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by Maisuri Tadjuddin Chalid

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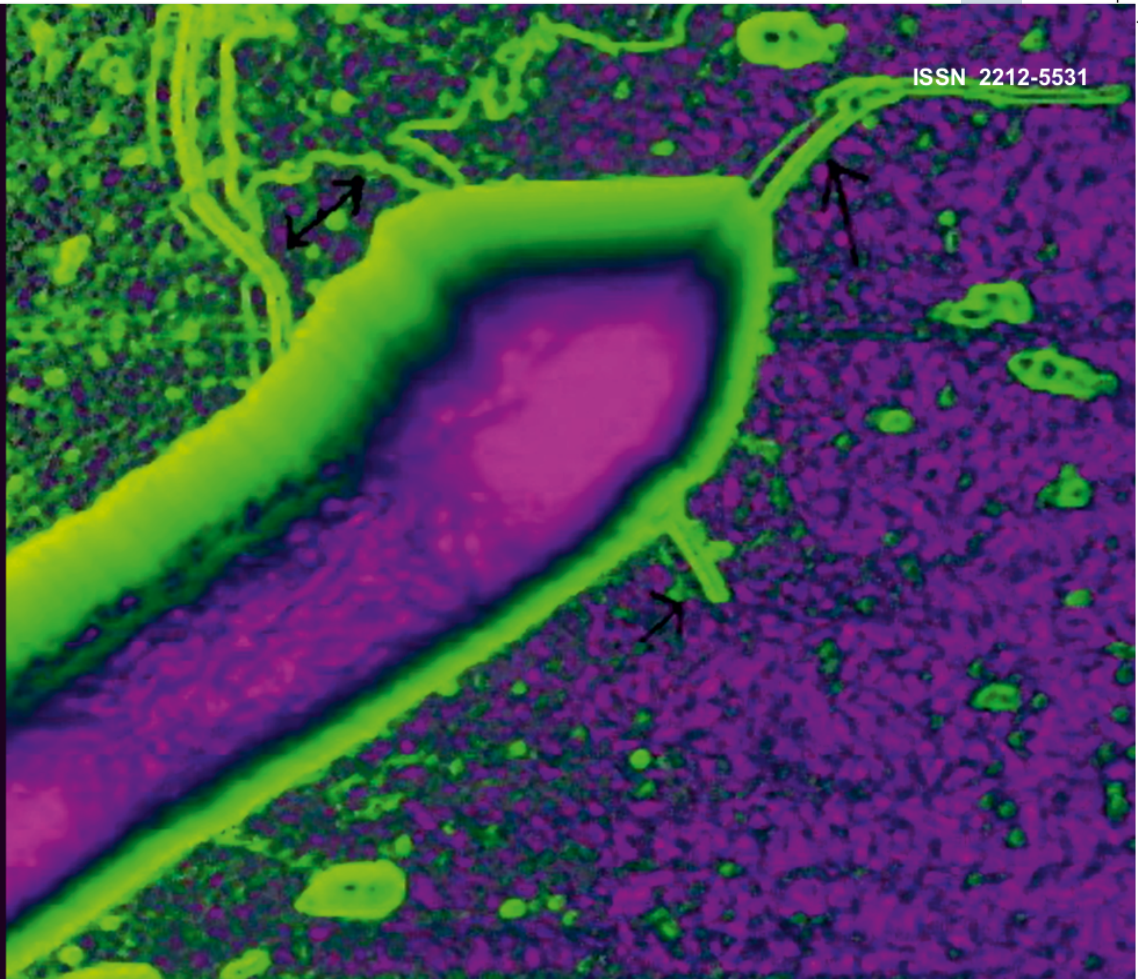
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Tuberculin Test versus Interferon Gamma Release Assay in Pregnant Women with Household Contacts of Tuberculosis Patients

Maisuri Tadjuddin Chalid¹, Dian Puspawaty¹, Andi Mardiah Tahir¹, Hidayah Najdah², Muhammad Nasrum Massi³

Departments of ¹Obstetrics and Gynecology, ²Clinical Microbiology, Faculty of Medicine, Hasanuddin University Hospital, Universitas Hasanuddin, ³Postgraduate Program, Faculty of Medicine, Universitas Hasanuddin, Makassar, South Sulawesi, Indonesia

