雑誌

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Elevated anti-tuberculous glycolipid antibody titres in healthy adults and tuberculosis patients in Thailand

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SUMMARY

OBJECTIVE: To evaluate immunoglobulin G (IgG) and immunoglobulin A (IgA) responses to tuberculous-glycolipid antigen (TBGL-IgG and -IgA) in pulmonary tuberculosis (TB) patients and healthy controls in Thailand.

DESIGN: Anti-TBGL antibody titres and other TB related markers were measured in the serum samples of 24 adults with pulmonary TB (PTB), 28 healthy adults (HA), 23 children with TB and 24 healthy children.

RESULT: Both TBGL-IgG and -IgA titres were significantly higher only in adult PTB cases compared to controls ($P < 0.001$ for all). TBGL-IgG was highly sensitive (92%) in PTB patients, but frequent positive proportions of TBGL-IgG (46%) and -IgA (36%) in HAs were the cause of low specificities of TBGL-IgG (54%) and -IgA (64%); that of TBGL-IgG+IgA (75%) was the highest. Antibody titres were positively correlated in TBGL-IgG+IgA double-positive HAs (HA+, 7/28, $P < 0.01$), but not in HA− ($P > 0.05$). Serum IgG and IgA levels were not correlated with TBGL-IgG or -IgA levels ($P > 0.05$). KL-6 and leptin levels were normal and were not different between HA+ and HA−, indicating absence of active TB in HAs.

CONCLUSION: Enhanced TBGL-IgG+IgA responses in HAs could indicate latent TB infection. Careful follow-up studies in HAs could clarify the significance of elevated TBGL antibodies as early disease markers.

KEY WORDS: anti-tuberculosis glycolipid IgG; TBGL; IgA; TB-endemic country; latent TB infection

MYCOBACTERIUM TUBERCULOSIS is a leading global health problem that caused an estimated 9.27 million new cases of tuberculosis (TB) infection and more than 2 million deaths worldwide in 2007.[1] The alarming increase in the incidence of multidrug-resistant TB, particularly among human immunodeficiency virus (HIV) infected patients,[1] and the development of the immune reconstitution syndrome after the initiation of highly active antiretroviral treatment (HAART),[2] have rendered the situation more critical. Conventional microscopy, which has a variable range of sensitivity of 20–60% in detecting tubercle bacilli, is widely used by resource-limited countries,[3] which harbour more than 90% of the world's TB infection.[1] However, approximately 20% of TB cases are not microbiologically proven, even with the more expensive fluorescence microscopy.[3,4] Moreover, a bacteriologically confirmed diagnosis of TB in paediatric groups is much more difficult, as children seldom produce sputum. There is therefore an urgent need to develop an early diagnostic approach to identify both paediatric and adult TB patients.

Cord factor (trehalose-6-6-dimycolate; TDM), which composes a major part of the mycobacterial cell wall, has been identified as the most immunogenic glycolipid; it is produced mainly by virulent *M. tuberculosis* as well as by atypical mycobacteria.[5,6] Tuberculous-glycolipid antigen (TBGL) consists of purified TDM from H37Rv.[7] The immunoglobulin G (IgG) response to TBGL antigen (TBGL-IgG) has been proposed as a useful tool for TB serodiagnosis (sensitivity and specificity >80%) in Japan, a non-TB-endemic country (incidence rate 20 per 100 000 population).[7,8] Although IgG and immunoglobulin A (IgA) responses to purified TB antigens and a commercial serological assay were demonstrated to have limited significance for the serodiagnosis of pulmonary tuberculosis (PTB) in a meta-analysis and systemic review by Steingart et al., of the lipid antigens, cord factor showed particularly high reactivity.[9,10] IgA responses against the mycobacterial fusion protein MT10.3: MPT64 was recently demonstrated to have higher sensitivity for the diagnosis of extra-pulmonary TB in a TB-endemic country.[11] Although the diagnostic efficacy

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of TBGL-IgA was not evaluated in prior studies, a significant association between TBGL-IgG and -IgA was reported in active TB patients.[12] However, IgG or IgA responses against TBGL antigen have not been evaluated for their diagnostic ability in TB-endemic countries.

As TBGL-IgG titres were found to be associated with C-reactive protein and cavity formation,[12] other markers related to TB pathology, including KL-6 and leptin, could have some role in promoting inflammation in PTB. A high-molecular-weight mucinous glycoprotein expressed on type-II pneumocytes, KL-6 was reported to be elevated in the serum of patients with interstitial pneumonia[13] and PTB with extensive radiographic changes.[14] Leptin, a cytokine-like hormone produced by the bronchial epithelial cells and type-II pneumocytes in addition to adipose tissue,[15] was reported to be low in the serum of PTB patients.[16]

The purpose of the present study was to evaluate IgG and IgA antibody (Ab) responses to the TBGL antigen in adult and children TB patients and healthy controls in Thailand. The relationship of TBGL antibodies to KL-6 and leptin was also assessed.

MATERIALS AND METHODS

Subjects

A case-control study was conducted between April 2007 and October 2008. Adult cases (age >16 years) were 24 newly diagnosed active PTB patients receiving care at the Chest Disease Institute (CDI), Nonthaburi, who were enrolled before or within 2 weeks of receiving anti-tuberculosis treatment. All of the PTB patients were positive for sputum acid-fast bacilli (AFB) stain and culture for *M. tuberculosis*.

Twenty-three children (age ≤12 years) diagnosed with TB and receiving care at the Queen Sirikrit National Institute of Child Health (QSNICH), Bangkok, were enrolled as child TB cases (CTB) before receiving anti-tuberculosis treatment. They were diagnosed with active TB based on the presence of two or more features suggestive of probable TB, including history of close TB contact, positive tuberculin skin test (TST) response (>10 mm diameter), chest X-ray (CXR) findings suggestive of TB, and histopathological features related to TB according to the diagnostic criteria of the World Health Organization (WHO) provisional guidelines for the diagnosis of paediatric TB.[17] Diagnosis was confirmed by positive culture of tubercle bacilli.

Subjects with underlying malignancy, metabolic disorders, HIV/AIDS (acquired immune-deficiency syndrome) or other active pulmonary diseases were excluded from the study.

Healthy adult individuals with no concomitant pulmonary symptoms, normal CXR and negative HIV serology were recruited from among blood donor subjects as healthy adult controls (HA). Volunteer healthy child controls (HC) were selected from among paediatric patients without respiratory symptoms and with normal CXR from the surgical department of the QSNICH.

Blood samples were collected from all enrolled participants. Serum samples were separated and stored in −20°C for further study.

This study was approved by the ethics committees of all the participating institutes in Thailand and Japan. Written informed consent was obtained from all enrolled participants. The study was conducted according to the recommendations of the Helsinki Declaration.

