

# Interferon Gamma Release Assay After BCG Vaccination Among Newborns and Family Members Living in an Overcrowded Area in Jakarta, Indonesia

Francisca Sriotami Tanoerahardjo (✉ [sriotami@gmail.com](mailto:sriotami@gmail.com))

National Institute of Health Research and Development, Ministry of Health Republic of Indonesia

Indri Rooslamati

National Institute of Health Research and Development, Ministry of Health Republic of Indonesia

Natalie Kipuw

National Institute of Health Research and Development, Ministry of Health Republic Indonesia

Hadiyanto

School of Medicine and Health Sciences, Atma Jaya Catholic University of Indonesia

Soegianto Ali

School of Medicine and Health Sciences, Atma Jaya Catholic University of Indonesia

Edhyana Sahiratmadja

Faculty of Medicine, Universitas Padjadjaran <https://orcid.org/0000-0001-9092-5848>

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## Research Article

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# Abstract

## Introduction

Active tuberculosis (TB) patient is a potential source of *Mycobacterium tuberculosis* transmission in an overcrowded and poor area. Family members living in the same house may have been infected or latent tuberculosis infection (LTBI) may develop. The aim of the study was to explore LTBI among newborns and their family members living in an overcrowded area in Jakarta, Indonesia.

## Methods

A prospective analytical study was conducted among newborns from October 2016 to March 2017. Interferon gamma release assay (IGRA) was examined before BCG vaccination and after 12 weeks. In parallel, TB active case finding was performed among family members of the newborns.

## Results

Of 135 newborns, only 117 (86.7%) came for BCG vaccination. Of 346 family members screened, 8 (2.3%) were detected as untreated active pulmonary TB, confirmed by positive sputum and/or MTB culture. Family members living in the same house with active TB individuals ( $p=0.011$ , OR 2.69) as well as being males ( $p = 0.025$ ; OR 1.68) had a significant higher risk of having a positive IGRA.

## Conclusions

Untreated pulmonary TB infection in overcrowded areas infects the surrounding neighbors, resulting in latent TB infection. An active program for detecting pulmonary TB cases and preventive measures need to be taken seriously to contain the potential spreading of the infection.

## Background

Tuberculosis (TB) is an airborne disease, caused by *Mycobacterium tuberculosis* (MTB).<sup>[1]</sup> In an overcrowded area, active TB patients are a potential source of MTB transmission that spreads the infection to the surrounding people by coughing and sneezing.<sup>[2]</sup> Also, young children living closely with infected parents or family members are at high risk of contracting the disease. Interestingly, active TB individuals are sometimes afraid to report to the health facilities due to lack of knowledge about the disease and stigma in the community.<sup>[3]</sup> Therefore, education and awareness as well as an active TB case finding program is necessary.

Furthermore, the immunity of newborns is still immature, making them more prone, and resulting in a latent TB infection (LTBI) that may progress to active TB disease.<sup>[4]</sup> They may later serve as a reservoir for future transmission.<sup>[5]</sup> Recently, interferon gamma release assays (IGRA) has been proposed to detect latent TB infection next to tuberculin skin test (TST). The latent infection of MTB is defined by a positive result of IGRA or TST in the absence of active TB.<sup>[4]</sup> Early diagnosis and treatment of LTBI are therefore considered to be the most effective strategy for reducing the incidence of TB in the population. However, study in a high TB-burden setting comparing TST and IGRA in diagnosing LTBI has shown that neither was able to predict the active TB during the follow up.<sup>[6]</sup> Therefore, further exploration is required.

To prevent the disease, BCG vaccine has been given to children in TB endemic countries, including Indonesia. This vaccine is mandatory based on the National TB Program (NTP) administered to newborns up to 12 weeks old. Interestingly, BCG efficacy studies have shown a range of 0 to 80% effectiveness.<sup>[7]</sup> Furthermore, despite the high coverage of BCG vaccination nationwide and the direct observed therapy system (DOTS) program, Indonesia still belongs to high TB burden countries.<sup>[8]</sup> This study aimed to explore the IGRA test among newborns and their family members living in an overcrowded area of Jakarta, Indonesia.

## Materials And Methods

### Study design

A prospective analytic study was conducted to explore the IGRA test among newborns living in an overcrowded district of Penjaringan and Tambora, Jakarta. The IGRA was performed before BCG vaccination and after 12 weeks. Furthermore, a cross-sectional analytic study was conducted to examine IGRA among the family members.

