ORIGINAL ARTICLE

Pulmonary tuberculosis diagnostic test using fluorescence immunoassay-based interferon gamma release assay with IchromaTM IGRA-TB

Bastiana Bermawi, PhD [1], Diyan Wahyu Kurniasari, Clin.Path[1], Ayunda Imaniar, Clin.Path[2], Nurul Hidayatih, BSc[3], Diana Arum Lisnawati, BSc[3], Ihsan Fahmi Rofananda, MD[3], Prihatini Prihatini, PhD[1]

[1]Department of Clinical Pathology, Faculty of Medicine, Universitas Nahdlatul Ulama Surabaya, Indonesia, [2]Department of Clinical Pathology, Jemursari Islamic Hospital, Surabaya, Indonesia, [3]Research and Community Service Unit, Faculty of Medicine, Universitas Nahdlatul Ulama Surabaya, Indonesia

ABSTRACT

Introduction: Tuberculosis (TB) is a serious global health problem in Indonesia, which is the country with the second-highest TB burden after India. Accuracy in TB diagnosis is the key to effective treatment and decreased transmission rate. One of the latest diagnostic methods is interferon gamma release assay (IGRA), which measures the interferon-γ release associated with Mycobacterium tuberculosis (MTB) infection. This study aims to determine the diagnostic value of IGRA-TB using IchromaTM IGRA-TB diagnostic kit (sensitivity, specificity, positive predictive value [PPV], and negative predictive value [NPV]), compared to Ziehl-Neelsen (AFB) staining, nucleic acid amplification-based test (Xpert-MTB) and chest-X Ray as the gold standard in TB diagnosis.

Materials and Methods: A cross-sectional observational study design was used. Patients were recruited through purposive sampling from pulmonology outpatient clinic and inpatient ward at Jemursari Islamic Hospital (RSI Jemursari), Surabaya from July 2023 to December 2023. All enrolled patients should have been previously tested positive or negative for pulmonary TB using AFB staining, Xpert MTB and chest x-ray. Blood samples of the patients were collected and processed using the IchromaTM IGRA-TB diagnostic kit. The results were then compared with gold standard methods for calculating the IGRA-TB diagnostic value.

Results: A total of 56 adult patients were enrolled in this study. The sensitivity, specificity, PPV, NPV and accuracy rate of IGRA-TB using IchromaTM IGRA-TB diagnostic kit were 80.56%, 85%, 90.62%, 70.83% and 82.14%, respectively.

Conclusion: IchromaTM IGRA-TB showed reasonably high diagnostic sensitivity and specificity, indicating that this method can be further utilised as a diagnostic and screening tool for pulmonary TB.

KEYWORDS:
Pulmonary tuberculosis, IGRA-TB, diagnostic value

INTRODUCTION

Tuberculosis (TB) remains a serious global health problem today. In 2019, it was estimated that there were over 10 million TB patients worldwide, with majority of cases were concentrated in Southeast Asia (44%), Africa (25%), and the Western Pacific (18%). Indonesia is ranked second as nation with the highest TB burden after India.[1] Rapid diagnosis of TB poses a challenge in clinical practice, especially in countries with a high TB burden, where delays in the diagnosis and initiation of TB treatment is a common occurrence.[2] Efforts to diagnose TB quickly and accurately are crucial for effective treatment and controlling the transmission of this disease in the community.[3,4]

There are various methods to confirm TB diagnosis, including chest X-ray, AFB staining, bacterial culture, nucleic acid amplification test and gene-based tests. The diagnosis of TB typically involves examinations such as Ziehl-Neelsen Acid-Fast Bacilli (AFB) stain and bacterial culture, which require 2 to 6 weeks for accurate results.[5] Although there is no perfect TB diagnostic test, diagnostic technology continues to evolve, becoming more accurate, simple and suitable for point-of-care treatment.[4] Advancements in testing including tests that are sensitive and specific, as well as affordable, rapid and usable by healthcare workers with minimal training at decentralised levels.[6]

A newer, modern solutions in TB diagnosis is a method called Interferon Gamma Release Assay (IGRA), which is specific to MTB antigens. IGRA-TB is an in vitro laboratory test that measures the release of interferon-γ (IFN-γ), an inflammatory cytokine released by T-cells and macrophage after Mycobacterium tuberculosis (MTB) exposure (Meldau et al). One of the methods of IGRA is by using IchromaTM IGRA-TB (Bioditech, Chuncheon, South Korea), which is an automated fluorescence lateral flow assay (LFA)-based IGRA.[7] This method is more practical and time-effective compared to the more widely available commercial IGRA method, which uses enzyme-linked immunosorbent assay (ELISA) or the enzyme-linked immunospot (ELISpot) assay technique.[7,8]

LFA-based IGRA has the potential to become a comparative method or a new gold standard for faster and more accurate

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Corresponding Author: Bastiana Bermawi
Email: dr.bastiana@unusa.ac.id

TB diagnosis, especially in low-resource settings. This study aims to determine the diagnostic value of IGRA-TB using IchromaTM IGRA-TB diagnostic kit (sensitivity, specificity, positive predictive value [PPV] and negative predictive value [NPV]), compared to AFB staining, nucleic acid amplification-based test (Xpert-MTB) and chest-X ray as the gold standard in TB diagnosis in Indonesia.

