Original Research Paper

Analysis of Interferon-Gamma Release Assay Results for Latent Tuberculosis Infection Diagnosis at a Referral Clinical Laboratory in South Korea

[1]Tae Soung Kim, [2]Ga Yeon Kim, [3]Young Ki Lee and [3]Jae Kyung Kim

[1]*Department of Clinical Pathology, Graduate School of Dankook University, Cheonan, Republic of Korea*
[2]*Department of Public Health, Graduate School of Dankook University, Cheonan, Republic of Korea*
[3]*Department of Biomedical Laboratory Science, College of Health and Welfare, Dankook University, Cheonan, Republic of Korea*

Abstract: The world health organization states that individuals with Latent Tuberculosis Infection (LTBI) are infected with Tuberculosis (TB) bacteria but do not show symptoms of active disease. However, they are at possibility of developing vigorous TB and potentially transmitting the illness to others. In 2018, South Korea reported a 14.8% positivity rate in LTBI screening, with higher rates observed among older individuals, those with lower incomes and women. Furthermore, approximately one in four people worldwide has LTBI. LTBI within certain groups is often the result of prolonged, cumulative and close contact with infected individuals. In this study, we aimed to analyze the current scenario regarding LTBI in South Korea, where large groups are being tested utilizing the Interferon-Gamma Release Assay (IGRA). From 2019-2022, 219,459 cases with IGRA results obtained from a referral clinical laboratory in Yongin city were investigated. This research was accepted by the Institutional Review Board (IRB) of Dankook university. The specimens were collected in QuantiFERON® gold plus Tubes (TB) (Qiagen GmbH, Hilden, Germany) and interferon-gamma activity was measured using an enzyme-linked immunosorbent assay (Dynex technologies, Inc., Chantilly, Virginia, USA). multinomial logistic regression analysis was performed to study the association among age, sex and LTBI confirmed by IGRAs. The p-value for sex was less than 0.001, indicating a significantly higher positivity rate in women than that in men. Additionally, the positivity rate decreased in both sexes from 2019-2022. Notably, the highest positivity rates were observed in 50-59-year age set, with men at 1.32% and women at 2.33%. As testing frequency increased, the probability of detecting positive results also increased. Continuous IGRA testing is recommended for individuals in the 50-59-year age group. Regular and extensive testing is recommended for healthcare workers, military personnel, prisoners and students. Therefore, the pros and cons of IGRA can be analyzed and it is believed that it can be helpful for public health.

Keywords: Interferon-Gamma, Interferon-Gamma Releasing Assay, Latent Tuberculosis Infection, Mycobacterium Tuberculosis, Quantiferon Tb Gold Plus

Introduction

According to the world health organization, individuals with Latent Tuberculosis Infection (LTBI) are infected with Tuberculosis (TB) bacteria but do not show symptoms of active TB. However, individuals with LTBI are at a possibility of having a vigorous TB and can potentially infect others (WHO, 2019). According to global estimates, one in four individuals has LTBI; therefore, expanding effective LTBI treatment is necessary to reduce the global incidence of TB, as outlined in the "end TB strategy," along with the rapid expansion of TB-preventive treatment in high-risk populations (WHO, 2019). In South Korea, the rate of

positive results for LTBI screening was 14.8% (12,660 of 857,765 individuals tested) in 2018, with higher positivity rates observed among older individuals, those with lower income and women. The treatment initiation rate in positive cases, which represents the proportion of individuals who begin treatment upon receiving a positive result, stands at only 31.7% (MHW, 2019). Although TB is preventable, action toward of LTBI is underutilized. To achieve the goal of TB elimination, it is crucial to prioritize screening of high-risk groups, especially individuals from countries with high TB incidence rates and provide patient-centered support (Bruxvoort *et al.*, 2023). The prevalence of LTBI is significantly increased (or higher) among contacts within households where pulmonary TB is present (Sangma *et al.*, 2023). Furthermore, workplace-associated LTBI may result from work-related interactions and continued, accumulative and near interaction with sick colleagues (Ruamtham *et al.*, 2023). Additionally, it is particularly important to detect LTBI in high-risk populations, including those in crowded settings such as correctional facilities and Healthcare Workers (HCWs).