Abstract

Background: Pregnant women who live in tuberculosis (TB)-affected households are more likely to develop latent TB infection (LTBI), which often escape treatment. This study aims to determine if Interferon-gamma release (IGRA) is reliable in screening for LTBI in pregnant women, compare to the tuberculin skin test (TST). **Methods:** It was a cross-sectional study that involved 60 pregnant women with TB contact history as a proxy for LTBI and 30 pregnant women without contact history. Latent TB was detected using the TST 5 tuberculin units and IGRA using the QuantiFERON Gold Plus TB Test kit (QFT-Plus). The sensitivity and specificity of the two diagnostic methods and the agreement between them were estimated using SPSS version 20.0. **Results:** The sensitivity 95% (95% confidence interval [CI]: 86.08%–98.96%) and specificity 26.7% (95% CI: 12.28%–45.89%) of TST were compared to that of the IGRA with 60% (95% CI: 46.54%–72.44%) and 73.3% (95% CI: 54.11%–87.72%) sensitivity and specificity, respectively in detecting LTBI in pregnancy. Although there was a significant difference ($P < 0.05$) between TST and IGRA, the agreement was fair (kappa 0.39; 95% CI: 0.24–0.45). **Conclusion:** TST assay is more sensitive than IGRA; however, the specificity of IGRA was superior to the TST method. In this study, a fair agreement of TST and IGRA was observed for detecting latent TB infection in pregnant women with household contact with TB patients.

Keywords: Interferon-gamma release assay, latent tuberculosis, pregnancy, tuberculin test

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INTRODUCTION

Worldwide, tuberculosis (TB) remains a health problem. TB is the leading cause of a single infectious agent, which rank above HIV/AIDS and is the tenth leading cause of death globally. The incidence of new TB cases is estimated at 10 million cases globally in 2019. Indonesia ranks third highest worldwide and contributes to 8.4% of global TB cases after India.^[1] Individuals with active TB whose sputum is smear-positive are the main source of *Mycobacterium tuberculosis* transmission in communities through respiratory droplets released into the air.^[2,3] A condition in which individuals are infected with *M. tuberculosis* without clinical manifestations of active TB called latent TB infection (LTBI). These individuals are at risk for developing active TB disease,^[4] pregnant women are particularly at high risk of developing TB.^[5]

Pregnant women with LTBI are more likely to progress developing the active tuberculous disease than men. It has long been observed by obstetricians that pregnancy is associated with a higher risk of active TB and also the more rapid progression of TB disease compared with the non-pregnant state.^[6]

Pregnancy may increase susceptibility to the risk of contracting infectious diseases including TB due to changes

Address for correspondence: Dr. Maisuri Tadjuddin Chalid, Department of Obstetrics and Gynecology, Faculty of Medicine, Hasanuddin University Hospital, Universitas Hasanuddin, Perintis Kemerdekaan Street Km. 10, Tamalanrea, Makassar 90245, South Sulawesi, Indonesia. E-mail: maisurichalid@gmail.com


ORCID:

Maisuri Tadjuddin Chalid: <https://orcid.org/0000-0001-8790-4915>

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in cell-mediated (Th1) immunity that protects against TB and body-mediated (Th2) immunity that leads to changes in the levels of pro-inflammatory cytokines.^[17] Transmission, infection, and clinical manifestations of TB during pregnancy is similar to that of its counterpart despite some typical symptoms of TB.^[8] In addition, pregnant women who develop TB are more difficult to diagnose because TB symptoms are physiologically similar to pregnancy symptoms.^[9] If left untreated, or if the diagnosis is late, TB during pregnancy may lead to poor outcomes for both mother and child. An increased risk of preeclampsia, vaginal bleeding, early pregnancy loss, and pre-term labour are the complications faced by infected pregnant women,^[8] whereas low birth weight and small gestational age are faced by neonates.^[10] Therefore, TB antenatal screening for pregnant women is an important strategy to identify and diagnose LTBI for improved pregnancy outcomes and reduction of TB transmission.

