Research Square

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Real-time PCR for the rapid identification of latent tuberculosis infection using host-serum biomarkers

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Abstract

Background

Latent tuberculosis infection (LTBI) is a major health challenge globally, which constitutes a large reservoir for future active disease and transmission. Currently, diagnostic tests, including the tuberculin skin test and interferon-γ release assays, have limited ability to distinguish LTBI from active disease and to predict disease progression. Therefore, this study aimed to evaluate host-derived serum biomarkers using a real-time qPCR assay for the rapid identification of LTBI.

Methods

The study population consisted of 143 individuals, including the primary study population (n = 93), which was categorized into 3 groups: active tuberculosis (n = 20), latent tuberculosis infection (n = 54), and contact tracing (n = 19), and an additional 50 individuals from close contacts of ATB for further validation of the study results. Serum samples were separated, and total RNA was extracted, followed by cDNA synthesis. The analysis of five host gene expression (*CXCL10*, *L6*, *CVIL*, *LIMD2*, and *ARSA1*) was done using SYBR Green-based real-time qPCR, with *B2M* as the housekeeping reference gene for normalization. Relative gene expression was calculated using the 2^−ΔΔCt method, and statistical analyses were performed based on data distribution.

Results

CXCL10 was the most highly detected gene among the three study groups (ATB: 100%, CT: 89.5%, LTBI: 92.6%) and the most consistently detected gene across the independent validation cohort (n = 50). Significant differential expression was observed for *CXCL10* (p = 0.019), *L6* (p = 0.001), and *CVIL* (p = 0.001) genes for the LTBI and ATB groups. *CXCL10*, *L6*, and *CVIL* were upregulated in LTBI and downregulated in ATB, suggesting their potential to distinguish infection states. *LIMD2* and *ARSA1* did not show consistent or statistically significant differences.

Conclusions

This study uses a molecular-based RT-qPCR assay to identify potential biomarkers that accurately differentiate LTBI and ATB patients. The genes, including *CXCL10*, *L6*, and *CVIL*, showed significant differences between the ATB and LTBI groups, suggesting that these genes could serve as potential biomarkers for early identification of LTBI. These results indicate that host biomarkers could serve as potential markers for developing a rapid, minimally invasive approach, particularly in resource-limited settings.

Background

Tuberculosis (TB) is a major infectious disease globally, which is caused by *Mycobacterium tuberculosis* (Mtb). According to the reported data, an estimated 10.8 million people are infected with TB, and among them, 1.25 million deaths were reported globally in 2023 [1]. Alternatively, due to the disruptions caused by the COVID-19 pandemic, the incidence rate increased by 4.6% from 2020 to 2023, reversing the gradual decrease in TB cases observed in the previous decade [2]. Disease burden is higher in low- and middle-income countries, as 98% of the global burden of TB occurs in developing countries, including India, Indonesia, China, Philippines, and Pakistan, which collectively contribute 56% of the global TB burden, although there have been significant advances in diagnostic and treatment options [3, 4]. There are still problems with the diagnostic delay of TB patients, the lack of case detection, and disease transmission. Specifically, around 2.7 million patients with TB disease are still not diagnosed, representing a significant challenge for disease management [2].

One of the most critical features of TB is latent tuberculosis infection (LTBI), which is defined as a state of persistent immune response to stimulation by *Mycobacterium tuberculosis* antigens with no evidence of clinically manifest TB disease [5]. Around 23–25% of the global population, nearly 1.7 billion individuals, are infected with LTBI, which constitutes a large reservoir for future active disease and transmission [6]. Although patients with LTBI do not show signs or symptoms of active disease, approximately 5–10% of individuals will develop active disease during their lifetime, with the highest risk occurring in the first few years following infection [7, 8].

Currently, interferon-γ release assays (IGRAs) and the tuberculin skin test (TST) are the most widely used tests for detecting LTBI; however, despite their ability to detect immune sensitization to Mtb antigens, these tests have several important limitations. These tests are unable to accurately differentiate between LTBI and ATB, and cannot predict which LTBI patients are more susceptible to getting the active disease [9, 10]. Several factors may influence the performance of these tests, including immunosuppression, concurrent illness, and Bacillus Calmette-Guérin (BCG) vaccination status. Considering these limitations, there is an urgent need to identify novel biomarkers that can accurately identify LTBI before progression to active disease and to develop rapid, reliable, and minimally invasive diagnostic tools.

Over the last decade, high-throughput technologies, including transcriptomics, proteomics, and metabolomics, have enabled in-depth analysis of host immune response to Mtb and facilitated the discovery of molecular markers associated with different stages of infection [11].

Host gene expression patterns can be identified through blood transcriptomic signatures, which are associated with progression from LTBI to ATB. A 16-gene blood RNA signature capable of predicting progression to active disease up to 12 months before diagnosis was identified in a landmark prospective cohort study [12]. Simplified versions of this signature, including the four-gene correlate-of-risk signature (RISK4) and the 6-gene transcriptomic signature (RISK6), have been developed and show promise in identifying patients at increased risk of developing active disease [13, 14]. These host transcriptomic

signatures differ across infection states by whether a person has ATB, LTBI, or is healthy in multiple cohorts, although their performance varies by population and assay platform [15].