### Study participants

From October 2016 to March 2017, pregnant women (n = 132) who were at their last month of antenatal care were approached and informed about the study. They were further informed about the mandatory BCG vaccination for newborns up to 3 months old according to the National Program. Before the BCG vaccination, mothers were consented and peripheral blood was drawn using a wing needle into a heparinized tube for the IGRA test (QuantiFeron, NZ). With the mother's consent, the IGRA test was repeated after 12 weeks of BCG vaccination.

In a parallel study, during the home visit of the newborns, active TB case detection was directed to all individuals living in the same house with the babies, including history taking and chest X-ray. However, only individuals with abnormal chest X-ray were examined for acid fast bacilli in their sputum and further cultured in LJ medium. In addition, family members older than 15 years old were tested for IGRA, including the mothers of newborns. The research flow was shown in Fig. 1.

### Ethical Clearance

The study protocol was granted by the Ethical Committee of National Institute of Health Research and Development, Ministry of Health Republic of Indonesia no. LB.02.01/5.2/KE.397/2015

### Statistical Analyses

The frequency of the IGRA test before and after BCG vaccination was described in percentage. Furthermore, chi-square was adopted for the IGRA test among family members, living with or without active TB individuals and stratified by gender. A p-value < 0.05 was considered statistically significant.

## Results

### BCG vaccination among newborns

A total of 132 pregnant women that gave birth to 135 babies were identified, of whom 3 mothers were delivered of twins, consisting of boys 64 (47.4%) and girls 71 (52.63%) with a birth weight of 3050 gram (median; range 1700–4100 gram). However, only 117 of 135 (86.6%) newborns came for BCG vaccination (Fig. 1). Some mothers (n = 18) refused to let their babies vaccinated.

At the time of BCG vaccination, the babies were weighed 4040 gram (median; range 2350–6100 gram) and were 39 days old (median; range 11–82 days). The characteristic of the mothers of newborns was analysed based on BCG vaccination attendance (Table 1). The result showed that the older age mothers were significantly more likely to allow BCG vaccinations for the newborns than younger mothers ( $p < 0.034$ ). However, the longer education of the mothers showed no significant difference ( $p = 0.193$ ) between mothers who came and did not come for the BCG vaccination. Furthermore, the location of delivery, either the hospital or the primary health care, was not significantly different ( $p = 0.063$ ).

Table 1  
Characteristic of the mothers of newborns from an overcrowded area whether they come for BCG vaccination

	BCG vaccination		<i>p</i> value
	Come (n 117)	Not come (n 18)	
Age, years old;			
median (min-max)	29 (17–40)	26 (18–33)	0.034*
Education <sup>a</sup> ; n			
≤ 6 years education	31	-	0.193
6–9 years	40	8	
9–12 years	46	10	
Place of delivery; n			
Hospital	46	3	0.063
Primary Health	71	15	
Note. * <i>p</i> was statistically significant (Chi-square); <sup>a</sup> Basic education ≤ 9 years vs. education > 9 years;			

## IGRA test result among newborn babies

From a total of 135 newborn babies, 133 had IGRA tests before BCG vaccination, however, only 97 babies came for IGRA after BCG. Furthermore, preBCG IGRA showed positive in 3 of 133 babies, however, these results turned to be negative 12 weeks after BCG, and they did not live with active TB family members. Interestingly, one baby who did not live with active TB family members turned out to be IGRA positive (Table 2).

Table 2  
The IGRA test result among newborns living in an overcrowded area before and after BCG vaccination

<b>BCG vaccination</b>				
	Before	After	N	%
IGRA result	No consent	-	2	1.5
	Negative	n.d	36	26.7
	Negative	Negative	93	68.9
	Negative	Positive	1	0.7
	Positive	Negative	3	2.2

Note. n.d. not determined; no IGRA test after BCG.

## IGRA results among family members

The active TB case finding showed that 8 out of 346 (2.3%) family members had active TB, confirmed by positive sputum examination and/or MTB culture. These individuals were not on TB therapy. Special attention was given to the 7 babies who had lived with active pulmonary TB parents or other family members (Table 3). However, 5 out of 7 babies were still IGRA negative after BCG; whereas others did not come for the IGRA test.