TBGL antibodies

TBGL-IgG and -IgA titres were measured using the Determiner TBGL-antibody ELISA kit (Kyowa Medex, Tokyo, Japan), an in vitro enzyme-linked immunosorbent assay (ELISA) kit for the quantitative measurement of TBGL-IgG and -IgA in serum or plasma. Antibody titres for both antibodies were expressed as U/ml. Samples were classified as TBGL-IgG-positive if TBGL-IgG serum levels were ≥2 U/ml.[7] An arbitrary cut-off value of ≥2 U/ml for TBGL-IgA was used as per the unpublished data of our previous study.[12]

ELISA assay

Serum leptin and sIL-2Rα levels were determined by sandwich ELISA using the Quantikine Human Leptin Immunoassay kit and the Quantikine Human IL-2 sRα Immunoassay kit (both from R&D Systems, Minneapolis, MN, USA) for the quantitative determination of the human leptin and sIL-2Rα concentrations respectively in serum or plasma according to the manufacturer's guidelines. Serum KL-6 levels were measured using an ELISA kit (Sanko-junyaku, Tokyo, Japan).

Measured laboratory markers

We assessed the whole blood profile as well as the serum levels of IgG and IgA and hepatic enzymes (aspertate amino-transferase [AST] and alanine amino-transferase [ALT]).

Statistical analysis

Data were analysed using Statcel 2 (OMS Publishing Inc, Saitama, Japan). We compared sensitivity and specificity using the χ^2 test for proportions. Values are presented as median and range. Differences in titres of different variables between two groups were analysed using the Mann-Whitney *U*-test. Correlations between each variable were evaluated using Spearman's rank correlation coefficient. A two-tailed $P < 0.05$ was considered significant.

RESULTS

Subjects

The demographic and clinical characteristics of the enrolled case participants are shown in Table 1.

Table 1 Demographic and clinical characteristics of study participants

Variable	Adult PTB cases (n = 24) n (%)	Healthy adults (n = 28) n (%)	Child TB patients (n = 23) n (%)	Healthy child controls (n = 24) n (%)
Male:female*	23:1	19:9	12:11	19:9
Age, years, median [range]	36.5 [20–50]	35.5 [21–52]	2 [0.5–12]	3.5 [0.6–12]
TST responses (>10 mm/<10 mm/0–5 mm)	ND	ND	19/1/3	ND
Sputum AFB stain and culture positive	24 (100)	ND	1 (4)	ND
Chest X-ray				
Normal	—	28 (100)	—	24 (100)
Pulmonary infiltration	8 (33.3)	—	11 (47)	—
Infiltration+fibrosis	1 (4.1)	—		—
Miliary infiltration		—	2 (8.6)	—
Hilar lymphadenopathy		—	9 (39)	—
Consolidation/cavity/calcification	1/1/1 (4 in each)	—	0/0/3 (13)	—
Diagnosis		—		—
PTB	24 (100)	—	21 (91)	—
EPTB		—	2 (9)	—

* Frequency.
PTB = pulmonary tuberculosis; TST = tuberculin skin test; ND = not done; AFB = acid-fast bacilli; EPTB = extra-pulmonary TB.

Among the 58 adult participants screened, 24 microbiologically confirmed PTB cases with male predominance (96%) and 28 age-matched HA subjects (male 68%) were included in the analysis; six PTB cases were eventually excluded due to HIV co-infection. In contrast, *M. tuberculosis* infection was not confirmed in 23 CTB cases except one; 19 (83%) children had positive TST responses (>10 mm diameter), including 12 who had a history of TB contact through family members. Although the TST response was <10 mm (range 0–10 mm) in the other four cases, they also had a history of TB contact. On CXR, 21 had pulmonary infiltration and/or hilar lymphadenopathy and other abnormalities relevant to PTB. Two others had massive pleural effusion and features of non-necrotising granulomatous pruritis suggestive of extra-pulmonary TB. Twenty-four age-matched children with no TB-related symptoms and normal CXR findings were enlisted for analysis as controls (HC).

Anti-TBGL antibodies and their correlations

In the adult participants, the TBGL-IgG and -IgA titres were elevated in respectively 22/24 (92%) and 17/24 (63%) PTB cases and 13/28 (46%) and 10/28 (36%) HAs. TBGL-IgG and -IgA titres were significantly higher in the PTB group than in the controls (P < 0.001 for both; Figure 1A, Table 2). The sensitivities of the TBGL-IgG and -IgA assay were 92% and 63%

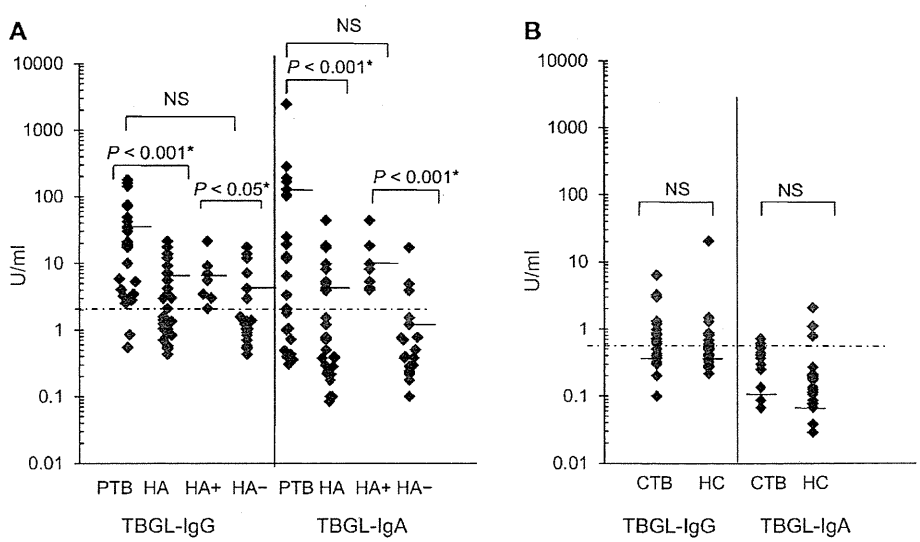

Figure 1 TBGL-IgG and TBGL-IgA titres in **A**) adult and **B**) child participants. Dashed lines indicate the cut-off value of ⩾2 U/ml for both antibodies. Solid bars indicate mean values. *Indicates significant difference. NS = not significant; PTB = adult pulmonary TB patients; HA = healthy adult controls; HA+ = HAs with high TBGL-IgG and -IgA titres; HA− = HAs with low TBGL-IgG or -IgA titres or both; TBGL = tuberculous glycolipid; Ig = immunoglobulin; CTB = child TB patients; HC = healthy child controls.