Patients with LTBI are asymptomatic and non-infectious; however, they are at a possibility of having vigorous TB (Pagaduan and Altawallbeh, 2023). Therefore, it is important to prevent the incidence and transmission of TB. Additionally, treatment of LTBI has been exposed to lessen the possibility of progression to active TB (Jonas *et al.*, 2023; Saint-Pierre *et al.*, 2023).

The Interferon-Gamma Release Assay (IGRA) has shown moderate compassion and high specificity in detecting LTBI. A use of QuantiFERON® TB Gold Plus (QFT-Plus) is appropriate for LTBI discovery in countries burdened with TB (Matucci *et al.*, 2023; Rudeeaneksin *et al.*, 2023). The global estimate is that one in four individuals have LTBI. An analysis of the current scenario of LTBI in South Korea, which screens numerous groups through IGRAs using the QFT-Plus tube kit to achieve the goal of TB eradication, was deemed necessary and was performed.

Materials and Methods

Samples for testing originating from hospitals across the country (including primary hospitals with 30 beds or fewer, secondary hospitals with 30-300 beds and tertiary hospitals with more than 300 beds) were sent to a referral clinical laboratory in Yongin city equipped for LBTI testing. We analyzed trends in sex-specific positivity rates based on age using IGRA results recorded from January 01, 2019, -December 31, 2022. Overall, 219,459 patients are involved: 193,866 tested negative, 22,662 tested positive and 2,931 showed indeterminate results (QuantiFERON®-TB Gold Plus, 2023). Samples were collected using QFT-Plus tubes (Qiagen GmbH, Hilden, Germany) and cultured in 16-24 h at 37±1°C. Interferon-gamma (IFN-γ) activity was measured using a QuantiFERON TB gold plus (Qiagen GmbH. Hilden, Germany) reagent and the DS-2 (Dynex technologies, Inc., Chantilly, Virginia, USA) enzyme-linked immunosorbent assay (ELISA) system (QuantiFERON®-TB Gold Plus, 2023), after the samples are cultured in 16-24 h at 37±1°C.

QuantiFERON®-TB gold plus: Fourth-generation test reagent, Dynex DS2: Testing equipment, positive: Latent Tuberculosis Infection (LTBI) positive, negative: LTBI negative, indeterminate: Undetermined. [Interpretation of results] positive: If Nil value is ≤8.0 and both TB1 Ag-Nil and TB2 Ag-Nil values are ≥0.35 and ≥25% of Nil value. Indeterminate: If Nil value is ≤8.0 and TB Ag-Nil value is <0.35 and Mitogen-Nil value is <0.5; if Nil value is ≤8.0 and TB Ag-Nil value is ≥0.35 and <25% of Nil value and Mitogen-Nil value is <0.5; and if Nil value is >8.0. Negative: If Nil value is ≤8.0 and both TB1 Ag-Nil and TB2 Ag-Nil values are <0.35 and Mitogen-Nil value is ≥0.5; if Nil value is ≤8.0 and both TB1 Ag-Nil and TB2 Ag-Nil values are ≥0.35 and <25% of Nil value and Mitogen-Nil value is ≥0.5.

The results were subjected to secondary data analysis utilizing IBM SPSS Statistics (version 29.0; IBM Corp., Armonk, NY, USA) and the association between age, sex and LTBI, confirmed through IGRA, was investigated. For this purpose, multinomial logistic regression analysis was performed on the significance probability value (Fig. 1).