Collectively, this evidence supports the concept that LTBI exists as a spectrum of disease, which gradually progresses from latent infection through incipient and subclinical phases to active disease, rather than as a single dormant state [16, 17]. Additionally, Host-serum biomarkers reflect immune activation and inflammatory responses during infection, characterized by the release of cytokines and chemokines such as interferon -γ (IFN-γ), C-C motif chemokine ligand 10 (*CXCL10*/IP-10), tumor necrosis factor-α (TNF-α), and interleukins (IL). These markers play important roles in granuloma formation and immune signaling and can therefore be used to differentiate between latent infection, ATB, and uninfected individuals [18, 19].

Recent studies have also demonstrated that using biomarker panels rather than a single biomarker can significantly improve diagnostic accuracy. For example, combinations of cytokines and chemokines, such as IFN-γ, CXCL9, CXCL10, and IL-2 receptor, have shown high sensitivity and specificity for differentiating between disease states [20].

The real-time quantitative polymerase chain reaction (RT-qPCR) is an important method for the rapid and quantitative measurement of host gene expression biomarkers. Targeting these immune signatures has demonstrated strong diagnostic performance, enabling sensitive detection of immune responses following Mtb-specific antigen stimulation [21]. These molecular tests provide more advantages over conventional methods to improve the diagnosis and accurate differentiation. Despite these advances, there is currently no single biomarker or biomarker panel that satisfies the requirements for accurately differentiating LTBI from early or active disease across a variety of populations, and biomarker discovery studies exhibit significant variability due to variations in study design, populations, and analytical platforms [11]. Therefore, integrating host biomarkers with RT-qPCR represents a promising tool for rapid and accurate differentiation of LTBI. Hence, in this study, we assessed the utility of SYBR green-mediated, RT-qPCR for the rapid detection of specific host genes, including ribosomal protein L6 (*L6*), LIM domain-containing protein 2 (LIMD2), ezrin (*EZR*/*CVIL*), *CXCL10*, and ArsA arsenite transporter ATP-binding homolog 1 (*ARSA1*), also known as guided entry of tail-anchored proteins factor 3, ATPase (*GET3*), present in serum samples, for early identification of LTBI.

Methods

Study setting, population, and ethical clearance

The study population consists of 143 individuals, including the primary study population (n = 93), which was categorized into 3 groups: ATB (n = 20), LTBI (n = 54), and contact tracing (CT) (n = 19), and an additional 50 individuals from close contacts of ATB for further validation of the study results. Blood samples were collected under aseptic conditions from all study participants at the Chest Clinic, Kandy, Sri Lanka, following ethical approval from the National Hospital, Kandy, Sri Lanka (NHK/ERC/18/2021).

ATB patients were recruited as patients with pulmonary symptoms after following either bacteriological confirmation or a clinical presentation consistent with TB and less than one month of anti-TB therapy. LTBI patients were recruited as those with a positive Mantoux test and a standard Chest X-ray (CXR), without clinical symptoms or evidence of ATB or other non-tuberculosis respiratory infections. TB household contacts were selected from the CT list of ATB patients with negative Mantoux test results.

Laboratory analysis

Sample collection, processing and RNA extraction

The whole blood was collected into serum separator tubes (BD Vacutainer SST™ Venous Blood Collection Tube Serum Tube/Separator Gel Additive) (3 ml) and kept at room temperature for 30–45 mins until serum separation. Serum was isolated using the refrigerated centrifuge, HERMLE Z326K at 3,200 rpm for 20 mins at 4 ºC. The serum samples were aliquoted (250 µl) and stored at -80 ºC until further processing. Total RNA was extracted using the guanidinium thiocyanate-phenol-chloroform method, as described in previous literature [22].

RNA quantification and cDNA synthesis

RNA concentration was determined using the QuantiFluor® RNA System with low-concentration calibration on a QuantiFluor™-ST fluorometer. Complementary DNA (cDNA) synthesis was performed using the GoScript™ Reverse Transcription System (Promega). The reverse transcription (RT) master mixture (15 µL) and the RNA reaction mixture (5 µL) were prepared separately on ice. For RT master mixture: 7.8 µL nuclease-free water, 4 µL 5X PCR buffer, 1.2 µL 25 mM $MgCl_2$, 1.0 µL PCR nucleotide mix (0.5 mM each dNTP), and 1.0 µL reverse transcriptase were added. For the RNA reaction mixture, 3 µL RNA sample and 1 µL each of oligo(dT) and random primers were added. The RNA mixture was loaded into the thermal cycler, heated to 70°C for 5 minutes, immediately chilled on ice for 5 minutes, and then combined with 15 µL of RT master mixture. cDNA synthesis was carried out at 25°C for 5 minutes (annealing), 42°C for 60 minutes (extension), and 70°C for 15 minutes (enzyme inactivation). Synthesized cDNA was stored at -20°C until further analysis.