Table 2 Measured parameters and comparison between adult PTB patients and healthy adult controls

Parameter	Adult PTB cases median [range]	Healthy adults median [range]*	*P* value
TBGL-IgG, U/ml	18.7 [0.5–179]	1.5 [0.4–21.4]	<0.001
TBGL-IgA, U/ml	4.9 [0.3–2448]	0.7 [0.08–43.7]	<0.001
Serum IgG, mg/dl	1961 [1433–2835]	1441 [1032–2051]	<0.01
Serum IgA, mg/dl	519 [411–695]	223 [143–861]	<0.01†
KL-6, U/ml	530 [231–1897]	225 [129–592]	<0.001†
Leptin, ng/ml	0.63 [0.13–5.3]	7.7 [0.3–21.6]	<0.001†
sIL-2Rα, ng/ml	2.8 [0.81–15.5]	0.54 [0.1–0.9]	<0.001†
Haemoglobin, gm/dl	12.5 [9.2–14.9]	13.1 [11.1–17.1]	<0.01†
WBC, 10^3/μl	10 [6.8–16.4]	7 [4.6–10.2]	<0.001†
Neutrophil, 10^3/μl	7.08 [5.04–13.78]	3.7 [2.07–6.9]	<0.001†
Lymphocyte, 10^3/μl	1.74 [0.88–3.2]	2.46 [1.85–3.6]	<0.01†
Monocyte, /μl	580 [248–1096]	393 [222–684]	<0.01†
AST, U/ml	25 [15–158]	21 [15–55]	NS
ALT, U/ml	18.5 [7–67]	15.5 [7–75]	NS

*Healthy adults with high titres of both TBGL-IgG and -IgA.
†Significant difference between the two groups ($P < 0.05$).
PTB = pulmonary tuberculosis; TBGL = tubercular-glycolipid; Ig = immunoglobulin; WBC = white blood cells; AST = aspartate aminotransferase; NS = not significant; ALT = alanine aminotransferase.

Table 3 Comparison between TBGL-IgG, TBGL-IgA and combined TBGL-IgG+IgA for their utility in the diagnosis of active pulmonary TB in adults

	TBGL-IgG %	TBGL-IgA %	TBGL-IgG+IgA %	*P* value*
Sensitivity	92	63	63	0.019†
Specificity	54	64	75	0.057

*Statistical difference between TBGL-IgG and TBGL-IgG+IgA groups.
†Significant difference.
TBGL = tuberculous glycolipid; Ig = immunoglobulin.

for the diagnosis of active TB, and the specificities were 54% and 64% (Table 2). Simultaneous detection of both TBGL-IgG and -IgA improved specificity (75%, $P = 0.057$), although sensitivity was significantly lower ($P = 0.019$) than for TBGL-IgG alone (Table 3). To elucidate the cause of high TBGL antibodies in HAs, we therefore further categorised them into two groups: HAs positive for both TBGL-IgG and -IgA (HA+ 7/28, 25%) and others (HA− 21/28, 75%). TBGL-IgG and -IgA titres in the HA+ group were significantly higher than in the HA− group ($P < 0.05$ and $P < 0.01$, respectively) and were not different from those in the PTB groups ($P > 0.05$ for all, Figure 1A). The levels of two antibodies were positively correlated in the HA+ subjects ($r = 0.843, P = 0.01$) and among the PTB patients ($r = 0.664$, $P < 0.00005$), but not in the HA− group (Figure 2). TBGL-IgG and -IgA titres were not correlated with those of serum IgG and IgA in the PTB, HA or HA+ groups ($P > 0.05$ for all). No correlation was observed between TBGL-IgG/IgA levels and KL-6 or leptin levels in patients or controls.

In contrast, among the paediatric subjects, only 3/23 (13%) CTB cases and 1/28 (3%) HC had high TBGL-IgG titres, demonstrating the very limited sensitivity (10%) of the assay for the diagnosis of paediatric TB patients. Neither TBGL-IgG nor -IgA titres were significantly different between paediatric cases and controls (Figure 1B).

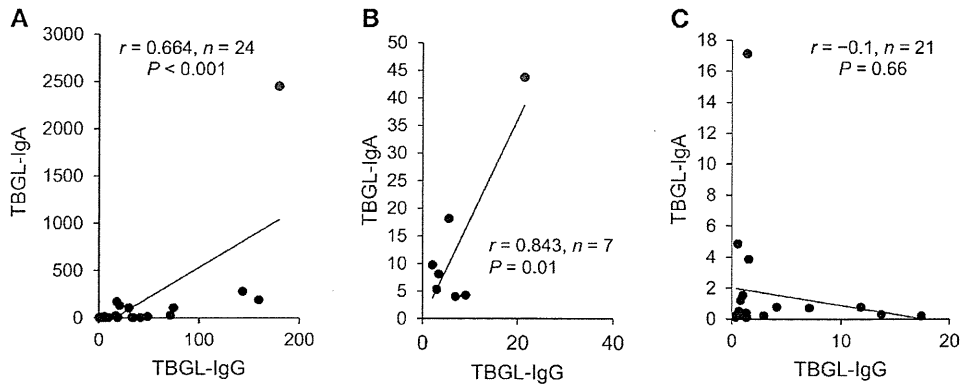

Figure 2 Correlation between TBGL-IgG and -IgA titres. An association was found in **A**) adult PTB patients and **B**) HA+ subjects (healthy adults with high TBGL-IgG and -IgA titres), but not in **C**) HA− subjects (healthy adults with low TBGL-IgG or -IgA titres or both). TBGL = tuberculous glycolipid; Ig = immunoglobulin.

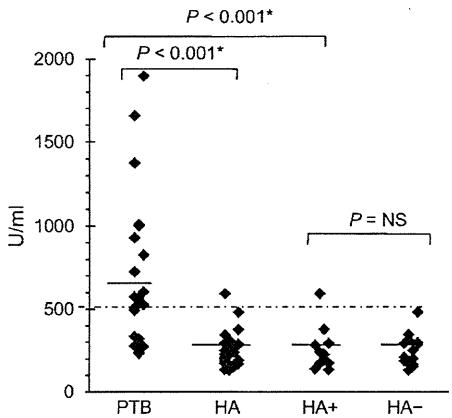

Figure 3 KL-6 titres in adult participants. Dashed line indicates the cut-off value of ⩾500 U/ml. Solid bars indicate mean values. *Indicates significant difference ($P < 0.05$). NS = non-significant; PTB = adult PTB patients; HA = healthy adult controls; HA+ = HAs with high TBGL-IgG and -IgA titres; HA− = HAs with low TBGL-IgG or -IgA titres or both; TBGL = tuberculous glycolipid; Ig = immunoglobulin.

KL-6, leptin and sIL-2Rα serum levels, and various laboratory markers

Serum KL-6 levels were significantly higher in PTB cases than in HAs ($P < 0.01$; Figure 3, Table 2) and were elevated (>500 U/ml) in 14/24 (58%) PTB patients. In contrast, significantly lower leptin titres were found in PTB patients than in HA subjects ($P < 0.001$). Serum IgG, IgA, sIL-2α levels and white blood corpuscle and monocyte counts were significantly higher, whereas the lymphocyte count was significantly lower in PTB cases than in HAs (Table 2). There were no significant differences in measured serum IgG, IgA, KL-6, leptin or other parameters between the HA+ and HA− groups (Table 4).

DISCUSSION

We evaluated TBGL-IgG and -IgA levels in paediatric and adult TB patients and healthy controls in Thailand, a TB-endemic country (TB incidence rate 142/100 000 population).[1]

Poor TBGL-IgG and -IgA reactivity was observed in the paediatric TB patients, consistent with previous findings of low antibody responses among child TB suspects against protein antigens, including purified protein derivative (PPD), 38kDa and HSP60.[18] Low TBGL-Ab titres cannot be explained by low serum IgG or IgA, as these were significantly higher in the CTB than in the HC group (data not shown). Although *M. tuberculosis* infection was not confirmed in most of the CTB cases, their clinical and radiological findings were strongly suggestive of active TB, and all responded well to anti-tuberculosis treatment. The cause of the low antibody responses in children is not clear. However, the underdeveloped immune system in young children might play a vital role against the development of specific adaptive immune responses against TB.