MATERIALS AND METHODS

This study employed a cross-sectional observational method. Ethical approval for this research was granted by the Ethics Committee of RSI Jemursari Surabaya (Approval Number JS.A.SKR.2360.06.23).

Participants of the Study

Patients were recruited using purposive sampling from pulmonology outpatient clinic and inpatient ward at RSI Jemursari Surabaya from July 2023 to December 2023. Inclusion criteria were individuals aged ≥18 years diagnosed with TB based on clinical characteristics, radiology, TCM, and positive AFB for the TB group. Meanwhile, respondents aged ≥18 years with clinical symptoms such as cough, shortness of breath, weight loss resembling TB symptoms were included into the non-TB group. Individuals with HIV, cancer, uncontrolled diabetes and chronic TB were excluded. The research protocol was explained to participants at the respiratory outpatient clinic of RSI Jemursari Surabaya, and participants provided written informed consent for clinical examination and blood sample collection for IGRA-TB testing.

Blood Sample Preparation and IchromaTM IGRA-TB Testing

Procedures of IchromaTM IGRA-TB test was carried out following the manufacturer's (Bioditech, Chuncheon, South Korea) protocol. Blood sample collection is conducted at the RSI Jemursari Surabaya laboratory. Three millilitres of venous blood samples were collected from each participant (1 ml in the Nil tube, 1 ml in the Antigen tube, and 1 ml in the Mitogen tube. The three IGRA tubes (Nil, Antigen, and Mitogen) were then incubated at a temperature of 37^0C for 18–24 hours. After incubation, the samples in the IGRA tubes were centrifuged for 15 minutes at a speed of 2000-3000 RCF (G). The detection buffer in the detector tube was prepared by adding 100 µL diluent to the dried detection buffer containing anti IFN-γ and anti-chicken IgY, both of which were fluorescently labelled. After diluting the detection buffer in the detector tube with the diluent, 50 µL of the culture supernatant taken from the Nil tube was added, homogenised, and then 100 µL of the mixture was taken and dripped onto the nitrocellulose membrane on the cartridge. After incubating at room temperature for 15 minutes, the cartridge was moved and the fluorescence intensity was measured at 613 nm wavelength using the IchromaTM II instrument (Bioditech, Chuncheon, South Korea). The protocol was repeated for the culture supernatant in the antigen and mitogen tubes.

Analysis of IGRA-TB Diagnostic Value

The obtained data were used to calculate the diagnostic values of IGRA-TB, including sensitivity, specificity, PPV, NPV and the accuracy of the IGRA-TB test compared to the gold standard TB examinations, namely TCM, AFB and radiology.

RESULTS

A diagnostic test study of IGRA-TB was conducted on 56 participants which has been previously assessed as TB-positive or TB-negative by gold-standard tests. In this study, the median age of TB-positive participant was 47.5 years old (range: 19–79), meanwhile in the TB-negative participant, median age was 53 years old (range: 29–67). The majority of respondents belonged to the ≥ 60 years age group. The majority of participants were female in both TB-positive and TB-negative group (55.6% and 55%, respectively), Demographic characteristic of the respondents are displayed in Table I.

Out of the 56 samples obtained from patients at the respiratory outpatient clinic of RSI Jemursari Surabaya, 32 were determined as IGRA-TB positive, and 24 were IGRA-TB negative. From the analysis using diagnostic tests with the gold standard TB examination, the sensitivity, specificity, PPV, NPV and accuracy rate of IGRA-TB using IchromaTM IGRA-TB diagnostic kit were 80.56%, 85%, 90.62%, 70.83% and 82.14%, respectively.