The Institutional Review Board (IRB) of Dankook university (IRB No. DKU 2023-04-002-002) approved this research and was shown in accordance with the principles of the Declaration of Helsinki. As data were analyzed retrospectively and patients' personal information was not used, the requirement for informed agreement was renounced by IRB of Dankook university.
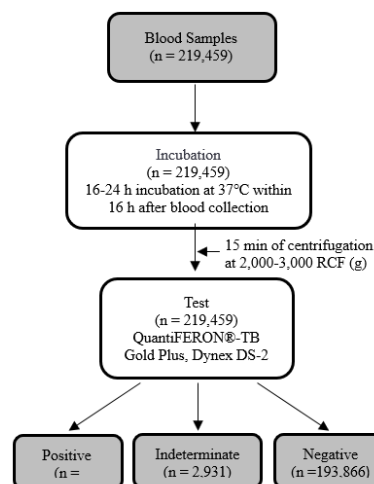

Fig. 1: Flow chart of the study

Results

According to the IGRA data reported from 2019-2022, the trend of sex-specific positivity rates by age group for men showed that the rates increased from 0-9 years 50-59 years of age and then decreased. The positivity rate was 0.03% for those aged 10-19 years, 0.54% for those aged 30-39 years, 1.32% for those aged 50-59 years and 0.45% for those aged ≥70 years. For women, the positivity rates increased from 0-9 years 50-59 years, followed by a decrease from 60-69 years. The rate was 0.01% for those aged 0-9 years, 1.13% for those aged 30-39 years, 2.33% for those aged 50-59 years and 0.40% for those aged ≥70 years. The p-value for the overall age was <0.001, indicating an important link among age and positive IGRA results. Both men and women aged 50-59 years displayed the maximum positivity rates, followed by a decline after 59 years (Table 1).

Regarding annual sex-specific IGRA positivity from 2019-2022 for men, the rate decreased from 4.28% in 2019 3.48% in 2022. This rate decreased from 7.35% in 2019 5.49% in 2022 for women. The p-value for sex was <0.001, indicating a significant difference in the positivity rates between men and women. Both sexes had a decreasing trend in the positivity rates from 2019-2022 (Table 2).

From 2019-2022, a continuous growth in the percentage of positive results, rising from 1.77% in 2019 3.23% in 2022. Similarly, indeterminate results also increased, going from 0.17% in 2019 0.48% in 2022, while the percentage of negative results saw a significant rise from 13.29% in 2019 32.30% in 2022. This trend of increasing percentages for positive, indeterminate and negative results is depicted in Fig. 2.

Table 1: Interferon-gamma release assay positivity rates from 2019 to 2022 according to age and sex

Age (years)	Positivity rate (%)		
	Men	Women	Overall
0-9	0.00	0.01	0.01
10-19	0.03	0.04	0.05
20-29	0.15	0.50	0.65
30-39	0.54	1.13	1.23
40-49	0.88	1.92	2.34
50-59	1.32	2.33	3.10
60-69	0.93	1.09	1.97
≥70	0.45	0.40	1.00

The p-value for age was <0.001, indicating a significant association with the interferon-gamma release assay results

Table 2: Comparison of interferon-gamma release assay positivity rates from 2019-2022

Year	Positivity rate (%)		
	Men	Women	Overall
2019	4.28	7.35	11.63
2020	4.03	6.83	10.86
2021	4.30	6.67	10.97
2022	3.48	5.49	8.98

The significance probability value for sex was <0.001, indicating that female sex had a significant effect on the interferon-gamma release assay results
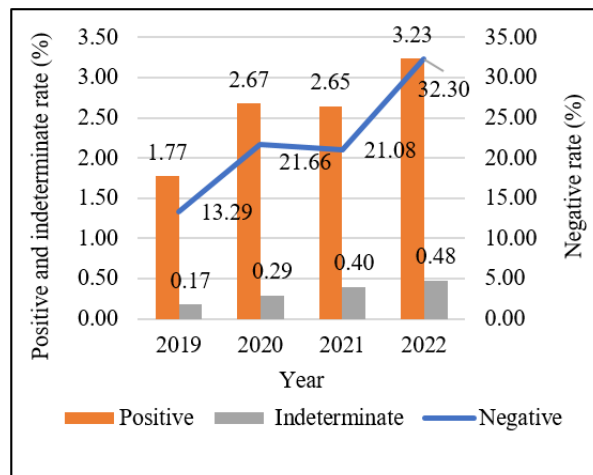
Fig. 2: Distribution of positive, indeterminate and negative interferon-gamma release assay results during the study period (2019-2022)