In contrast, TBGL-IgG detection in adult PTB patients was revealed to be highly sensitive (92%), in line with a previous report from Japan.[8] However, increased proportions of positive TBGL-IgG in HAs were accountable for the low specificity (54%), and therefore diminished its usefulness as an active TB diagnostic marker in Thailand. The diagnostic ability of TBGL-IgA was also inadequate, showing lower sensitivity and specificity in the current study. However, the specificity was higher than that of TBGL-IgG. Julean et al. also demonstrated high IgA specificity against four trehalose-containing mycobacterial lipid antigens, including cord factor, in a clinical study.[19]

Table 4 Comparison of clinical and laboratory markers between HA+ and HA−

Parameter	HA+ ($n = 7$) median [range]	HA− ($n = 21$) median [range]	*P* value
Male:female*	5:2	14:7	—
Age, years	38 [23–49]	33 [21–51]	—
TBGL-IgG, U/ml	5.5 [2.1–21.4]	1.3 [0.4–17.4]	<0.05†
TBGL-IgA, U/ml	8 [3.9–43.7]	0.3 [0.08–17.1]	<0.001†
Serum IgG, mg/dl	1367 [1281–1943]	1465 [1032–2051]	—
Serum IgA, mg/dl	192 [166–370]	238 [143–861]	—
KL-6, U/ml	227 [132–592]	223 [129–480]	—
Leptin, ng/ml	8.7 [1.14–19.9]	7.5 [0.3–21.6]	—
sIL-2Rα, ng/ml	0.53 [0.1–0.77]	0.55 [0.1–0.9]	—
Haemoglobin, g/dl	13.1 [12.2–5]	13.7 [11.1–17.1]	—
WBC, 10^3/μl	6.4 [5.5–8.1]	7.3 [4.6–10.2]	—
Neutrophil, 10^3/μl	3.46 [2.3–4.5]	4.1 [2–6.9]	—
Lymphocyte, 10^3/μl	2.5 [2–3.1]	2.4 [1.8–3.6]	—
Monocyte, /μl	402 [384–486]	360 [222–684]	—
AST, U/ml	17 [15–23]	21 [15–55]	—
ALT, U/ml	14 [9–24]	16 [7–75]	—

*Frequency.
†Significant difference between the two groups ($P < 0.05$).
HA+ = healthy adults with high titres of both TBGL-IgG and -IgA; HA− = healthy adults with low titres of either TBGL-IgG or -IgA or both; TBGL = tuberculous glycolipid; Ig = immunoglobulin; WBC = white blood cells; AST = aspartate aminotransferase; ALT = alanine aminotransferase.

Elevated TBGL-IgA titres may therefore reflect infection more specifically.

Elevated TBGL-IgG levels were also found in healthy older (>40 years, 17%) and younger adults (<40 years, 5%) in Japan (a non-endemic country); the possibilities of latent TB infection (LTBI) in the TBGL-IgG positive group have already been described by Maekura et al.[8] In this study in Thailand, positive proportions of TBGL-IgG were higher in healthy adults (46%, mean age 34 years) and that of TBGL-IgA was also high (36%). As TBGL-IgG and -IgA titres were not associated with those of serum IgG and IgA, high TBGL antibody titres in endemic HAs cannot be explained by non-specific hyperglobulinaemia. Moreover, none of the HAs had a history of TB. Cross-antibody reactions to other respiratory infections can be excluded, as the HAs were free from respiratory symptoms and had normal CXR findings at the time of enrolment, and bacille Calmette-Guérin vaccination status does not influence antibody production against TDM in adults.[20] It was considered that non-tuberculous mycobacteria (NTM) infection may be responsible for the elevated TBGL-Ab titres in HAs. However, TBGL-IgG titres were reported to increase only in active NTM diseases.[8] Although leptin titres were low in some HAs, none of the TB-related markers, including leptin, KL6 and sIL-2Rα, were different between the HA+ and HA− groups, indicating absence of active disease in HA+. Significant elevations of sensitive TBGL-IgG ($P < 0.05$) and specific TBGL-IgA titres ($P < 0.01$) in HA+ compared to HA− subjects, and the correlation between TBGL-IgG and -IgA titres only in the former group, might be suggestive of the enhancement of TB-specific antibody responses in that group. Although we could not confirm LTBI in HA+ individuals by PPD or an interferon gamma (IFN-γ) release assay (IGRA), a significant association between the QuantiFERON®-TB Gold assay (one of the IGRAs) and the TBGL-IgG assay in healthy adults was documented in our very recent study in the Philippines.[21]

Of note, an increased risk of progression to active TB was correlated with high antibody reactivity to some TB antigens in HIV patients[22,23] and with elevated IFN-γ production to early secreted antigenic target-6 in those with household TB contacts,[24] as the adaptive immune system can recognise antigens produced by early *M. tuberculosis* replication that are thought to be initiated months before the development of active TB.[22,23] However, no follow-up study was undertaken in our HA+ subjects to elucidate risk of active TB.

Taken together, we found that reduced specificity of TBGL-Ab in adult TB patients is due to enhanced humoral immune responses against TBGL in HAs, and that the high TBGL-IgG+IgA reactivity in HA+ controls might be specific and indicative of LTBI. Further extensive evaluation of control subjects from different population groups, including healthy subjects and patients with other pulmonary diseases, and careful follow-up studies, may clarify whether HA+ subjects are at greater risk of development of active TB than in HA− subjects. This might be helpful for the identification of potential markers for early TB diagnosis and the prevention of progressive disease.

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RÉSUMÉ

OBJECTIF : Evaluer chez les patients atteints de tuberculose pulmonaire (TBP) et chez les sujets-contrôle sains en Thaïlande des réponses en IgG et en IgA à l'égard de l'antigène tuberculeux-glycolipide (TBGL-IgG et -IgA).

SCHÉMA : Les titres d'anticorps anti-TBGL ainsi que d'autres marqueurs liés à la TB ont été mesurés dans le sérum de 24 adultes avec une TBP, 28 adultes sains (HA), 23 enfants avec une TBP et 24 enfants sains.

RÉSULTATS : Les titres tant de TBGL-IgG que de TBGL-IgA ne sont significativement plus élevés que dans les cas de TBP adultes comparés aux contrôles ($P < 0{,}001$ pour l'ensemble). Le test TBGL-IgG est très sensible (92%) chez les patients TBP, mais des proportions relativement élevées de TBGL-IgG (46%) et de TBGL-IgA (36%) chez les HA sont les causes d'une faible spécificité respectivement de TBGL-IgG (54%) et de TBGL-IgA (64%). La spécificité la plus élevée est celle de TBGL-IgG+IgA (75%). Les titres d'anticorps sont en corrélation positive chez les HA doublement positifs pour TBGL-IgG+IgA (HA+ 7/28 ; $P < 0{,}01$) mais non chez les HA− ($P > 0{,}05$). Les taux sériques d'IgG ou d'IgA ne sont pas en corrélation avec les taux de TBGL-IgG ou de TBGL-IgA ($P > 0{,}05$). Les taux de KL-6 et de leptine sont normaux et ne sont pas différents entre les HA+ et les HA−, ce qui indique l'absence d'une TB active chez les sujets HA.