Real-time PCR analysis

Five host gene transcripts, including *L6*, LIMD2, *CVIL*, *CXCL10*, and *ARSA1*, were analyzed by RT-qPCR in 20 ATB, 19 CT, and 54 LTBI samples, along with an additional 50 CT samples for validation (primer sequences are withheld due to intellectual property considerations). Real-time PCR assays were performed on the Rotor-Gene Q real-time PCR system (QIAGEN), and gene amplification was monitored by measuring SYBR Green fluorescence. Each reaction carried out using 25 µL of master mixture consisted of 4 µL 5X PCR buffer, 2 µL dNTP mix (2 mM each), 0.8 µL 25 mM $MgCl_2$, 1µL of 2X SYBR Green, 0.125 µL Taq DNA polymerase (5 U/µL; QIAGEN), 0.5µL of each primer (10µM), and 5µL of 100-

fold diluted cDNA, and final volume adjusted with nuclease-free water. The amplification process is initiated by an initial denaturation at 94°C for 1 minute, followed by 40 cycles of amplification (denaturation at 94°C for 30 seconds, annealing (withheld due to IP), and extension at 72°C for 30 seconds). After cycling, melting curves were analyzed over the temperature range of 65°C to 95°C in 0.5°C increments, with a 90-second hold at each step and fluorescence acquisition in the green channel. A final cooling step was performed at 25°C for 30 seconds.

Relative gene expression analysis

The expression levels of selected genes in the ATB, LTBI, and CT groups were evaluated by RT-qPCR, with *B2M* as the endogenous reference gene for normalization. The Comparative Ct (2^−ΔΔCt) method was used for the relative gene expression analysis, using the CT group as the calibrator. At every step, the Ct value of the target gene is first normalized to the Ct value of B2M to obtain the ΔCt value: $\Delta Ct = CtTarget - CtB2M$. The ΔCt values used for the statistical analysis were chosen to ensure accurate analysis, as Ct values are inherently logarithmic. Data normality was assessed to determine whether it followed a normal distribution. Based on the distribution of the data, appropriate statistical tests were used for further analysis. One-way ANOVA followed by Tukey's post-hoc test was used for normally distributed data, while non-normally distributed data were analyzed using Kruskal-Wallis test followed by Mann-Whitney U tests. A p-value < 0.05 was considered statistically significant.

Results

Participant selection and demographic data analysis

The 93 participants were primarily included in this study, comprising active tuberculosis (n = 20), latent tuberculosis infection (n = 54), and contact tracing (n = 19). Among them, the majority are female (n = 67). The male-to-female ratio is 1:3. Participants' ages range from 12 to 70 years, with the majority in the 35–64 age group. The 19% of children were under 16 years of age, and the 6% of elderly people were over 64 years old. Considering the ATB group, the female percentage is 80%, and in the LTBI group, the female percentage is 60%. Additional variables such as height, weight, and household contact status were also collected (Table 1). Among the LTBI participants, 92% were reported a history of close contact with ATB patients.

Table 1
Socio-demographic and anthropometric characteristics of active tuberculosis (ATB), latent tuberculosis infected (LTBI), and contact tracing (CT) groups

Characteristics	Total population (N = 93)	ATB (N = 20)	LTBI (N = 54)	CT (N = 19)
Socio-demographic				
Age (years)				
≤15	18 (19)	2 (10)	10 (19)	6 (18.8)
16–34	28 (30)	6 (30)	15 (28)	7 (37)
35–64	41 (44)	10 (51)	25 (46)	6 (32)
≥65	6 (6)	2 (10)	4 (7)	0(0)
Gender				
Male	26 (28)	4 (20)	22 (40)	0 (0)
Female	67 (72)	16 (80)	32 (60)	19 (100)
Anthropometric				
Weight (kg)				
≤25	6 (6)	2 (10)	2 (4)	2 (11)
26–39	18 (19)	5 (25)	9 (17)	4 (21)
40–54	35 (38)	8 (40)	21 (39)	6 (32)
55–69	24 (26)	3 (15)	15 (28)	6 (32)
≥70	10 (11)	2 (10)	7 (13)	1 (5)
Height (cm)				
110–140	12 (13%)	4 (20%)	6 (11%)	2 (11%)
141–155	30 (32%)	8 (40%)	16 (30%)	6 (32%)
156–170	40 (43%)	6 (30%)	25 (46%)	9 (47%)
≥ 171	11 (12%)	2 (10%)	7 (13%)	2 (11%)

Analysis of SYBR green-mediated real-time PCR assay

Total RNA was extracted from separated serum samples, and cDNA was synthesized for the RT-qPCR analysis. Candidate genes were selected based on the available literature and previously published transcriptomic datasets [23]. The presence of the gene was detected by the fluorescent dye SYBR green, and the specificity was identified by the analysis of melting curves (Fig. 1). Gene expression analysis

was conducted based on SYBR Green-mediated RT-qPCR results. The *B2M* gene was used as the endogenous reference gene because of its stable expression across study groups. The study revealed that detection rates for the selected host-specific genes varied (Table 2); *CXCL10* showed the highest detection rate among the three study groups (ATB: 100%, CT: 89.5%, LTBI: 92.6%) and was also the most consistently detected gene across the independent validation cohort (n = 50). In contrast, *LIMD2* and *ARSA1* genes showed relatively low detection rates, particularly in the ATB group.