DISCUSSION

In this study carried out in Indonesia, a TB-endemic country, LFA-based IGRA using IchromaTM IGRA-TB showed a reasonable sensitivity and specificity (80.56% and 85%, respectively). IGRA is a diagnostic method which indirectly assessed MTB infection by measuring IFN-γ release from T-cells, following the stimulation by antigens such as ESAT-6 and CFP-10.[9] These antigens were encoded in gene located in RD1 of the MTB complex gene, thus specific to the MTB complex infection.[9,10]

Currently, WHO recommends IGRA and tuberculin skin test (TST) as diagnostic methods in latent tuberculosis infection (LTBI). IGRA has shown remarkable sensitivity and specificity for diagnosing LTBI (91–94% sensitivity and 95–96% specificity).[11,12] TSTs have low specificity in populations vaccinated with BCG and low sensitivity in immunosuppressed individuals. Indonesia has a mandatory BCG vaccination program during childhood, thus IGRA is more suitable.[7,13,14]

Widely-available IGRA test kit is based on ELISA or ELISpot assay. An example of the ELISA-based kit is QuantiFERON®-TB Gold InTube (Cellestis Ltd., Carnegie, Australia) whereas the T-SPOT®.TBTM test (Oxford Immunotec Inc., Abingdon, UK) is based on the ELISpot assay. Meanwhile, the IchromaTM IGRA-TB is a fluorescence LFA, in which sandwich immunodetection method is used. Detector antibodies in buffer bind to antigens in the sample, forming antigen-antibody complexes and then migrate onto nitrocellulose matrix where it will be captured by the other immobilised-antibodies on a test strip. More antigens in the sample means more antigen-antibody complexes, and it leads to a stronger fluorescence signal by detector antibodies, which is captures by the instrument for Ichroma™ IGRA-TB tests to show latent TB-positive in the sample.[7,9]

Several studies comparing the diagnostic accuracy between LFA-based IGRA test using IchromaTM IGRA-TB test and ELISA-based test have shown that there is a high agreement

Table I: Demographic characteristic of participants

Parameter	TB-positive patients (n = 36)	TB-negative patients (n = 20)
Age (years)		
< 20	2 (5.6%)	0
20 – 29	7 (19.4%)	2 (10%)
30 – 39	5 (13.9%)	3 (15%)
40 – 49	7 (19.4%)	2 (10%)
50 – 59	8 (22.2%)	5 (25%)
≥ 60	7 (19.4%)	8 (40%)
Gender		
Male	16 (44.4%)	9 (45%)
Female	20 (55.6%)	11 (55%)

TB: tuberculosis

Table II: IGRA-TB diagnostic test results compared to the gold standard TB examinations

IGRA-TB	Gold-standard tests		Total
	Positive	Negative	
Positive	29	3	32
Negative	7	17	24
Total	36	20	56

IGRA: interferon-gamma release assay; TB: tuberculosis

between both test. Considering the simpler and less-time, cost and labour consuming process of IchromaTM IGRA-TB than ELISA-based test, the IchromaTM IGRA-TB has potential use as confirmatory test in low-resource settings.[8,15]

Some problems are related to IGRA accuracy in diagnosing TB, and this may explain the lower sensitivity and specificity found in this study. Some mycobacterial species in MTB complex such as M. kansasii, M. marinum and M. szugai possess CFP-10, ESAT-6 and TB 7.7 in their DNA sequence, therefore IGRA can show a false-positive owing to these species.[10] IGRA also show some false negative and indeterminate results of IGRAs in patients with active TB in some studies. The frequency of indeterminate results in IGRAs with active TB ranged from 1–20% and that of false-negative results ranged from 17–19%. All indeterminate results were due to low mitogen response in almost all studies. Factors such as Asian race, age > 65 years, sex, corticosteroid use, autoimmune disease, inpatient setting and number of comorbidities of anaemia, lymphocytopenia and hypoalbuminemia were significantly correlated with independent predictors of indeterminate results.[10] Another study also shows that indeterminate results could be attributed to the tube factor of IchromaTM IGRA-TB.[13] False-negative results were significantly correlated with elderly patients, female sex, acid-fast bacilli smear-negative, HIV co-infection and inflammatory diseases.[10]

It is also noted that IGRA cannot be used to discriminate LTBI and active TB, as IFN-γ release is an immune response caused by MTB infection, and the IFN-γ levels overlaps between LTBI dan active TB patients.[16,17] Another study shows that to discriminate between LTBI and active TB, interleukin (IL)-2 could be used as potential biomarker as it level was significantly higher in individuals with LTBI compared with patients with active TB.[18]

To the extent of our knowledge, this study presents a novelty as this is the first study assessing diagnostic value of LFA-based IGRA in diagnosing pulmonary TB. LFA-based IGRA using IchromaTM IGRA-TB has shown potential as sensitive and specific pulmonary TB diagnostic method, albeit it is more suitable in diagnosing LTBI. There are several limitations of this study. First, we use cut-off value for determining TB-positive or TB-negative as written in manufacturer's instruction, and this value may be lower or higher in places in different TB endemicity. This study is a single-centred study, and the number of samples are relatively low, therefore cautions are required to generalise the findings to the general population.