CONCLUSION : Les réponses renforcées TBGL-IgG+IgA chez les HA pourraient indiquer une infection TB latente. Une étude soigneuse du suivi chez les sujets HA pourrait clarifier la signification du taux élevé d'anticorps TBGL comme marqueur précoce de la maladie.

RESUMEN

OBJETIVO: Se buscó evaluar la respuesta en IgG e IgA al estímulo con el antígeno glicolipídico de tuberculosis (TBGL) en pacientes con tuberculosis pulmonar (TBP) y en testigos sanos en Tailandia.

MÉTODO: Se cuantificaron los anticuerpos anti-TBGL y otros marcadores relacionados con *M. tuberculosis* en el suero de 24 adultos con TBP, 28 adultos sanos (HA), en 23 niños con TB y 24 niños sanos.

RESULTADOS: La cuantificación de TBGL-IgG y -IgA dio resultados significativamente más altos en comparación con los testigos, solo en los adultos con TBP ($P < 0{,}001$ en todos). La determinación de TBGL-IgG fue muy sensible (92%) en los adultos con TBP, pero las frecuentes proporciones positivas de TBGL-IgG (46%) y TBGL-IgA (36%) en los adultos sanos condicionaron una baja especificidad de estas mediciones (TBGL-IgG 54%; TBGL-IgA 64%); la especificidad más alta se obtuvo al combinar ambas determinaciones, TBGL-IgG+IgA (75%). Las concentraciones de ambos anticuerpos se correlacionaron en forma positiva en el subgrupo de adultos sanos con ambos títulos (TBGL-IgG+IgA) positivos (HA+ 7/28; $P < 0{,}01$) pero no en los HA con uno solo de los títulos positivos (HA−, $P > 0{,}05$). Ni la concentración sérica de IgG ni la concentración de IgA se correlacionaron con las concentraciones de TBGL-IgG o de TBGL-IgA ($P > 0{,}05$). Las concentraciones de KL-6 y de leptina fueron normales y no mostraron diferencias entre los subgrupos de HA+ y HA−, lo cual indica la ausencia de TB activa en los HA.

CONCLUSIÓN: Un aumento de las respuestas en IgG e IgA al antígeno TBGL en los HA podría estar en favor de una infección tuberculosa latente. Un cuidadoso estudio de seguimiento de los HA podría definir la significación de una alta concentración de anticuerpos contra el TBGL como marcador temprano de TB.

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Clinical Study

Frequent Detection of Anti-Tubercular-Glycolipid-IgG and -IgA Antibodies in Healthcare Workers with Latent Tuberculosis Infection in the Philippines

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Anti-tubercular-glycolipid-IgG (TBGL-IgG) and -IgA (TBGL-IgA) antibodies, and the QuantiFERON-TB Gold test (QFT) were compared in healthcare workers (HCWs, $n = 31$) and asymptomatic human immunodeficiency virus-carriers (HIV-AC, $n = 56$) in Manila. In HCWs, 48%, 51%, and 19% were positive in QFT, TBGL-IgG, and -IgA, respectively. The TBGL-IgG positivity was significantly higher ($P = 0.02$) in QFT-positive than QFT-negative HCWs. Both TBGL-IgG- and -IgA-positive cases were only found in QFT-positive HCWs (27%). The plasma IFN-γ levels positively correlated with TBGL-IgA titers ($r = 0.74$, $P = 0.005$), but not TBGL-IgG titers in this group, indicating that mucosal immunity is involved in LTBI in immunocompetent individuals. The QFT positivity in HIV-AC was 31% in those with CD4+ cell counts > 350/μl and 12.5% in low CD4 group (< 350/μl). 59 % and 29% were positive for TBGL-IgG and -IgA, respectively, in HIV-AC, but no association was found between QFT and TBGL assays. TBGL-IgG-positive rates in QFT-positive and QFT-negative HIV-AC were 61% and 58%, and those of TBGL-IgA were 23% and 30%, respectively. The titers of TBGL-IgA were associated with serum IgA ($P = 0.02$) in HIV_AC. Elevations of TBGL-IgG and -IgA were related to latent tuberculosis infection in HCWs, but careful interpretation is necessary in HIV_AC.

1. Introduction

Although the incidence of tuberculosis has been falling since 2002, there were still 8.8 million incident cases of TB, 1.1 million deaths from TB, and an additional 0.35 million deaths from HIV-associated TB in 2010 [1]. The high rate of latent TB infection (LTBI) is one of the factors that make it difficult to achieve global control and eliminate TB [2]. The recent introduction of the immune-based interferon-γ release assay (IGRA) made a great impact on facilitating the diagnosis of LTBI [3] and clarified the high rate of infection in TB-high-risk populations including healthcare workers (HCWs) [4]. Attempts to detect LTBI in HIV-infected individuals were also facilitated by the development of IGRA, although their higher rates of pseudonegative IGRA response due to low CD4+ T cell counts and diminished Th1 immunity cannot be ignored [5]. Trehalose 6,6-dimycolate (TDM), which constitutes a major part of the mycobacterial cell wall, was identified as the most immunogenic glycolipid and is produced predominantly by virulent MTB as well as by atypical mycobacteria [6]. Tubercular-glycolipid antigen (TBGL) consists of TDM purified from virulent mycobacterial strain H37Rv [7, 19]. The immunoglobulin-G to tubercular-glycolipid antigen (TBGL-IgG) has been proposed to be a useful marker for the serodiagnosis of active pulmonary tuberculosis (PTB) in Japan [7]. However, frequent elevated titers (17%) were also found in healthy elderly control people (age: >40 yrs) in the same study,

and the possibility of LTBI was suggested by Maekura and colleagues [7]. Although IgA antibody to TBGL antigen (TBGL-IgA) was not evaluated earlier as a biomarker, strong association was revealed between the TBGL-IgG and-IgA titers in PTB cases [8]. Frequent positivity for TBGL-IgG (46%) and -IgA (36%) in healthy adults was also observed in our very recent study in Thailand, a TB-endemic country [9]. The TBGL-IgG-positive responses were not related to BCG vaccination [10]. Since both cellular-mediated and humoral immunity are necessary for an effective immune response against MTB, we aimed to clarify the relationship between the TBGL-IgG and -IgA responses with QuantiFERON-TB Gold In-Tube (QFT) assay system, in healthcare workers (HCWs) in a hospital of the Philippines.

Infection of human immunodeficiency virus (HIV) has substantially boosted the occurrence of tuberculosis (TB) disease worldwide [1]. The devastating association between HIV and TB is responsible for one of four TB-related deaths [11]. The East-Asian countries are predominantly TB endemic [1]. Similarly to Sub-Saharan Africa, the rapid, progressive increase of HIV infections in East-Asian countries may further accelerate TB infection in HIV/AIDS patients [12]. To clarify how HIV infection may alter immune responses in LTBI, newly diagnosed, asymptomatic, non-TB HIV-infected individuals were studied.