Table 3

The IGRA test of newborn babies who live with untreated pulmonary active TB family members

ID	ID no.	IGRA test		ID no.	Relation	IGRA	Other family members	IGRA Result in house	
no.	Baby	Before BCG	After BCG	TB active	to baby	test	With age and IGRA result	Positive	Negative
123	123003	Negative	n.d*	123001	Father(39)	Positive	-	2	-
				123002	Mother (33)	Positive			
444	444003	Negative	n.d**	444005	Grand Ma (42)	Positive	Mother (17) (+)	2	-
121	121003	Negative	Negative	121006	Cousin (19)	Positive	Mother (36) (-), Aunty (40) (-)	1	2
142	142003	Negative	Negative	142001	Father (39)	Positive	Mother (36) (+), Aunty (36), (+)  Grand Pa (75) (+), Grand Ma (73) (+)  Uncle (32) (-), Sister (15) (-)	5	2
148	148003	Negative	Negative	148001	Father (24)	Positive	Grand Pa (64) (+), Grand Ma (45) (+)  Mother (20) (-), Grand Pa (48) (-),  Grand Ma (64) (-)	3	3

**Note.** \* n.d. not determined; did not come for BCG vaccination

ID	ID no.	IGRA test		ID no.	Relation	IGRA	Other family members	IGRA Result in house	
411	411003	Negative	Negative	411004	Grand Ma (52)	Positive	Father (36) (+), Grand Pa (55) (+) Mother (28) (-), Sister (5) (-)	3	2
117	117003	Negative	Negative	117004	Grand Ma (73)	Negative	Father (39) (+) Mother (32) (+)	2	1
<b>Note.</b> * n.d. not determined; did not come for BCG vaccination									

Furthermore, there was a significant statistical difference ( $p < 0.011$ ) in the IGRA test result of family members living with active TB individuals compared to those without active TB. There was 2.69 times higher chance of positive IGRA in the individual living with active TB (95% CI 1.22–5.94) (Table 4). Interestingly, there was a statistical difference in gender ( $p < 0.025$ ); whereas males had a higher chance of having positive IGRA compared to females (OR 1.68; CI95% 1.07–2.64).

Table 4  
The IGRA test result among family members living in the same house with active TB individuals and stratified with gender

	IGRA (+)	IGRA (-)	<i>p</i> value	OR (95%CI)
	N	N		
<b>Family members</b>				
With active TB	17	11	0.011*	2.69 (1.22–5.94)
Without active TB	116	202		
<b>Gender</b>				
Male	55	63	0.025*	1.68 (1.07–2.64)
Female	78	150		
<b>TOTAL; n</b>	133	213		
(%)	(32.6)	(67.4)		
Note. TB; tuberculosis, IGRA; Interferon Gamma Release Assay				
* <i>p</i> was statistically significant when $p < 0.05$ (Chi-square)				

## Discussion

Indonesia is a TB endemic country and the BCG vaccine has been prioritized to prevent TB disease. It is mandatory according to the National Program given to newborn babies up to 12 weeks, however, only 86.6% newborn babies came for BCG vaccination in our study. Despite the home visit from the cadre or health employee of the primary health care, some mothers refused to take the baby for routine vaccination. Furthermore, the older mothers were significantly more likely to attend the primary health care for BCG vaccination compared to the younger age ( $p < 0.034$ ). In addition, a study in Malaysia has shown that several factors may play a role, including the assumption that vaccines have no effect, doubts about its contents, religious influence, and personal belief.<sup>[9]</sup> Therefore, education and awareness programs for pregnant women during their antenatal care need to be strengthened. Furthermore, the basic education of mothers and the birthplace have no significant associations with the willingness to allow vaccination for their children. The reason why mothers refuse vaccination for their babies needs further exploration.

As Indonesia ranks second in the global TB prevalence list, a national TB emergency is required, therefore, an active TB case detection program plays an important role.<sup>[10]</sup> This study has shown that active TB cases (2.3%) were identified and not all the individuals were on TB therapy (Table 3). Furthermore, TB is a chronic disease with clinical symptoms, which include coughing for over 3 weeks. Also, the health-promoting behaviour of people with chronic cough appears to be a burden. Some of the reasons include stigma in the community, lack of awareness, and knowledge of TB.<sup>[11]</sup> Moreover, there is a spectrum of TB infection, ranging from latent to active TB. Individuals who are able to contain MTB infection are known as latent TB and may also harbour a low-grade, subclinical infection. These individuals are at a higher risk of reactivation.<sup>[12]</sup> The reason behind infected individuals who do not visit health care providers need further clarification.

This study explored latent TB infection among newborns and family members living in the same house in an overcrowded area. Various studies have explored the usefulness of IGRA for the diagnosis of LTBI.<sup>[13]</sup> This study, particularly showed that family members living with active TB have 2.69 times higher chance of positive IGRA (95% CI 1.22–5.94) compared to those without (Table 4). In addition, it is well known that the chance of being infected with MTB among individuals living in the same house is higher.<sup>[1]</sup> Also, positive IGRA among individuals who do not live in the same house with TB patients indicates that the infection is transmitted outside the house as well. The isolation of TB patients in a sanatorium or other institution in Indonesia is not possible, however, greater measures are required to reduce MTB transmission.

