sharing vs. not sharing a house with a TB patient (adjusted Prevalence Ratio (aPR): 1.52, 95% CI 0.48–4.77), experiencing vs. not any TB signs and symptoms (aPR: 1.65, 95% CI 0.47–5.77), previously being vs. not being diagnosed with TB (aPR: 1.66, 95% CI 0.13–20.8), previously on vs. not being on TB treatment (aPR: 0.46, 95% CI 0.03–6.12), previously on vs. not being any TPT (aPR: 1.35, 95% CI 0.56–3.26), substance use vs. no substance use (aPR: 1.00, 95% CI 0.43–2.35), having vs. not having a suppressed viral load (aPR: 1.00, 95% CI 0.94-1.00) and being 33 years of age and below vs. being above 33 (aPR: 1.62, 95% CI 0.71–3.12), *see* Table 3.

Discussion

The study results revealed a sensitivity, specificity, PPV, and NPV of anti-MPT51 of 32.6%, 56.1%, 61.7%, and 27.7%, respectively, for diagnosing LTBI. These results are similar to findings by Wanchu et al. among HIV-positive individuals without TB in India, which revealed a sensitivity of 32% [19, 24]. MPT51 is an adhesin of *M. tb*, likely expressed during active replication of the bacteria, as suggested by its presence in culture filtrates of *M. tb* [26–28]. It is, therefore, conceivable that antibody reactivity against MPT51 reflects increasing *M. tb* infection activity [20] and a heightened risk of progressing to active TB. The low sensitivity and specificity of 27.7% and 56.1%, respectively, would not lead to replacing IGRA as an LTBI test; however, a positive MPT51 antibody test is still informative as the presence of these antibodies predicts a higher likelihood of progression to active TB as has been demonstrated in earlier studies [18, 20].

Among the factors tested, none was independently associated with an increased risk of antibody reactivity against MPT51. This could have resulted from most participants having a suppressed viral load as they all were on antiretroviral therapy (ART). A study by Achkar et al., 2010 showed that worsening immunosuppression with concomitantly reduced capacity to control *M. tb* replication translates into a high risk for progression to TB, increasing the chance of detecting antibodies against MPT51 [20].

The other factors, such as currently or previously being on TPT, having been diagnosed with TB before or having taken any TB treatment, sharing a house with a TB patient, and substance use, had never been evaluated before.

Limitations

Long-term monitoring of our study participants was not done, making it impossible to determine the prognostic value of the antibody responses. Long-term monitoring would allow serial assessments of antibody reactivity over time to determine whether it increases, wanes, or persists.

Table 3 Logistic analysis for factors associated with antibody reactivity against MPT51

Variable	Category	MPT51 positive *n*/*N* (%)	Multivariate analysis	
			Adjusted Prevalence Ratio	*P*-value
Sex	Female	34/82(41.5)	Ref	
	Male	13/48(27.1)	0.52	0.12
			[0.23–1.19]	
Age	33 and below	22/68(32.4)	Ref	
	Above 33	25/62(40.3)	1.62	0.23
			[0.71–3.72]	
BMI	Underweight	1/8(12.5)	Ref	
	Normal	33/85(38.8)	3.56	0.26
			[0.39–32.44]	
	Overweight	10/26(38.5)	3.49	0.29
			[0.34–36.16]	
	Obesity	3/11(27.3)	1.83	0.64
			[0.15–22.66]	
Sharing a house with a TB patient	No	39/112(34.8)	Ref	
Not sharing a house with a TB patient	Yes	8/18(44.4)	1.52	0.48
			[0.48–4.77]	
Experienced any TB signs and symptoms	No	40/114(35.1)	Ref	
Not Experienced any TB signs and symptoms	Yes	7/16(43.7)	1.65	0.43
			[0.47–5.77]	
Previously diagnosed with TB	No	34/89(38.2)	Ref	
Not previously diagnosed with TB	Yes	13/41(31.7)	1.66	0.69
			[0.13–20.8]	
Previously on TB treatment	No	33/86(38.4)	Ref	
Not previously on TB treatment	Yes	14/44(31.8)	0.46	0.56
			[0.03–6.12]	
Been on any TB prophylaxis	No	15/46(32.6)	Ref	
Never been on TB prophylaxis	Yes	32/84(38.1)	1.35	0.5
			[0.56–3.26]	
Substance use	No	33/92(35.9)	Ref	
No substance use	Yes	14/37(37.8)	1	0.99
			[0.43–2.35]	
Viral Load	suppressed	47/127(37.0)	Ref	
	non-suppressed	0/3(0)	1	0.129
			[0.94-1.00]	

Even though we intended to have high statistical power, we only managed to collect samples from 146 participants due to financial constraints. This could have affected the study's results.

Abbreviations

TB	Tuberculosis
HIV	Human Immunodeficiency Virus
AIDS	Acquired Immune Deficiency Syndrome
ART	Antiretroviral Therapy
M. tb	*Mycobacterium tuberculosis*
MPT51	Mycobacterial Protein 51
WHO	World Health Organization
TPT	Tuberculosis Preventative Therapy
ELISA	Enzyme-Linked Immunosorbent Assay
IGRA	Interferon Gamma Release Assay
LTBI	Latent Tuberculosis Infection.
IDI	Infectious Disease Institute
PR	Prevalence Ratio
PLHIV	People Living with HIV

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Author contributions
JVN contributed to the conception, proposal development, sample collection and analysis, data collection and interpretation, and drafted the manuscript. CM generated the suitable in-house ELISA for MPT51 and performed sample analysis. SN contributed to the data analysis and interpretation. OJS and JM contributed to the conception, proposal development, and data interpretation and substantively revised the work. All authors reviewed and approved the final manuscript.

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Data availability
Data is provided within the manuscript. The datasets generated and analyzed in the current study are available from the corresponding author.

Declarations

Ethical approval and consent to participate
The study was conducted in compliance with the Declaration of Helsinki and Good Clinical Practice Guidelines. Ethical approval was obtained from the School of Biomedical Sciences Research Ethics Committee (Approval number: SBS-2022-265) under the College of Health Sciences at Makerere University and the IDI REC. Administrative clearance was obtained from the IDI management for conducting the research at the IDI adult HIV clinic. Informed written consent was obtained from all study participants before any study procedure, and their information was treated with confidentiality.

Consent for publication
Not Applicable.

Competing interests
The authors declare no competing interests.

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