To understand the health condition of the individuals, we measured two TB-related biomarkers. Leptin, a cytokine-like hormone produced by bronchial epithelial cells and type II pneumocytes in addition to adipose tissue, exhibits a Th1-bias immune response [13]. Osteopontin (OPN) is a member of extracellular matrix proteins that is synthesized within the immune system by activated T cells, NK cells, dendritic cells, and macrophages. Involvement of OPN in Th1 immune responses has been reported [14]. OPN deficiency was found to be associated with the dissemination of mycobacterial disease, and its expression correlated with an effective immune and inflammatory response against mycobacteria in rodents as well as in human [15, 16]. Elevated levels of circulatory plasma OPN [17] and low levels of leptin [18] were reported to be associated with active tuberculosis; these biomarkers served as a negative evidence of active disease.

2. Materials and Methods

2.1. Study Subjects. A case-control study was conducted between March and October of 2010 in adult participants (age > 18 years) in the Philippines. Thirty-one healthy, adult healthcare workers (HCWs) without any concomitant symptoms or chest radiographic findings relevant to active TB and who had negative HIV serology were recruited from San Lazaro Hospital (SLH), Manila, Philippines. Fifty-six newly diagnosed, asymptomatic HIV carriers (HIV-AC) without any clinical symptoms relevant to tuberculosis were randomly selected from among patients receiving care at the outpatient department of the SLH. None of the subjects took any anti-HIV therapy. Subjects with AIDS-defining events, currently active tuberculosis, or any symptoms relevant to tuberculosis, other than active pulmonary diseases, underlying malignancy or metabolic disorders were excluded from the study. The exclusion criteria for active tuberculosis were based on both clinical findings and chest X-ray (CXR) findings in the HCWs. The study was approved by the ethics committee of SLH and the Tohoku University Hospital. We obtained written informed consent from all the participants. Three mL of blood was obtained directly (one mL in each tube) from each participant to perform the QFT assay. Simultaneously, plasma was separated from blood by centrifugation after treatment with EDTA and was aliquoted to CryoTubes for storage at −80°C until further utilization. All the procedures were conducted in accordance with the Helsinki declaration.

2.2. TBGL-Antibody Assay. TBGL-IgG antibody and -IgA antibodies were measured using the Determiner TBGL Antibody ELISA kit (Kyowa Medex, Tokyo, Japan), an in vitro enzyme-linked immunosorbent assay for the quantitative measurement of anti-TBGL-IgG and -IgA in plasma. This assay employs glycolipid antigens purified from *M. tuberculosis* H37Rv (TBGL antigen) coated on a 96-well plate. The details of the assay were described in our previous study [8]. The antibody titers for TBGL-IgG and -IgA were expressed as U/mL. Positive TBGL-IgG titers were determined according to the cutoff index proposed by Kishimoto et al. [19]. The samples were classified as positive when the serum levels of anti-TBGL-IgG were ≥2 U/mL. An arbitrary cutoff value of ≥2 U/mL for TBGL-IgA was used according to the unpublished data of our previous study [8].

2.3. QuantiFERON-TB Gold In-Tube (QFT). The QFT test was performed using fresh whole blood in accordance with the manufacturer's instruction (Cellestis, Australia). The results were interpreted using specific software provided by Cellestis. The result was scored positive if the IFN-γ concentration in the tube TB-specific antigen containing was >0.35 IU/mL after subtracting the value of the nil control (IFN-γ-nc) and at least >25% of NC value. If the net IFN-γ response (TB Ag minus nil) was <0.35 IU/mL for the antigens and the response to the mitogen-positive control was >0.5 IU/mL, the response was considered as test negative. An intermediate result was recorded if the net IFN-γ response was <0.35 IU/mL for the antigen and <0.5 IU/mL for the mitogen and/or was above 8 IU/mL for the NC.

2.4. Leptin and OPN Elisa Assay. Plasma leptin levels were determined by sandwich ELISA using Quantikine Human Leptin Immunoassay kit (R&D Systems) for the quantitative determination of the human leptin concentrations in plasma according to the manufacturer's guidelines. Plasma OPN concentrations were determined using Human OPN Elisa kit (Immuno-Biological Laboratories, Takasaki, Japan) according to the manufacturer's guidelines, and values were expressed as ng/mL.

2.5. Clinical Data. We measured different laboratory markers including complete red blood cell counts, the number of white blood cells with their differential counts, levels of

hemoglobin, and serum levels of IgG and IgA. The number of CD4+ T cell counts and HIV RNA load of HIV-AC were also determined.

2.6. Statistical Analysis. The data of quantitative variables are summarized as median and range. Categorical variables were computed as frequency and percentage. The data were analyzed using Stat Flex software, version 5 (Artech Co., Ltd: http://www.statflex.net/index.html) and Statcel 2 (OMS Publishing Inc. Saitama, Japan). The ability of each single marker to discriminate HIV from HCW by receiver operating characteristic (ROC) curve and the area under curve (AUC) was also analyzed. The percentage of overall agreement between QFT and TBGL-IgG/IgA ELISA assays was calculated, and a Cohen's Kappa coefficient was used to assess the level of agreement. The significance of association for categorical variables was estimated by Fisher's exact test, whereas correlations between continuous variables were evaluated by Spearman's rank correlation coefficient. The differences in significance between continuous variables were compared by the Mann-Whitney U test. A 2-tailed P value of <0.05 was considered significant.

3. Results

3.1. Characteristics of Study Participants. A total of 31 HCWs and 56 newly diagnosed HIV-AC were enrolled in the current study. Basic demographic and clinical characteristics of the study participants are shown in Table 1. The participating HIV-AC were relatively young ($P = 0.03$) with a significant male predominance ($P < 0.0001$) compared to the HCWs. Although lymphocyte counts were comparable between the two groups, total counts of WBC, neutrophils, and monocytes were significantly lower in HIV-AC.

3.2. QFT and TBGL-Antibody Assays in HCWs. Forty-eight percent (15/31) of the HCWs showed positive reactions in the QFT assay indicating high incidences of LTBI (Table 1). The median age of the QFT-positive responders from among the HCWs were significantly higher than those of the QFT-negative group ($P = 0.002$). TBGL-IgG and TBGL-IgA were positive in 51% and 19% of HCWs, respectively (Table 1).

Eleven of 15 (73%) QFT-positive HCWs had positive TBGL-IgG responses (categorical agreement 73%), whereas 5 of 16 (31%) QFT-negative subjects had positive TBGL-IgG responses (categorical agreement 68.7%). The overall κ value was 0.42, indicating a moderate association between the two assays (overall agreement: 71%; 95% CI: 0.10~0.73). The TBGL-IgG-positive proportions were also significantly different between QFT-positive and QFT-negative groups of HCWs ($P = 0.02$). Although the number of positive TBGL-IgA responders was small in HCWs and failed to show any significant difference ($P = 0.072$), the TBGL-IgG+IgA double-positive response was shown only by QFT-positive HCWs and none of the QFT-negative HCWs had double-positive reactions ($P = 0.043$) (Figure 1) (Table 2).