The individual that is known to have close household contacts with pulmonary TB or are suspected to have active TB are particularly at high risk to develop latent TB in medium to high TB burden countries, where most of the cases result from a recent transmission.^[11,12] There is a large ongoing risk of developing TB after initial contact, particularly during the first year. In low and middle-income countries, the prevalence of TB in household contacts is 3.1%.^[12] Screening TB patients from close household contacts are expected to reach a 2% decrease in TB prevalence every year.^[13] Active screening among household contacts is an effective way to improve TB case detection.^[14] Diagnosis and treatment of LTBI are the most effective strategy to control TB transmission. This strategy prevents the risk of the progression of active TB disease that may otherwise accelerate the eradication of TB.^[15] Until recently, the available methods to predict the progression of active TB from LTBI are the tuberculin skin test (TST) and interferon-gamma (IFN- γ) release assays (IGRAs).^[16]

For more than a century, TST is the main diagnostic tool for TB which relies on delayed-type hypersensitivity response to purified protein derivative (PPD) and is often used to estimate the average burden of TB infection in a population.^[17] However, false-positive TST results can occur due to nontuberculous mycobacterial infection and Bacille Calmette–Guerin (BCG) vaccination,^[18-21] particularly if the BCG is received after infancy.^[22] Active TB, HIV infection, malnutrition, impaired immunity, immunosuppressant drugs such as prednisolone and methotrexate therapy also affect skin test sensitivity.^[23-25] The immunocompromised person, children under five years, and older people may result in false negatives.^[26]

The IGRA test is based on the production of IFN- γ , a key cytokine involved in cellular immunity,^[27-29] released by T-cells and natural killer (NK) cells as a specific response to *M. tuberculosis*,^[30] while absent in BCG and most other mycobacteria, and thus, makes the IGRA assay more accurate than TST.^[31] This assay requires costly equipment and consumables. The IGRA positive rate for household contacts

of TB patients ranges from 42 to 65%.^[32-35] Most of the TST or IGRA-positive LTBI patients remain not reactivated after latent infection, and the TB risk was not significantly different between the two groups.^[36] In this study, we evaluate TST and IGRA in detecting latent TB infection in pregnant women with household contact history to TB patients.

METHODS

Study population and design

Between July and November 2018, a cross-sectional study was conducted at Makassar city, South Sulawesi Province of Indonesia. Pregnant women which have a household contact history with TB patients were traced through interviews with 271 TB patients who regularly seek treatment at the Pulmonary Hospital and several primary health cares and recruited consecutively 79 pregnant women who are their family members, and only 70 agreed to be subject in this study.

Pregnant women with a contact history with TB patients for more than three months or who exhibit clinical symptoms of TB and did not suffer from any other lung diseases such as bronchial asthma, bronchitis, or lung tumours were considered eligible for this study. Pregnant women were excluded if they had a severe reaction to TST; underwent treatment against TB before or during the current pregnancy; experienced shortness and chest pain; their physical examination results showed a decrease in the sound of breathing; and their blood samples were spilt during collection, experienced cell lysis, or were not sufficient in volume for further analysis.

In the same period, 57 pregnant women were recruited consecutively for the no contact history group in the parallel primary health centers, which finally reached 40 subjects eligible to be enrolled.

All pregnant women were screened by clinical and laboratory examination and were questioned regarding their history of exposure to TB patients and BCG vaccination. All pregnant women were tested for HIV and excluded if they were positive. Nutritional status of pregnant women was categorised according to pre-pregnancy body mass index which was computed as reported weight (kg) divided by square of measured height (m) and categorized into four groups as overweight (<18.5 kg/m²), normal (≥ 18.5 and <25 kg/m²), overweight (≥ 25 and <30 kg/m²) and obese (≥ 30 kg/m²).^[37]

Tuberculin skin test

The TST was performed using 5 tuberculin units (TU) of PPDRT23 (Staten Serum Institute, Copenhagen, Denmark) in a total volume of 0.1 ml injected intradermally. The diameter of skin induration was measured 72 hours after injection. To minimize imprecise reading of TST, the BCG scar result was measured by the same person who has attended standardized training. The mean diameter of scar size was calculated from diameters perpendicular to each other. Induration size ≥ 5 –15 mm was considered positive.