CONCLUSION

Our results showed that the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy rate of interferon gamma release assay (IGRA)-tuberculosis (TB) using IchromaTM IGRA-TB diagnostic kit for pulmonary TB were 80.56%, 85%, 90.62%, 70.83% and 82.14%, respectively. High sensitivity and specificity for this method suggests that it may be useful for pulmonary TB diagnosis, especially in lower-resource settings. Further studies are required to implement lateral flow assay (LFA)-based IGRA as point-of-care TB diagnostic method.

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REFERENCES

1. Bastiana, B. and Arimbi, M.R. 2022. Sputum smear conversion as prognostic determinant of timely complete therapy on pulmonary tuberculosis. Indonesian J Clin Pathology Med Lab 2022; 28(8): 219-24.
2. Getnet F, Demissie M, Assefa N, Mengistie B, Worku A. Delay in diagnosis of pulmonary tuberculosis in low-and middle-income settings: systematic review and meta-analysis. BMC Pulm Med 2017; 17(1): 202.
3. Treatment Action Group. 2020. Panduan Activist Untuk Alat Diagnostik Tuberculosis. Treatment Action Group [cited January 2024]. Accessed from: https://npin.cdc.gov/publication/panduan-activist-untuk-alat-diagnostik-tuberculosis-activist%E2%80%99s-guide-tuberculosis
4. Kon, OM. Tuberculosis in clinical practices. Nature 2021; 29–52.
5. World Health Organization. 2011. Early detection of tuberculosis: an overview of approaches, guidelines and tools [cited Jan 2024]. Accessed from: https://apps.who.int/iris/handle/10665/70824#sthash.TN5snAtB.dpuf.
6. García-Basteiro AL, DiNardo A, Saavedra B, Silva DR, Palmero D, Gegia M, Migliori GB, et al. Point of care diagnostics for tuberculosis. Pulmonol 2018; 24(2): 73-85.
7. Bioditech Med Inc. 2021. IchromaTM IGRA-TB Guide. Bioditech Med Inc, South Korea
8. Lee HH, Choi DH, Kim JR, Kim YG, Jo KW, Shim TS. Evaluation of a lateral flow assay-based IFN-γ release assay as a point-of-care test for the diagnosis of latent tuberculosis infection. Clin Rheumatol 2021; 40(9): 3773-81.
9. Susilawati TN and Larasati R. A recent update of the diagnostic methods for tuberculosis and their applicability in Indonesia: a narrative review. Med J Indones 2019; 28(3): 284-91.
10. Kobashi Y. Current status and future landscape of diagnosing tuberculosis infection. Respir Investig 2023; 61(5): 563-78.
11. Sotgiu G, Saderi L, Petruccioli E, Aliberti S, Piana A, Petrone L, et al. QuantiFERON TB Gold Plus for the diagnosis of tuberculosis: a systematic review and meta-analysis. J Infect 2019; 79(5): 444-53.
12. Pourakbari B, Mamishi S, Benvari S, Mahmoudi S. Comparison of the QuantiFERON-TB Gold Plus and QuantiFERON-TB Gold In-Tube interferon-g release assays: a systematic review and meta-analysis. Adv Med Sci 2019; 64(2): 437-43.
13. Kweon OJ, Lim YK, Kim HR, Kim TH, Lee MK. Performance evaluation of newly developed fluorescence immunoassay-based interferon-gamma release assay for the diagnosis of latent tuberculosis infection in healthcare workers. J Microbiol Immunol Infect 2022; 55(2): 328-31.
14. Ministry of Health of Republic of Indonesia. 2022. Strategi Komunikasi Nasional Imunisasi 2022-2025. Ministry of Health of Republic of Indonesia, Jakarta, Indonesia
15. Hur YG, Hong JY, Choi DH, Kim A, Park SY, Kwon M, et al. A feasibility study for diagnosis of latent tuberculosis infection using an IGRA point-of-care platform in South Korea. Yonsei Med J 2019; 60(4): 375-80.
16. Higuchi K, Harada N, Fukazawa K, Mori T. Relationship between whole-blood interferon-gamma responses and the risk of active tuberculosis. Tuberculosis 2008; 88(3): 244-8.
17. Kobashi Y, Shimizu H, Ohue Y, Mouri K, Obase Y, Miyashita N, et al. Comparison of T-cell interferon-g release assays for Mycobacterium tuberculosis-specific antigens in patients with active and latent tuberculosis. Lung 2010; 188(4): 283-7.
18. Mamishi S, Pourakbari B, Marjari M, Bahador A, Mahmoudi S. Discrimination between latent and active tuberculosis: the role of interleukin-2 as biomarker. J Infect 2015; 70(4): 429-31.