Discussion

The present study provides valuable insights regarding the trend of sex-specific IGRA positivity rates by age group. For men, the positivity rates showed an increasing trend from an average of 0-9 years 50-59 years, followed by a decrease until the age of ≥70 years. The positivity rates were 0.03% for an average of 10-19 years, 0.54% for 30-39 years, 1.32% for 50-59 years and 0.45% for ≥70 years. For women, the positivity rates showed an increasing trend from an average of 0-9 to 50-59 years, followed by a decrease from 60-69 years. The positivity rates were 0.01% for an average of 0-9 years, 1.13% for 30-39 years, 2.33% for 50-59 years and 0.40% for ≥70 years.

The average age of individuals with a positive result for LTBI obtained utilizing the QuantiFERON® TB Gold In-Tube (QFT-GIT) test in Taiwan was 58.2 years and the tendency of QFT-GIT positivity augmented after 50 years of age (Chang *et al.*, 2022). In line with the results obtained from this study, it was noted that there were notably high positivity rates among individuals aged ≥50 years.

The probability of LTBI in individuals aged 50 years and older in this study was statistically important (p<0.001), indicating a noteworthy association. Both men and women exhibited the highest positivity rates in the age group of 50-59 years, with rates gradually decreasing after the age of 60-69 years. A study conducted in China using the QFT-GIT showed a 10.80% LTBI positivity rate (Zhao *et al.*, 2023). In this study, the annual positivity for LTBI based on IGRA results from 2019-2022 showed a decreasing trend among men, but the rate for 2021 (4.30%) was higher than the rates for 2019 (4.28%) and 2022 (3.48%). Among women in Taiwan, the annual positivity rates were 7.35% in 2019, 6.67% in 2021 and

5.49% in 2022. These rates were found to be lower than those reported in a study conducted in China.

The p-value for sex was <0.001, indicating a significantly higher positivity rate in women for the IGRA results. Both men and women had a decreasing annual positivity rate from 2019-2022. From 2019-2022, the distributions of the positive, indeterminate and negative IGRA results showed an increasing trend. Analysis of the distribution of positive results revealed it was 1.77% in 2019, 2.67% in 2020, 2.65% in 2021 and 3.23% in 2022, with a total of 10.32%. Although it was slightly lower than 10.80% from China's preceding study, there was not a significant difference.

According to a systematic analysis and meta-analysis of a global research conducted by authors from Germany, the prevalence of LTBI was the lowest in North American and pacific countries and the top in Eastern Mediterranean countries (Peters *et al.*, 2020). Additionally, HCWs in Semarang, Indonesia, exhibit a high prevalence of LTBI (Erawati and Andriany, 2020). Furthermore, a study in Poland indicated that the average IFN-γ concentration in IGRA principles was meaningfully developed in the LTBI group than in children with active TB, suggesting its potential use as a selective test for children and youths unprotected to infectious TB (Druszczynska *et al.*, 2023). An IGRA screening strategy is also effective in children in areas with low TB prevalence (Calzada-Hernández *et al.*, 2023). Tailored interventions are required to prescription and accomplishment for patients with LTBI (Bruxvoort *et al.*, 2023).

Analysis of the IGRA results from 2019-2022, revealed that the age of 50-59 years had the maximum positivity rate among both men and women. Furthermore, the likelihood of detecting a positive result increase with the frequency of testing. Considering the decreasing trend in yearly positivity rates, continuous testing with IGRAs is necessary at healthcare institutions for individuals aged 50-59 years to monitor and manage LTBI cases effectively. Our 4-year analysis of IGRA results provides valuable insights into LTBI trends. There is a need for regular and expanded testing of high-risk groups, such as HCWs, military personnel, detainees and students.

Rifampin and isoniazid, used for LTBI treatment, can cause adverse effects such as hepatotoxicity and peripheral neuropathy (Jonas *et al.*, 2023; Kim *et al.*, 2023). It is important to ensure that the drugs used for treatment are safe for patients and that effective methods are developed for each step of LTBI testing and treatment (Yang *et al.*, 2023; Cola *et al.*, 2023). Additionally, there is a need for research on drug susceptibility for available pharmacotherapy.