58

Interferon-gamma release assay

IFN- γ IGRA by using QuantiFERON Gold Plus TB Test kit (QFT-Plus). Six ml of venous blood was obtained from participants and transferred to 0.8-1.2 uL of QFT Plus in 4 tubes (Nil, TB1, TB2, Mitogen). Four tubes were simultaneously shaken until covered in blood. These four tubes were incubated at 37°C for 16-24 h, followed by a 15-minute centrifugation to separate the plasma. Plasma samples were stored at -20°C until employed in the IFN- γ enzyme-linked immunosorbent assay (ELISA) procedure, according to manufacture protocol. The Optical Density (OD) was measured using an ELISA reader with a 450 nm filter and 620 nm to 650 nm reference filter. QuantiFERON-TB Gold Plus Analysis Software ver. 2.71 (Qiagen) was used to calculate IFN- γ from OD readings. All tested samples had a Nil value ≤ 8 IU/mL and a Mitogen-Nil difference ≥ 0.5 IU/mL indicating that all samples were valid. The difference value of Tb1-Nil and/or Tb2-Nil ≥ 0.35 IU/mL was considered positive.^[38]

Statistical analysis

The sensitivity and specificity of TST and IGRA were measured and having contact history as a proxy for having LTBI. The sensitivity and specificity of the two diagnostic methods and agreement between them were estimated using SPSS version 20.0 (SPSS Inc., Ammonk, NY, USA). Categorical data were compared using the Chi-squared test or Fischer-exact test. The agreement between those TST and IGRA was measured by Kappa (κ) test. κ test was interpreted as poor ($\kappa \leq 0.2$), fair ($0.21 \leq \kappa \leq 0.4$), moderate $0.41 \leq \kappa \leq 0.6$, good ($0.61 \leq \kappa \leq 0.8$), and very good ($0.81 \leq \kappa \leq 1$).^[39] A *P* value less than 0.05 was considered significant. The confidence interval (95% CI) was estimated according to the binomial distribution.

Ethics statement

Written informed consent was obtained from each pregnant woman in this study and ethics approval of the study was obtained from the Health Research Ethics Committee of Faculty of Medicine, University of Hasanuddin, Makassar (No. 517/H4.8.4.5.31/PP36-KOMETIK/2018, July 27, 2018).

Patient declaration of consent statement

The authors confirmed that they have collected all necessary consent papers from patients. All subjects have consented to the publication of their clinical information in the journal, are aware that their names and initials will not be published, and that all reasonable attempts will be done to protect their anonymity.

RESULTS

Initially, we interviewed 271 TB patients, whether they have pregnant women who lived in their house for more than 3 months, then we consecutively recruited 79 pregnant women, but only 70 subjects agreed to participate in this study. We searched for no contact subjects in the parallel primary health centers. From 65 subjects, only 57 subjects agreed, but through further interviews, we found that the "true" no contact subjects

narrowed to 40 pregnant women [Figure 1]. Nine women were lost in the follow-up process, which consisted of five women with contact history to TB patients and four women without contact history to TB patients. Five blood samples from the contact history group and six blood samples from the no contact history group experienced cell lysis and therefore were not eligible for further analysis. Thus, this study enrolled 60 pregnant women with a history of household contact with TB patients and 30 women without contact history.

Of these 90 women, 31.1% (28/90) were aged 25-28 years, 62.2% (56/90) were multiparous, 47.8% (43/90) were in the second trimester of pregnancy, and 70% (63/90) had normal body mass index (BMI). There was 43.3% (39/90) of the participants in this study whose TB patient contact were their parents and 44.4% (40/90) of participants had duration of exposure ranged from 4-6 months [Figure 1]. Characteristics of the samples are shown in Table 1.

Table 2 shows a significant correlation of both TST and IGRA with the duration of exposure to TB contacts and with history of TB (all *P* < 0.05), whereas nutritional status and trimester of pregnancy were not significant. There were 87.8% (79/90)

Table 1: Pregnant women characteristics based on contact history with tuberculosis patients