Table 2
RT-PCR results for the detection of selected Host-specific gene transcripts in serum

Patient groups	**Detection of Host-specific gene transcripts**				
	***CXCL10* (%)**	***L6* (%)**	***CVIL* (%)**	***LIMD2* (%)**	***ARSA1* (%)**
ATB (N = 20)	20 (100)	3 (15)	4 (20)	1 (5)	3 (15)
CT (N = 19)	17(89.5)	12 (63.2)	5 (26.3)	3 (15.8)	3 (15.8)
LTB (N = 54)	50 (92.6)	52 (96.3)	46 (85.2)	11 (20.4)	9 (16.7)

Differential expression analysis in the primary study cohort

Different expression patterns were observed across the ATB, LTBI, and CT study groups, particularly between the ATB and LTBI groups (Table 3). The *CXCL10* gene expression was slightly upregulated in the LTBI group and markedly downregulated in the ATB group ($log_2FC = -4.31$).

Similarly, *L6* and *CVIL* genes were relatively upregulated in the LTBI group and substantially downregulated in the ATB group, suggesting differential expression across the disease states. Additionally, *LIMD2* did not show consistent differential expression across the study groups, and *ARSA1* showed a trend toward downregulation, though not statistically significant. However, analysis of the independent validation cohort showed partially consistent expression patterns, with *CXCL10* remaining downregulated and other genes showing variable regulation, which supports their potential role in host response profiling.

Table 3
Relative expression of five selected genes in LTBI and ATB samples using the CT group as the calibrator

Gene	Patient Groups	Mean ΔCt	log_2FC	Regulation
CXCL10	LTBI	-5.49	0.27	Upregulated
	ATB	-0.91	-4.31	Downregulated
	CT	-5.22	–	–
L6	LTBI	-4.25	0.79	Upregulated
	ATB	12.43	-15.88	Downregulated
	CT	-3.45	–	–
CVIL	LTBI	-6.30	2.39	Upregulated
	ATB	8.52	-12.43	Downregulated
	CT			
LIMD2	LTBI	1.91	1.98	Upregulated
	ATB	–	–	Not determined (n = 1)
	CT	3.89	–	–
ARSA1	LTBI	0.12	-10.95	Downregulated
	ATB	0.003	-10.83	Downregulated
	CT	-10.83	–	–

Statistical analysis

Among the selected genes, significant differences were observed for *CXCL10* ($p = 0.019$), *L6* ($p = 0.001$), and *CVIL* ($p = 0.001$) among the study groups. Especially between LTBI and ATB. These results indicated the potential of these genes to distinguish different TB infection states. Additionally, *LIMD2* and *ARSA1* did not show statistically significant differences among study groups, suggesting that their expression patterns were less consistent among study groups.

Validation of gene expression in an independent cohort

The selected host genes were analyzed in an independent cohort (n = 50) comprising close contacts (CT) of ATB patients to validate the detection patterns observed in the primary study cohort. The samples were subjected to an RT-qPCR assay using target primers for selected genes (*CXCL10*, *L6*, *CVIL*, *LIMD2*, and *ARSA1*) and *B2M* as the endogenous reference gene. The *CXCL10* gene showed the highest detection frequency (86%), and the detection frequencies of *CVIL*, *LIMD2*, and *ARSA1* genes were comparatively lower, which is consistent with the findings of the primary study (Table 4). However, the detection frequency of the *L6* gene was reduced in the independent cohort (36%) compared to that in the

CT group of the primary study (63.2%), which may be due to the variation in the duration and intensity of exposure to the Mtb, along with host immune response among exposed individuals that influence the development of LTBI. Additionally, we identified 9 individuals in the independent cohort who were positive for both selected genes (*CXCL10*, *L6* and *CVIL*) suggesting that these individuals may have a higher possibility could be suspect as to developing LTBI.

Table 4
RT-PCR results for the detection of selected Host-specific gene transcripts in validated samples

Patient group	**Detection of Host-specific gene transcripts**				
	***CXCL10* (%)**	***L6* (%)**	***CVIL* (%)**	***LIMD2* (%)**	***ARSA1* (%)**
N = 50	43 (86)	18 (36)	12 (24)	11 (22)	6 (12)

Discussion

The eradication and clinical management of TB is primarily affected by the diagnosis gap between accurately differentiating LTBI in lower- and middle-income resource-limited countries. Currently, TST and IGRA tests are used as convenient tools to diagnose Latent TB. These tests detect immune sensitization but cannot detect a rapid disease response to the pathogen [9, 10]. Therefore, our study focused on identifying serum-based host biomarkers to develop a molecular approach for early identification of LTBI patients.

Among the evaluated biomarkers, *CXCL10* showed a significant differential expression between ATB and LTBI groups. *CXCL10* is a chemokine that plays a major role in granuloma formation. After infection, *CXCL10* supports the recruitment of activated T cells and monocytes to the infectious site, which facilitates the granuloma formation and maintenance [24, 25]. Additionally, recent studies have demonstrated that *CXCL10* is a highly robust marker for the differential diagnosis of TB disease states. Host-derived *CXCL10* levels are more sensitive for the discrimination between LTBI and ATB than the traditional IGRAs assay [26]. Furthermore, *CXCL10* was identified as a biomarker for predicting progression in high-risk household contacts, not only for differentiating disease status, in a combined biomarker panel with an AUC of 0.9 [27].