This study has some limitations. First, we only analyzed the results of IGRAs conducted at a single institution and the results were not compared with those obtained using other analytical methods. Second, this study did not consider the possibility of duplicate testing.

Therefore, the pros and cons of IGRA can be analyzed and it is believed that it can be helpful for public health.

Conclusion

The LTBI positivity rate is high in both men and women 50-59 years of age and the IGRA positivity rate increases with the frequency of testing. Continued IGRA testing is necessary for groups such as HCWs, soldiers and students. Furthermore, it is essential to compare and analyze the results of IGRAs and other testing methods as representative data in future studies conducted in Korea. This will allow for a thorough examination of the pros and cons of IGRA, with the potential to benefit public health.

Acknowledgment

Funding Information

Author's Contributions

Tae Soung Kim and Ga Yeon Kim: Made substantial contributions to the conception and designed of the study. These authors contributed equally to this study.

Young Ki Lee and Jae Kyung Kim: Made substantial contributions and acquisition and analysis of the data.

Ethics

The authors state that there is no conflict of interest. This research was accepted by the Institutional Review Board (IRB) of Dankook University (IRB No. DKU 2023-04-002-002) and was directed in accordance with the principles of the Declaration of Helsinki. As data were analyzed retrospectively and patients' personal information was not used, the requirement for obtaining informed agreement was renounced by the IRB of Dankook University.

References

Bruxvoort, K. J., Skarbinski, J., Fischer, H., Li, Z., Eaton, A., Qian, L., ... & Tartof, S. Y. (2023). Latent Tuberculosis Infection Treatment Practices in Two Large Integrated Health Systems in California, 2009-2018. In *Open Forum Infectious Diseases*, (Vol. *10*, No. 5, p. ofad219). US: Oxford University Press. https://doi.org/10.1093/ofid/ofad219

Calzada-Hernández, J., Anton, J., Martín de Carpi, J., López-Montesinos, B., Calvo, I., Donat, E., ... & Noguera-Julian, A. (2023). Dual latent tuberculosis screening with tuberculin skin tests and QuantiFERON-TB assays before TNF-α inhibitor initiation in children in Spain. *European Journal of Pediatrics*, *182*(1), 307-317. https://doi.org/10.1007/s00431-022-04640-3

Chang, A., Wu, C. Z., Lin, J. D., Lee, C. N., Tsai, K. Y., Wu, P. H., & Hsieh, A. T. (2022). Prevalence and risk factors for latent tuberculosis among diabetes patients in Taiwan: A cross-sectional study. *The Journal of Infection in Developing Countries*, *16*(04), 644-649. https://doi.org/10.3855/jidc.15839

Cola, J. P., Prado, T. N. D., Campos, B. A., Borges, B. J. P., Alves, B. M. C. S., Jezus, S. V. D., ... & Noia Maciel, E. L. (2023). Protocol for pragmatic randomized clinical trial to evaluate the completion of treatment of latent Mycobacterium tuberculosis infection with Isoniazid in the 300 mg formulation. *Plos One*, *18*(2), e0281638. https://doi.org/10.1371/journal.pone.0281638

Druszczynska, M., Seweryn, M., Wawrocki, S., Pankowska, A., Godkowicz, M., & Kowalewska-Pietrzak, M. (2023). The Interferon-Gamma Release Assay versus the Tuberculin Skin Test in the Diagnosis of Mycobacterium Tuberculosis Infection in BCG-Vaccinated Children and Adolescents Exposed or Not Exposed to Contagious TB. *Vaccines*, *11*(2), 387. https://doi.org/10.3390/vaccines11020387

Erawati, M., & Andriany, M. (2020). The prevalence and demographic risk factors for Latent Tuberculosis Infection (LTBI) among healthcare workers in Semarang, Indonesia. *Journal of Multidisciplinary Healthcare*, 197-206. https://doi.org/10.2147/JMDH.S241972