Furthermore, this study revealed that male has a higher chance of having positive IGRA compared to female (OR 1.68; CI95% 1.07–2.64) (Table 4). This is due to their frequent outdoor activities and contact with others. Interestingly, male is more susceptible to TB disease as shown in a previous genetic study in Indonesia.<sup>[14]</sup> The variation in Toll Like Receptor *TLR8* gene, located in chromosome X, has been related to the susceptibility to TB.<sup>[14]</sup> This finding suggests the role of host genetic factor in immune response differences. Moreover, since only half of the family members showed IGRA positivity, the immune response may play a role in the development of TB infection.<sup>[12]</sup>

Children under 5 years old, especially those who live with active TB family members have a relatively higher risk to develop active TB. This depends on various factors, such as the proximity of contact with the index case, the MTB virulence, the environmental conditions of the house, which include solar radiation and air circulation as well as several other factors.<sup>[5]</sup> It is therefore important to detect LTBI cases and treat them early. Previously, TST was used to screen TB in adolescents, who have contact with TB patients.<sup>[15]</sup> However, a study has shown that IGRA is quite sensitive in children younger than 15 years old. Interestingly, the IGRA sensitivity is reduced compared to TST in

children younger than 2 years old.<sup>[16]</sup> Furthermore, IGRA seems to offer alternative LTBI detection, especially babies living with TB parents.<sup>[17]</sup> This study has shown that the newborns were still negative for IGRA 12 weeks after BCG vaccination, suggesting that BCG may stimulate immune response and play a protective role against TB disease.<sup>[18]</sup>

Most importantly, the study traced active TB individuals with no treatment, therefore, an active case finding is needed in all family members living in the same house and using proper diagnostic tools for possible LTBI, followed by preventive therapy. Furthermore, education was given to the family members and active TB individuals who were also assigned for TB therapy. LTBI treatment requires a long period of therapy, for example, Isoniazid (INH) needs to be given for 6 or 9 months.<sup>[5]</sup> Interestingly, the population in Indonesia are predominantly intermediate acetylators.<sup>[19]</sup> Therefore, drug induced liver intoxication should be considered when giving positive IGRA individuals a long period of INH<sup>[20]</sup> and adherence as well as compliance, need to be well monitored. In addition, good education and awareness from the health care provider are required to ensure that positive IGRA individuals adhere to LTBI treatment for at least 6 months. This study further shows that 32.6% of the respondents are LTBI as indicted by IGRA positivity, compared to Singapore that detects LTBI for 12.7%.<sup>[21]</sup> This gives valuable information to the authority to set up preventive therapy for INH.

This study encountered several limitations, including the temporary address of some mothers, which was untraceable after delivery of the baby, as a result, some are lost to follow-up care. This issue needs to be raised to the authority to improve the population registration system. Also, education and awareness about TB and immunization need to be properly given, to ensure that they have their babies vaccinated. Furthermore, a longer cohort study will provide more information on how the IGRA positivity in babies develops after exposure to active TB individuals. The data of TB exposed babies and family members as well as others with IGRA positivity were given to the local health care providers for INH preventive therapy. Finally, good monitoring on this program requires the commitment of stakeholders.

## Conclusion

Untreated active pulmonary TB individuals in overcrowded areas have been detected in this study (2.3%) and may infect the surrounding neighbors, resulting in latent TB infection or IGRA positive (32.6%). Family members living in the same house with active TB individuals as well as being males have a significant higher risk of having a positive IGRA. Since latent TB infection in the overcrowded area have a major impact on future MTB transmission, active pulmonary TB case finding program and preventive measures need to be taken to contain the potential spreading of the infection. It is therefore, necessary to detect latent TB infection and to educate the community on how the MTB is spreading.

## Abbreviations

BCG: Bacillus Calmette-Guérin;

IGRA: Interferon Gamma Release Assay;

TB: Tuberculosis;

TST: Tuberculin Skin Test;

WHO: World Health Organization

# Declarations

## Ethics approval and consent to participate

Ethical approval for this study was granted by the the Ethical Committee of Ministry of Health Republic Indonesia LB.02.01/5.2/KE.397/2015

## Consent for publication

Not applicable

## Data availability

The data used for the present study are available with FST and can be provided on reasonable request.

## Competing interests

The authors declare that they have no competing interests.