Characteristics	Contact history		Total, n (%)
	Yes, n (%)	No, n (%)	
Age (years)			
17-20	11 (18.4)	7 (23.3)	18 (20)
21-24	20 (33.3)	6 (20)	26 (28.9)
25-28	20 (33.3)	8 (26.7)	28 (31.1)
29-32	6 (10)	6 (20)	12 (13.3)
33-36	3 (5)	3 (10)	6 (6.7)
Nutritional status* (prepregnancy BMI)			
Underweight	23 (38.3)	0	23 (25.6)
Normal	33 (55.0)	30 (100)	63 (70)
Overweight	4 (4.7)	0	4 (4.4)
Parity			
Primigravida	21 (35)	13 (43.3)	34 (37.8)
Multigravida	39 (65)	17 (56.7)	56 (62.2)
Trimester at enrolment			
First	9 (15)	5 (16.7)	14 (15.5)
Second	30 (50)	13 (43.3)	43 (47.8)
Third	21 (35)	12 (40)	33 (36.7)
Contact duration (months)			
Never	0	30 (100)	30 (33.3)
4-6	40 (66.7)	0	40 (44.4)
>6	20 (33.3)	0	20 (22.3)
Relationships with TB patients			
Husband	9 (15)	0	9 (10)
Parents	39 (65)	0	39 (43.4)
Sibling	12 (20)	0	12 (13.3)

*Nutritional status is categorised according to prepregnancy BMI: underweight (< 18.5 kg/m²), normal (≥ 18.5 and < 25 kg/m²), overweight (≥ 25 and < 30 kg/m²) and obese (≥ 30 kg/m²). TB: Tuberculosis, BMI: Body mass index

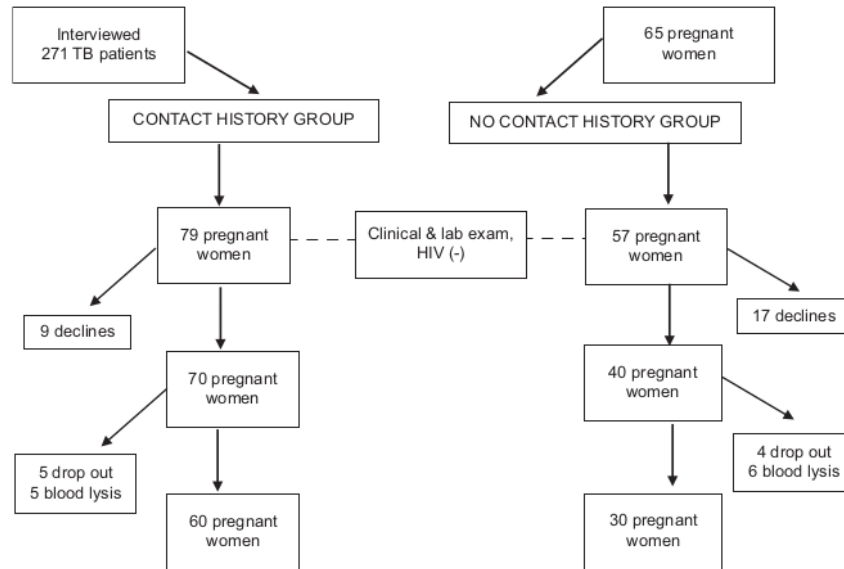


Figure 1: Subjects recruitment flowchart

Table 2: The relationship of pregnant women characteristic with tuberculin skin test and interferon-gamma release assay

Characteristic	TST		P	IGRA		P
	Positive, n (%)	Negative, n (%)		Positive, n (%)	Negative, n (%)	
Nutritional status (prepregnancy BMI)*						
Underweight	21 (91.3)	2 (8.7)	0.585	14 (60.9)	9 (39.1)	0.402
Normal	54 (85.7)	9 (14.3)		28 (44.4)	35 (55.6)	
Overweight	4 (100)	0		2 (50)	2 (50)	
Trimester at enrolment						
First	12 (85.7)	2 (14.3)	0.788	10 (71.4)	4 (28.6)	0.133
Second	37 (86)	6 (14)		21 (48.8)	22 (51.2)	
Third	30 (90.9)	3 (9.1)		13 (39.4)	20 (60.6)	
Contact history						
No	22 (73.3)	8 (26.7)	0.005	8 (26.7)	22 (73.3)	0.003
Yes	57 (95)	3 (5)		36 (60)	24 (40)	
Contact duration (months)						
Never	22 (73.3)	8 (26.7)	0.013	8 (26.7)	22 (73.3)	0.001
4-6	38 (95)	2 (5)		28 (70)	12 (30)	
>6	19 (95)	1 (5)		8 (40)	12 (60)	