In addition, significant positive correlation was observed between the concentrations of IFN-γ-nc and TBGL-IgA titers in the QFT-positive group ($r = 0.74$, $P = 0.005$) (Figure 2), but not in the QFT-negative group. There was no such association between IFN-γ-nc and TBGL-IgG levels in HCWs, although a tendency for a positive correlation was observed in the QFT-positive HCWs ($r = 0.43$, $P = 0.11$) (Figure 2). No association was observed in the net IFN-γ concentrations in antigen-stimulated QFT-plasma with TBGL-IgG or -IgA titers (data not shown). The plasma levels of OPN and leptin were not different between QFT-positive and QFT-negative HCWs (Table 2).

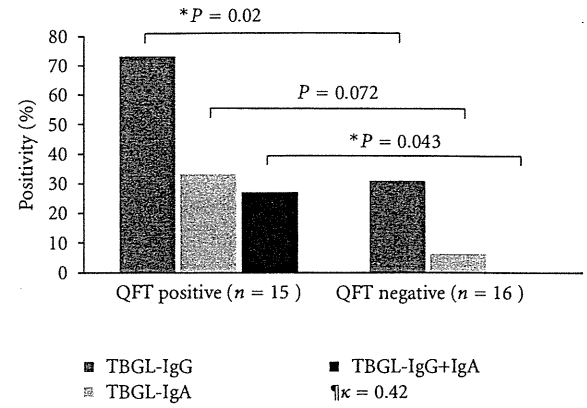

FIGURE 1: Positivity percentage of TBGL-IgG/IgA assay in QFT-positive/negative healthcare workers. The level of agreement between QFT and the TBGL-Ab assay was measured by Cohen's kappa (κ). ¶κ = 0.42; overall agreement 71%; 95% confidence interval: 0.1~0.73.* Significant difference ($P < 0.05$).

3.3. QFT and TBGL-Antibody Assays in HIV-AC. As shown in Table 1, only 13 of 56 (23%) HIV carriers showed positive reactions by QFT assay. The rate of positivity was closely associated with high median CD4+ T cell counts ($P = 0.012$) and younger age ($P = 0.036$) (Table 2). Seven of 56 (12.5%) HIV-AC who had lower mitogen responses (IFN-γ concentrations: median: 1.78 IU/mL; range: 0.38~6.73 IU/mL) than the rest (>10 U/mL) had negative responses by QFT assay. Their median CD4+ T-cell counts were 60/μL (range: 43~425/μL) (data not shown). Thirty-three of 56 (59%) and 16 of 56 (29%) HIV-AC were attributed with positive TBGL-IgG and TBGL-IgA responses, respectively (Table 1). The positive proportions of TBGL-IgG and -IgA responses were not significantly different between QFT-positive and -negative HIV-AC (Table 2). However, 6 of 7 QFT-negative low mitogen responders in HIV-AC were positive for both TBGL-IgG and -IgA assay (data not shown). The TBGL-IgA titers were significantly higher in the TBGL-IgG-positive HIV-AC ($P = 0.041$) (Table 3). In addition, TBGL-IgA-positive HIV-AC had significantly elevated titers of TBGL-IgG ($P = 0.042$), serum IgA ($P = 0.015$), and OPN ($P = 0.03$), (Table 3). Interestingly, the TBGL-IgA-positive proportion was inversely correlated with the CD4+ T-cell counts ($P = 0.018$), and the titers were significantly higher in the HIV-AC with CD4+ T-cell count < 350/μL

Table 1: Demographic and clinical data of study participants.

Variables	HCWs (n = 31)	HIV-AC (n = 56)	P
Demographic data			
Gender: male; n (%)	16 (51.6)	55 (96.5)	<0.0001*
Age year; median (range)	35 (19~62)	28 (19~48)	0.03*
Laboratory findings†			
Hemoglobin (g/dL)	13.2 ± 2.6	13 ± 1.49	0.36
RBC (million/μL)	4.96 ± 1.6	4.43 ± .55	0.069
WBC ($10^3/\mu$L)	7.5 ± 2.5	5.9 ± 1.9	0.01*
Neutrophil ($10^3/\mu$L)	4.4 ± 2.2	3.3 ± 1.2	0.048*
Lymphocyte ($10^3/\mu$L)	2.4 ± 0.6	2.2 ± 0.9	0.82
Monocyte (/μL)	562 ± 237	338 ± 182	<0.001*
CD4+ T-cell count (/μL)	ND	443 ± 286	NA
QFT assay positive; n (%)	15 (48)	13 (23)	0.03*
TBGL-IgG positive; n (%)	16 (51)	33 (59)	0.9
TBGL-IgA positive; n (%)	6 (19)	16 (29)	0.87
IFN-γ-nc (IU/mL)	0.42 ± 0.96	0.13 ± 0.11	<0.001*
TBGL-IgG (U/mL)	3.12 ± 3.36	3.94 ± 6.63	0.14
TBGL-IgA (U/mL)	1.68 ± 2.56	3.1 ± 6.64	0.012*
Serum IgG (mg/dL)	1409 ± 212	1391 ± 224	0.49
Serum IgA (mg/dL)	246 ± 92	319 ± 138	0.058
OPN (ng/mL)	14.4 ± 11	159 ± 191	<0.00001*
Leptin (ng/mL)	18.6 ± 13.9	7.2 ± 5.4	<0.001*

Abbreviations: HCWs, healthcare workers; HIV-AC, newly diagnosed cases of asymptomatic human immune-deficiency virus infection; OPN, osteopontin; ND, not determined; NA, not applicable.
† values were presented as mean ± SD unless indicated otherwise; IFN-γ-nc: levels of IFN-γ, measured in the nonstimulated QFT-plasma samples; P values for statistical differences between HCW and HIV-AC; * significant differences ($P < 0.05$).

Table 2: Comparison between QFT-positive and QFT-negative HCWs and HIV-AC.

Variables	HCWs			HIV-AC		
	QFT+ (n = 15)	QFT− (n = 16)	P	QFT+ (n = 13)	QFT− (n = 43)	P
Age; median (range)	45 (21~62)	23.5 (19~48)	0.002*	25 (19~45)	31 (21~35)	0.036*
Gender: male; n (%)	7 (46.6)	9 (47.4)	0.43	12 (92.3)	42 (97.67)	0.43
Work duration->10 yrs; n (%)	11(73.3)	6 (37.5)	0.098	NA	NA	NA
CD4+ count (/μL); median (range)	ND	ND	NA	611 (148~1466)	356 (13~1125)	0.012*
TBGL-IgG positive; n (%)	11 (73)	5 (31)	0.02*	8 (61.5)	25 (58.13)	0.545
TBGL-IgA positive; n (%)	5 (33)	1 (6)	0.072	3 (23)	13 (30)	0.415
TBGL-IgG+IgA positive; n (%)	4 (27)	0 (0)	0.043*	2(15.4)	10 (23.3)	0.42
IFN-γ-nc (IU/mL)†	0.3 ± 0.4	0.2 ± 0.13	0.9	0.21 ± 0.17	0.1 ± 0.07	0.0087*
Serum IgG (mg/dL)†	1450 ± 188	1368 ± 235	0.2	1306 ± 207	1414 ± 249	0.5
Serum IgA (mg/dL)†	268 ± 81	225 ± 101	0.32	330 ± 130	312 ± 138	0.68
OPN (ng/mL)†	14.5 ± 11.2	14.2 ± 11.2	0.87	115.4 ± 130	173.2 ± 203	0.43
Leptin (ng/mL)†	21.3 ± 13.3	15.9 ± 14.3	0.25	6.46 ± 4.12	7.448 ± 5.68	0.24

Abbreviations: HCWs, healthcare workers; HIV-AC, newly diagnosed cases of asymptomatic human immune-deficiency virus infection; OPN, osteopontin; ND, not determined; NA, not applicable.
†mean ± SD; IFN-γ-nc: levels of IFN-γ, measured in the nonstimulated QFT-plasma samples; P values for statistical differences between QFT-positive and QTF-negative groups; *significant differences ($P < 0.05$).