According to our findings, *CXCL10* was significantly upregulated in the LTBI group compared with the ATB group. Similarly, recent studies revealed that *CXCL10*, as a specific immune signature of household contacts, is predictive of incipient stages that transition from the latent infection to the active disease [27]. The increased expression in the LTBI group is possibly caused by a protective Th1-polarized immune response that successfully limits Mtb within granulomas. Antigen-specific T cells primarily mediate immunological control during latent infection, promoting granuloma formation and preserving bacterial containment at the infection site [28].

The significant differences observed in *L6* and *CVIL* between ATB and LTBI (p = 0.001) introduce less-conventional but highly promising markers into the diagnosis of TB. Ribosomal protein L6 is a key component of the 60S subunit, and it is essential for protein synthesis. Therefore, the level of *L6* expression can serve as an indirect indicator of a cell's translational capacity and underlying metabolic activity. In the context of an infection, changes in ribosomal protein expression reflect the host's defense mechanism, which produces antimicrobial peptides and cytokines through altered translational machinery [11].

CVIL is a component of the EZM (Ezrin-radixin-moesin) protein family that links the plasma membrane to the cell's actin cytoskeleton. This facilitates T-cell activation, leukocyte migration, synapse formation, and phagosomal maturation. It facilitates the destruction of microbes by linking the outer surface to the internal cell structure. Therefore, the significant upregulation of *CVIL* in the LTBI group compared with the ATB group suggests that a controlled, latent state is characterized by high cellular motility and efficient immunological synapse formation. The higher expression also revealed that the host’s structural proteins are actively maintaining the pathogen containment [29]. In contrast, the observed significant difference between the ATB and CT group (p = 0.039) is due to the interference of Mtb with the host cell’s internal structure by disrupting normal cell function, such as phagosomal-lysosome fusion by modulating actin-binding proteins. This allows the bacteria to survive within the cell instead of being destroyed [30, 31]

No statistically significant difference was observed in our study group for the *ARSA1* (p = 0.06) and *LIMD2* (p = 0.168) genes. Previous studies have shown that gene expression can be different due to host genetic and environmental factors [10]. Host response to Mtb bacilli is influenced by factors such as BCG vaccination status, nutritional conditions, and prior exposure to non-tuberculosis mycobacteria (NTM), which can alter immune-related gene expression and contribute to variability among infected individuals [32, 33].

Additionally, recent transcriptomic studies consistently reported that TB-related biomarkers are not reliable for single-use as diagnostic markers, because a single gene is rarely stable across different study populations and thus inconsistent as a marker. Therefore, these gene expression patterns are better reflected by multigene panels, which demonstrate coordinated activation of immune pathways rather than the isolated effect of a single gene [34]. This could explain the non-significant trend observed for the *ARSA1* and *LIMD2* genes, which were not relevant in all situations and whose utility may depend on the context being studied. However, the trend we observed, with the *ARSA1* gene becoming a significant marker, suggests that it remains useful when combined with a multigene panel rather than used alone, thereby increasing sensitivity, specificity, and reproducibility across geographically distinct populations [12, 34].

Moreover, the current study builds on previously established molecular diagnostic approaches that have shifted towards nucleic acid-based host response analysis as a rapid, minimally invasive diagnostic approach [12, 33, 35]. In our study, we used a serum-based RT-qPCR assay to develop multi-gene panels

for precise diagnosis and to conduct large-scale screening to identify early LTBI cases, thereby reducing active TB incidence, enabling early treatment, and minimizing health care costs in a developing country.

Another major observation in our study is the overlap of the gene expression between the LTBI and CT groups. Biologically, this confirms that TB is a continuum of disease rather than a single dormant stage that progresses to active disease depending on bacterial metabolic activity and the host's control of the infection [17]. The CT group is considered theoretically uninfected but is expected to show early immune activation due to recent exposure to the pathogen. The similar patterns observed in the *L6* and *CVIL* genes suggest that these genes can be used as markers of the response to the pathogen rather than the pathogen load itself. Additionally, these markers can be used to determine whether an individual will progress to ATB by assessing the host's immune status. Furthermore, the relative gene expression analysis of the independent validation cohort also showed a clear difference from the ATB group in the primary study, further confirming the potential utility of these host biomarkers to differentiate LTBI or early exposures from the ATB. Although some genes showed a variable expression pattern across the study population, the partially consistent trends observed support the usefulness of a multigene biomarker panel for detecting different states of TB.

Considering these factors, RT-qPCR offers several advantages over conventional TB diagnostic methods, including rapid detection of infected individuals and the ability to detect subclinical transcriptional changes. However, several limitations remain. These genes should be validated with large independent cohort before they are used as diagnostic tools. Additionally, gene expression analysis and statistical testing should be further validated using healthy controls who were not known to have exposure to Mtb as the calibrator group, rather than using the contact tracing group. Furthermore, we cannot definitively conclude the prognostic value of these markers for predicting progression to active disease. Longitudinal follow-up of the LTBI group would be necessary to validate these genes as the risk signatures for developing active disease.