Jonas, D. E., Riley, S. R., Lee, L. C., Coffey, C. P., Wang, S. H., Asher, G. N., ... & Kahwati, L. C. (2023). Screening for latent tuberculosis infection in adults: Updated evidence report and systematic review for the US Preventive Services Task Force. *JAMA*, *329*(17), 1495-1509. https://doi.org/10.1001/jama.2023.3954

Kim, S., Thal, R., & Szkwarko, D. (2023). Management of Latent Tuberculosis Infection. *JAMA*. https://doi.org/10.1001/jama.2022.24362

Matucci, T., Riccardi, N., Occhineri, S., Pontarelli, A., Tiseo, G., Falcone, M., ... & Sotgiu, G. (2023). Treatment of latent tuberculosis infection in incarcerated people: A systematic review. *Clinical Microbiology and Infection*. https://doi.org/10.1016/j.cmi.2023.02.016

MHW. (2019). Analysis of Korean national LTBI mass screening program and establishment of LTBI cohort. *Ministry for Health and Welfare*. https://www.mohw.go.kr/react/al/sal0301vw.jsp?PAR_MENU_ID=04&MENU_ID=0403&CONT_SEQ=348007

Pagaduan, J. V., & Altawallbeh, G. (2023). Advances in TB testing. *Advances in Clinical Chemistry*. https://doi.org/10.1016/bs.acc.2023.03.003

Peters, C., Kozak, A., Nienhaus, A., & Schablon, A. (2020). Risk of occupational latent tuberculosis infection among health personnel measured by interferon-gamma release assays in low incidence countries-A systematic review and meta-analysis. *International Journal of Environmental Research and Public Health*, *17*(2), 581. https://doi.org/10.3390/ijerph17020581

QuantiFERON®-TB Gold Plus. (2023). *QIAGEN* https://www.quantiferon.com/products/quantiferon-tb-gold/package-inserts/

Ruamtham, N., Chaiear, N., Krisorn, P., Faksri, K., & Chanpho, P., (2023). Latent tuberculosis infection one year after exposure among hospital laboratory workers. *Asia-Pacific Journal of Science and Technology*, *28*(2), 10. https://doi.org/10.14456/apst.2023.18

Rudeeaneksin, J., Srisungngam, S., Klayut, W., Bunchoo, S., Bhakdeenuan, P., & Phetsuksiri, B. (2023). QuantiFERON-TB Gold Plus and QuantiFERON-TB Gold In-tube assays for detecting latent tuberculosis infection in Thai healthcare workers. *Revista do Instituto de Medicina Tropical de São Paulo*, *65*. https://doi.org/10.1590/S1678-9946202365013

Saint-Pierre, G., Conei, D., Cantillana, P., Raijmakers, M., Vera, A., Gutiérrez, D., ... & Ramonda, P. (2023). Comparison of Two Tuberculosis Infection Tests in a South American Tertiary Hospital: STANDARD F TB-Feron FIA vs. QIAreachTM QuantiFERON-TB. *Diagnostics*, *13*(6), 1162. https://doi.org/10.3390/diagnostics13061162

Sangma, V. S. C., Jaggi, S., Saini, V., Aggarwal, D., Kumar, P., & Chander, J. (2023). Prevalence of latent tuberculosis infection in the household contacts of pulmonary tuberculosis, time to treat. *Monaldi Archives for Chest Disease*. https://doi.org/10.4081/monaldi.2023.2563

WHO. (2019). Update of WHO guidelines on the management of latent TB infection. *World Health Organization*.

Yang, H., Lee, J. S., & Kim, Y. (2023). Healthcare workers' acceptance of and adherence to latent tuberculosis treatment. *Occupational Medicine*, *73*(4), 186-192. https://doi.org/10.1093/occmed/kqad036

Zhao, R., Miao, W., & Li, B. (2023). The Relation Between Trace Elements and Latent Tuberculosis Infection: A study based on national health and nutritional examination survey 2011-2012. *Biological Trace Element Research*, *201*(3), 1080-1089. https://doi.org/10.1007/s12011-022-03240-4