*Nutritional status was categorised according to prepregnancy BMI underweight (<18.5 kg/m²), normal (≥18.5 and <25 kg/m²), overweight (≥25 and <30 kg/m²) and obese (≥30 kg/m²). BMI: Body mass index, TST: Tuberculin skin test, IGRA: Interferon-gamma release assay

of the pregnant women tested in this study had positive TST results, while only 48.8% (44/90) of them had positive IGRA results. Pregnant women with a contact history with TB patients in this study had 95% (57/60) positive results with TST, which is higher compared to 60% (36/60) positive results in the IGRA. However, our results also show that pregnant women without any contact history to TB patients were also detected as positive by both TST 73.3% (22/30) and IGRA 26.7% (8/30). We obtained a 95% (confidence interval [CI]: 86.08%–98.96%) sensitivity and 26.7% (CI: 12.28%–45.89%) specificity for TST with positive predictive value (PPV) and negative predictive value (NPV) were 72.15% (CI: 67.45%–76.41%)

and 72.73% (CI: 43.25%–90.32%), respectively. While for IGRA, we obtained a 60% sensitivity (CI: 46.54%–72.44%) and 73.3% (CI: 54.11%–87.72%) specificity with PPV and NPV were 81.82% (CI: 70.59%–89.40%) and 7.83% (CI: 38.59%–57.21%), respectively.

Seventy-nine of the pregnant women tested had positive TST results. Among these women, 39 (49.4%) tested positive and 40 (50.6%) tested negative with the IGRA. However, there were 5 (45.5%) cases where the women who tested as positive with the IGRA, tested negative with TST in contrast. The difference in the results of TST and IGRA were significant ($P < 0.05$),

and the agreement between those two diagnostic tests was fair (kappa 0.39; 95% CI 0.24–0.45) [Table 3].

DISCUSSION

There is no gold-standard diagnostic method for detecting latent TB infection that can be used as a basis to determine whether the TST or the IGRA were better in detecting LTBI. We assumed that having contact history is a proxy for having LTBI. A few studies have evaluated the use of IGRA for latent TB infection during pregnancy in both low- and high-burden areas. In this study, we evaluated the TST and IGRA to detect LTBI in pregnant women who had a history of household contact with TB patients. As a novel assay for detecting latent TB infection, the IGRA has been reported to show higher sensitivity and higher specificity than TST. However, even though we found similar results with previously reported studies regarding a higher specificity of IGRA compared to TST, our results indicated that the sensitivity of IGRA was lower than TST, and the agreement between the two methods was fair (kappa 0.39). Assuming that having a contact history as a proxy for LTBI is a limitation of this study. This implies that the definition of sensitivity and specificity was slightly different from the actual one. Studies in high-burden areas were a challenge in itself, because misclassification occurred in determining contact history and without contact history, although efforts have been made to minimize it by finding family members with active TB patients who were pregnant and have lived for more than 3 months, which means that the subject actually have contact history. However, determining the true non-contact history was quite difficult in high-burden areas.

In high-burden countries, such as Indonesia, BCG vaccination is a prevention strategy that has been included in the national immunization program for infants aged 4 weeks, but the protection of this vaccine declines in adolescence. The vaccination effects reduce ten years after injection, and therefore, would then no longer influence TST results. TST has a low specificity and a low sensitivity due to BCG vaccination and non-tuberculous mycobacteria, particularly amongst HIV-positive patients and pregnant women. Findings from PRACHITI, a cohort study in Pune, India revealed the both IGRA and TST were impacted by the phases of pregnancy, which demonstrated the influence of immunological alterations during pregnancy. This result may indicate that immune suppression reduces the tuberculin

sensitivity test (TST) and IGRA sensitivity (IGRA). Although the study reported a low concordance between IGRA and TST, it was unable to conclusively compare these two tests since it only investigated patients with positive IGRAs and did not compare them with subjects with negative IGRAs. (Bhosale 2020, utk ganti no 41).