Conclusion

This study concludes by identifying host-derived serum biomarkers that successfully distinguish the LTBI group from the ATB group in the studied population, which could serve as potential biomarkers for early identification of LTBI, including *CXCL10*, *L6*, and *CVIL*. These results imply that real-time insights into infection status are the promise of RNA-based diagnostic techniques. In high-burden settings, including these biomarkers in current diagnostic algorithms may lead to more accurate preventive actions that advance the objectives to end TB.

Abbreviations

TB
Tuberculosis
Mtb

Mycobacterium tuberculosis
LTBI
Latent tuberculosis infection
IGRAs
Interferon-γ release assays
TST
Tuberculin skin test
BCG
Bacillus Calmette-Guérin
RISK4
four-gene correlate-of-risk signature
RISK6
6-gene transcriptomic signature
IFN-γ
Interferon -γ
TNF-α
Tumor necrosis factor-α
CXCL10
C-C motif chemokine ligand 10
RT-qPCR
Real-time quantitative polymerase chain reaction
L6
Ribosomal protein L6
LIMD2
LIM domain-containing protein 2
EZR
Ezrin
ARSA1
ArsA arsenite transporter ATP-binding homolog 1
GET3
guided entry of tail-anchored proteins factor 3
ATPase
CT
Contact tracing
CXR
Chest X-ray
cDNA
Complementary DNA
EZM
Ezrin-radixin-moesin

NTM
Non-tuberculosis bacteria

Declarations

Competing interests

The authors declare that they have no competing interests.

Ethics declarations

Ethics approval and consent to participate

Approval for the study was obtained from the Ethics Review Committee of the National Hospital, Kandy, Sri Lanka (NHK/ERC/18/2021) and was conducted in accordance with the ethical principles of the Declaration of Helsinki.

Consent to participate

Written informed consent was obtained from all participants before enrollment in the study. For participants under 16 years of age, written informed consent was obtained from their parents or legal guardians.

Consent for publication

Not applicable

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Author Contribution

RR, DC, TB carried out the sample collection, molecular techniques and RR carried out the data analysis and drafted the manuscript. DNM concept, designed and supervised the study, and coordinated and corrected the manuscript. DM screened and recruited the patients and assisted in the sample collection. All authors read and approved the final manuscript.

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Data Availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

References

1. World Tuberculosis Day 2025: Yes! We Can End TB – Commit, Invest, Deliver! Sri Lanka. World Health Organization. 2025. Accessed March 18, 2026. https://www.who.int/srilanka/news/detail/24-03-2025-world-tuberculosis-day-2025--yes%21-we-can-end-tb--commit--invest--deliver%21
2. World Health Organization. Global Tuberculosis Report 2024. World Health Organization; 2024. p. 68. https://www.who.int/publications/i/item/9789240101531.
3. Chakaya J, Khan M, Ntoumi F, et al. Global Tuberculosis Report 2020 – Reflections on the Global TB burden, treatment and prevention efforts. Int J Infect Dis. 2021;113:S7–12. 10.1016/j.ijid.2021.02.107.
4. World Health Organization. Global Tuberculosis Report 2021. World Health Organization; 2021. p. 25. https://www.who.int/publications/i/item/9789240037021.
5. World Health Organization. Latent tuberculosis infection: updated and consolidated guidelines for programmatic management. 2018. Accessed March 18, 2026. https://www.who.int/publications/i/item/9789241550239
6. Houben RMGJ, Dodd PJ. The Global Burden of Latent Tuberculosis Infection: A Re-estimation Using Mathematical Modelling. Metcalfe JZ. ed PLOS Med. 2016;13(10):e1002152. 10.1371/journal.pmed.1002152.
7. Goletti D, Lee M, Wang J, Walter N, Ottenhoff THM. Update on tuberculosis biomarkers: From correlates of risk, to correlates of active disease and of cure from disease. Respirology. 2018;23(5):455–66. 10.1111/resp.13272.