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## Authors' contributions

FST, IR and ES made substantial contributions to the conception and design of the study. NK, H and SA made contributions to the data collection. All authors were involved in data interpretation. FST and ES drafted and revised the manuscript. NK, IR, H, and SA critically revised the manuscript. The final version of the manuscript was approved by all authors, and all authors are accountable to the accuracy and integrity of all parts of the paper.

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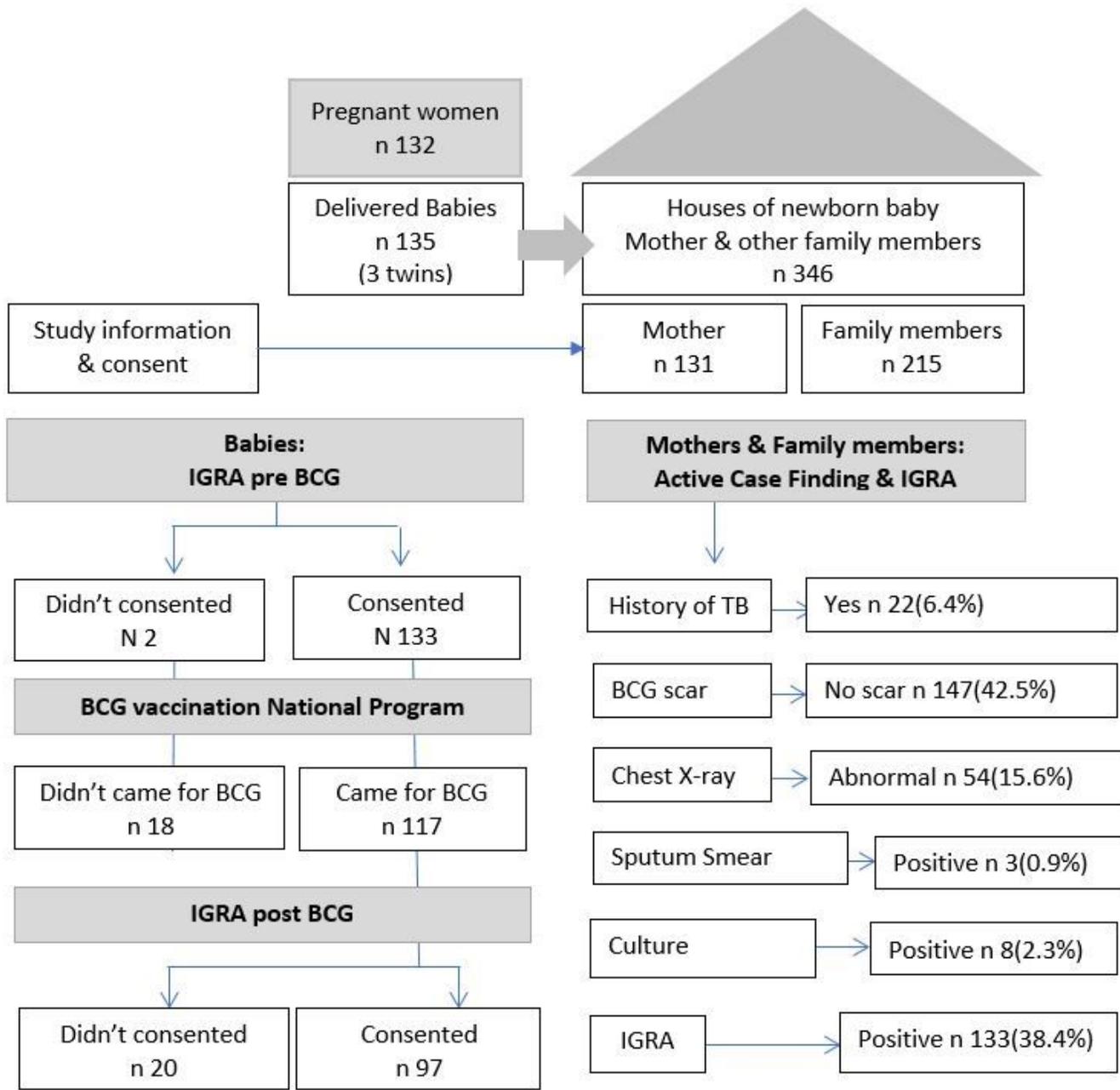
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## Figures



**Figure 1**

Research Flow on Interferon Gamma Release Assay (IGRA) study among newborn babies before and after BCG vaccination and family members who lived in the same house in overcrowded area in Jakarta, Indonesia.