Compared to TST, the IGRA has a very high specificity and is unaffected by prior BCG vaccination or non-tuberculous mycobacteria. This assay is more sensitive to detect *M. tuberculosis* infections in immunosuppressive patients than TST. However, the absence of a gold standard results in the difficulty of concluding as to whether the IGRA outperforms TST, or if there are higher false-positive results. A study of adult (non-pregnant) patients in Zambia reported the sensitivity of IGRA (Quantiferon-TB Gold Plus) among people living with HIV was comparable to that of HIV-negative individuals (85%, 95% CI: 75–93, and 80%, 95% CI: 64–91). Moreover, the fair agreement between TST and IFN- γ IGRA results were also reported by other previous studies. In several studies comparing IGRA (Quantiferon-TB Gold (QFT-G), the T-SPOT-TB (SPOT), and TST results for latent TB in pregnancy, the range of kappa values were from 0.2 to 0.93. Birku et al. found that the concordance of TST and QFT-G results in pregnant women varies according to the presence of HIV infection. Other reports on non-pregnant cases also report varying agreements, for example, in screening LTBI in children, the kappa value of both tests in the range of 0.46–0.74.

In the present study, the number of pregnant women that were TST-positive was higher than the number of those who were IGRA-positive. Our findings are contrary to the previous study in high-burden areas who observed that the number of IGRA positives were higher than that of TST positives, but in concordance to another recent report. Cases that were TST-positive but were IGRA-negative in the present study may occur due to impaired IGRA performance in pregnancy. Unfortunately, this study did not show that the pregnancy trimester had any significant effect on IGRA results. On the other hand, the different results between the two methods may also indicate false positives or false negatives in TST results. Various factors such as age, impaired immunity, the coexisting disease may interfere with its interpretation. False-negative TST may result in very young age <6 months and patients with cutaneous anergy, recent TB (8–10 weeks of exposure) or chronic TB infection, protein malnutrition and imprecise reading of induration or incorrect method of administration. Some nutritional studies in Indonesia showed a low average protein intake in pregnant mothers was associated with poor pregnancy outcomes. However, this condition might not be directly related to the false-negative TST result. False-positive TST results is increasingly observed phenomenon, which may be due to cross-reaction with non-tuberculous mycobacteria and prior administration of BCG vaccination. Besides, there is also the possibility of positive TST results due to pathergic reactions.

Table 3: Agreement between the results of tuberculin skin test and interferon-gamma release assay

	IGRA positive, n (%)	IGRA negative, n (%)	P	Kappa (95% CI)
TST positive (n=79)	39 (49.4)	40 (50.6)	0.007	0.39 (0.24-0.45)
TST negative (n=11)	5 (45.5)	6 (54.4)		

TST: Tuberculin skin test, IGRA: Interferon-gamma release assay, CI: Confidence interval

It has been suggested that nutrition plays a major role in developing the innate and Th1 immune responses that are appropriate for TB. Low-protein diets result in decreased lymphocyte proliferation, higher levels of immunoglobulin G, and decreased cytokines such as IL-2, TNF- α , and IFN- γ and other mycobactericidal substances that may impair the cell-mediated response important for the immune system to eliminate TB infection. Patients with TB typically experience weight loss or malnutrition due to poor protein intake, muscle catabolism generated by inflammation during infection, and gastrointestinal symptoms induced by acute-phase proteins, such as elevated TNF levels.^[62,63] Malnutrition may decrease immunity and increase the risk of the development of active TB and primary progressive disease. Active TB increases the risk of malnutrition and makes malnutrition worse.^[64] However, nutritional status was not associated with positivity for either test.

CONCLUSION

This study showed that both contact history and duration of exposure of pregnant women to TB patients affect TST and IGRA results. The sensitivity of the TST was higher than IGRA, while the specificity of IGRA was higher than TST. The agreement between the two methods was fair for detecting LTBI in pregnant women with household contacts. A rapid and affordable diagnostic tool is required to detect LTBI, particularly for high-risk person in high-burden areas.

Limitation

We are aware of the limitations of this study as there is no gold standard diagnostic test in detecting latent TB to date, so in this case, we used contact history as a proxy for having LTBI. Another limitation of this study was the insufficient information on the BCG vaccination during the childhood of pregnant women. Some of these women did not have a BCG vaccination record and could not confirm either whether they had BCG vaccination during their childhood or not. Therefore, we could not assess the influence of BCG vaccination on false-negative results in the present study.

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Conflicts of interest

There are no conflicts of interest.

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