8. Zhang H, Guan W, Zhou J. Advances in the Diagnosis of Latent Tuberculosis Infection. Infect Drug Resist. 2025;18:483–93. 10.2147/IDR.S504632.
9. Pai M, Denkinger CM, Kik SV, et al. Gamma Interferon Release Assays for Detection of Mycobacterium tuberculosis Infection. Clin Microbiol Rev. 2014;27(1):3–20. 10.1128/CMR.00034-13.
10. Dheda K, Davids M. Latent Tuberculosis Infection–associated Immunodiagnostic Test Responses as Biomarkers of Incipient Tuberculosis: Fruitful or Futile? Am J Respir Crit Care Med. 2020;201(8):895–8. 10.1164/rccm.201912-2425ED.
11. Walzl G, McNerney R, Du Plessis N, et al. Tuberculosis: advances and challenges in development of new diagnostics and biomarkers. Lancet Infect Dis. 2018;18(7):e199–210. 10.1016/S1473-3099(18)30111-7.
12. Zak DE, Penn-Nicholson A, Scriba TJ, et al. A blood RNA signature for tuberculosis disease risk: a prospective cohort study. Lancet. 2016;387(10035):2312–22. 10.1016/S0140-6736(15)01316-1.
13. Suliman S, Thompson EG, Sutherland J, et al. Four-Gene Pan-African Blood Signature Predicts Progression to Tuberculosis. Am J Respir Crit Care Med. 2018;197(9):1198–208. 10.1164/rccm.201711-2340OC.
14. Penn-Nicholson A, Mbandi SK, Thompson E, et al. RISK6, a 6-gene transcriptomic signature of TB disease risk, diagnosis and treatment response. Sci Rep. 2020;10(1):8629. 10.1038/s41598-020-65043-8.
15. Gupta RK, Turner CT, Venturini C, et al. Concise whole blood transcriptional signatures for incipient tuberculosis: a systematic review and patient-level pooled meta-analysis. Lancet Respir Med. 2020;8(4):395–406. 10.1016/S2213-2600(19)30282-6.
16. Barry CE, Boshoff HI, Dartois V, et al. The spectrum of latent tuberculosis: rethinking the biology and intervention strategies. Nat Rev Microbiol. 2009;7(12):845–55. 10.1038/nrmicro2236.
17. Drain PK, Bajema KL, Dowdy D, et al. Incipient and Subclinical Tuberculosis: a Clinical Review of Early Stages and Progression of Infection. Clin Microbiol Rev. 2018;31(4):e00021–18. 10.1128/CMR.00021-18.
18. Tebruegge M, Dutta B, Donath S, et al. Mycobacteria-Specific Cytokine Responses Detect Tuberculosis Infection and Distinguish Latent from Active Tuberculosis. Am J Respir Crit Care Med. 2015;192(4):485–99. 10.1164/rccm.201501-0059OC.
19. Cui Y, Li H, Liu T, et al. The Evolving Landscape of Host Biomarkers for Diagnosis and Monitoring of Tuberculosis. Biomedicines. 2025;13(9):2076. 10.3390/biomedicines13092076.
20. Korma W, Mihret A, Chang Y, et al. Antigen-Specific Cytokine and Chemokine Gene Expression for Diagnosing Latent and Active Tuberculosis. Diagnostics. 2020;10(9):716. 10.3390/diagnostics10090716.
21. Kim S, Lee H, Kim H, et al. Diagnostic Performance of a Cytokine and IFN-γ–Induced Chemokine mRNA Assay after Mycobacterium tuberculosis–Specific Antigen Stimulation in Whole Blood from Infected Individuals. J Mol Diagn. 2015;17(1):90–9. 10.1016/j.jmoldx.2014.08.005.

22. Chomczynski P, Sacchi N. The single-step method of RNA isolation by acid guanidinium thiocyanate–phenol–chloroform extraction: twenty-something years on. Nat Protoc. 2006;1(2):581–5. 10.1038/nprot.2006.83.
23. Magana-Arachchi D, Madegedara D, Bandara U. Whole Blood vs Serum-Derived Exosomes for Host and Pathogen-Specific Tuberculosis Biomarker Identification: RNA-Seq-Based Machine-Learning Approach. Biochem Genet. 2025;63(6):5528–50. 10.1007/s10528-024-11002-1.
24. Ruhwald M, Aabye MG, Ravn P. IP-10 release assays in the diagnosis of tuberculosis infection: current status and future directions. Expert Rev Mol Diagn. 2012;12(2):175–87. 10.1586/erm.11.97.
25. Antonelli A, Ferrari SM, Giuggioli D, Ferrannini E, Ferri C, Fallahi P. Chemokine (C-X-C motif) ligand (CXCL)10 in autoimmune diseases. (13(3)):272–80.
26. Whittaker E, Gordon A, Kampmann B, Is. IP-10 a Better Biomarker for Active and Latent Tuberculosis in Children than IFNγ? Redfield RJ. ed PLoS ONE. 2008;3(12):e3901. 10.1371/journal.pone.0003901.
27. Rajamanickam A, Ann Daniel E, Dasan B, et al. Plasma Immune Biomarkers Predictive of Progression to Active Tuberculosis in Household Contacts of Patients With Tuberculosis. J Infect Dis. 2025;231(3):696–705. 10.1093/infdis/jiae365.
28. Girmay G, Kiflie A, Alem M, Lemma M, Bewket G. Human-immunodeficiency virus infection associated with the impaired Th1 and pro-inflammatory cytokine response in latent tuberculosis-infected individuals: A comparative cross-sectional study. Bethunaickan R, ed. *PLOS ONE*. 2024;19(11):e0313306. 10.1371/journal.pone.0313306
29. Ivetic A, Ridley AJ. Ezrin/radixin/moesin proteins and Rho GTPase signalling in leucocytes. Immunology. 2004;112(2):165–76. 10.1111/j.1365-2567.2004.01882.x.
30. Guérin I, de Chastellier C. Disruption of the actin filament network affects delivery of endocytic contents marker to phagosomes with early endosome characteristics: the case of phagosomes with pathogenic mycobacteria. 2000;(79(10)):735–49.
31. Härtlova A, Herbst S, Peltier J, et al. LRRK2 is a negative regulator of *Mycobacterium tuberculosis* phagosome maturation in macrophages. EMBO J. 2018;37(12):e98694. 10.15252/embj.201798694.
32. Singhania A, Verma R, Graham CM, et al. A modular transcriptional signature identifies phenotypic heterogeneity of human tuberculosis infection. Nat Commun. 2018;9(1):2308. 10.1038/s41467-018-04579-w.
33. Berry MPR, Graham CM, McNab FW, et al. An interferon-inducible neutrophil-driven blood transcriptional signature in human tuberculosis. Nature. 2010;466(7309):973–7. 10.1038/nature09247.
34. Sweeney TE, Braviak L, Tato CM, Khatri P. Genome-wide expression for diagnosis of pulmonary tuberculosis: a multicohort analysis. Lancet Respir Med. 2016;4(3):213–24. 10.1016/S2213-2600(16)00048-5.
35. Keerthirathne TP, Magana-Arachchi DN, Madegedara D, Sooriyapathirana SS. Real time PCR for the rapid identification and drug susceptibility of Mycobacteria present in Bronchial washings. BMC Infect Dis. 2016;16(1):607. 10.1186/s12879-016-1943-y.

Figures

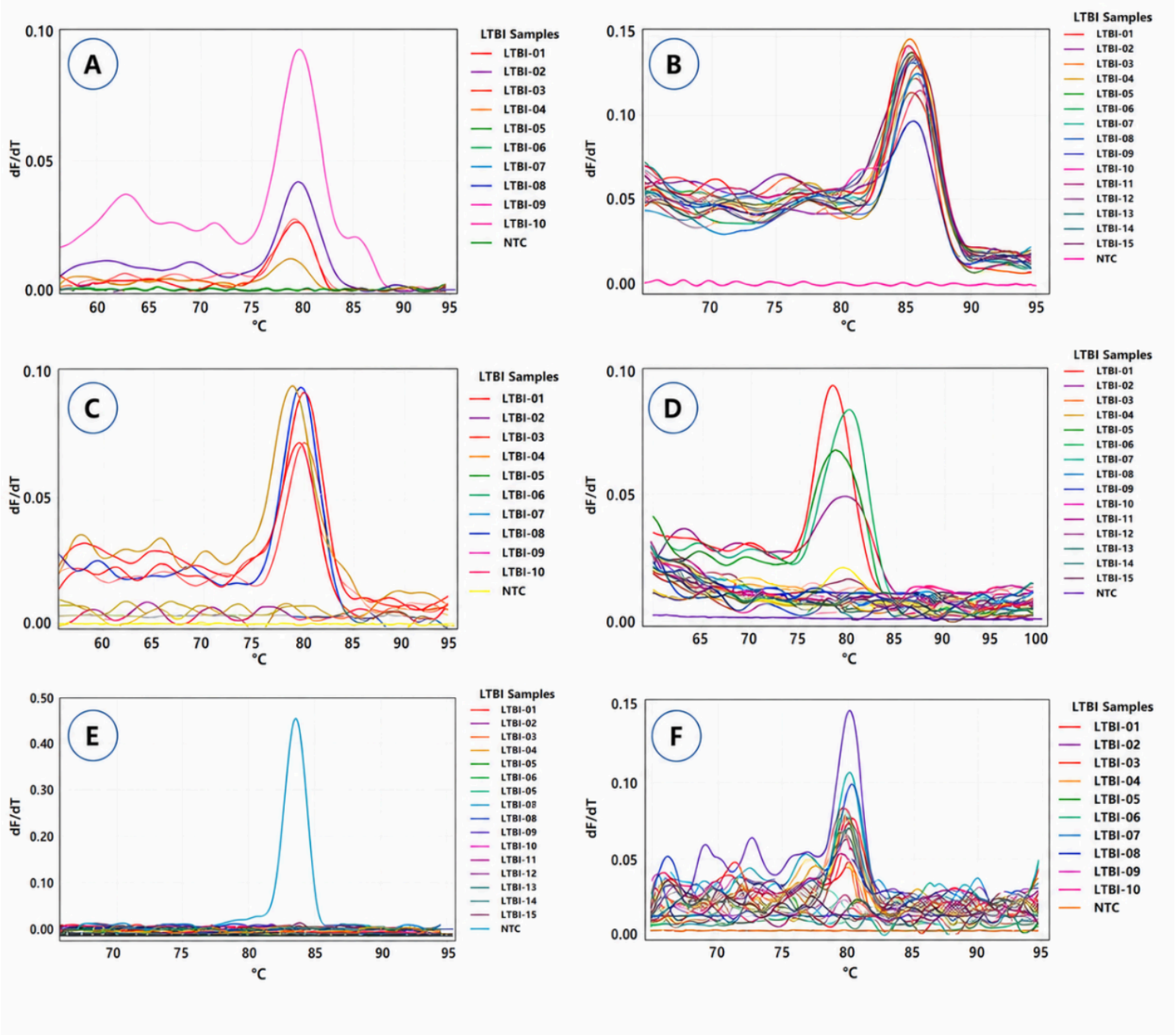


Figure 1

Confirmation of the presence of the reference gene and target genes (A) *B2M*, (B) *L6*, (C) *CXCL10*, (D) *ARSA1*, (E) *LIMD2*, and (F) *CVIL* by the melting curve generated by the SYBR Green-mediated real-time PCR