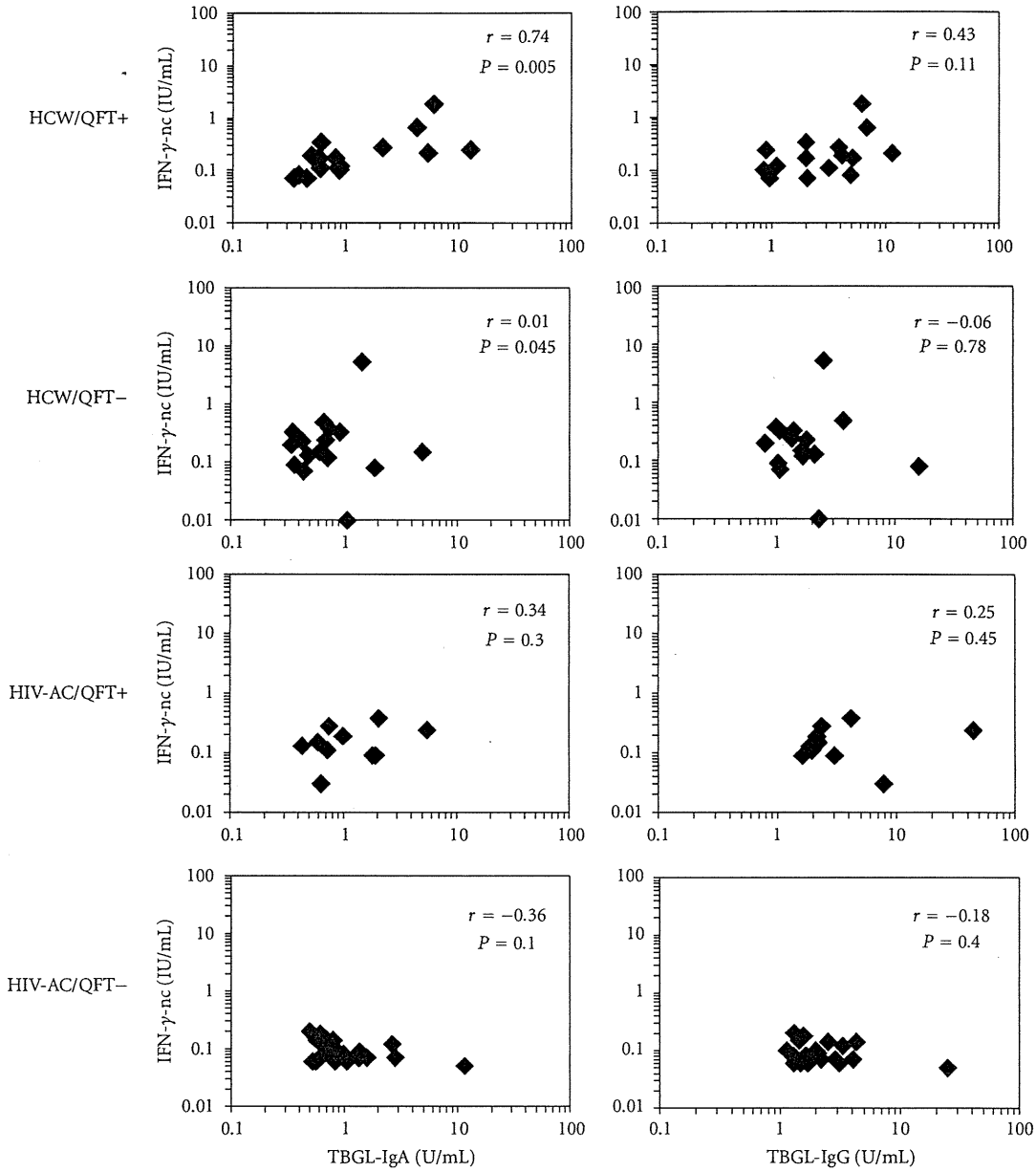

FIGURE 2: Correlations between TBGL-IgA or TBGL-IgG titers and IFN-γ concentrations measured in nonstimulated QFT-plasma samples (IFN-γ-nc) in QFT-positive/QTF-negative healthcare workers (HCWs) and asymptomatic HIV carriers (HIV-AC). The only significant positive correlation was observed between the IFN-γ-nc concentrations and TBGL-IgA titers in the QFT-positive HCW group ($r = 0.74$, $P = 0.005$).

(HIV-LCD) ($P = 0.048$) (Table 4). Furthermore, in the HIV-AC, a relatively higher proportion of double positive (TBGL-IgG+IgA) responders was found in the HIV-LCD group (29%) than in the HIV-HCD group (CD4+ count $\geq$ 350/μL) (16%), although the difference was not statistically significant ($P = 0.32$) (Table 4).

Moreover, the IFN-γ-nc concentrations were significantly lower in the QFT-negative HIV-AC ($P = 0.008$) (Table 2). No association was observed between the IFN-γ-nc concentrations and TBGL-IgG or -IgA titers in any group of HIV-AC (Figure 2). The plasma levels of OPN and leptin were not different between QFT-positive and QTF-negative HIV-AC (Table 2).

3.4. Comparison between the Serum Antibodies and TBGL Antibodies. The TBGL-IgG and -IgA had no correlation with the serum IgG and IgA in HCW and HIV-AC except for the

Table 3: Comparison between TBGL-IgG or TBGL-IgA-positive and -negative HIV-AC.

Variables	TBGL-IgG			TBGL-IgA		
	Positive (n = 33)	Negative (n = 23)	P	Positive (n = 16)	Negative (n = 40)	P
Age; median (range)	28 (19~48)	30 (19~41)	0.18	31.5 (19~48)	28 (19~45)	0.038*
Gender: male; n (%)	33 (100)	21 (91.3)	0.43	16 (100)	38 (95)	1
CD4 count (/μL); mean (range)	436 (13~1466)	450 (60~851)	0.45	346 (46~1125)	480 (13~1466)	0.06
QFT positive; n (%)	8 (24.2)	5 (21.7)	0.545	3 (19)	10 (25)	0.45
TBGL-IgA positive; n (%)	12 (36.4)	4 (17.4)	0.1	—	—	—
TBGL-IgG positive; n (%)	—	—	—	12 (75)	21 (52.5)	0.14
IFN-γ-nc (IU/mL)†	0.13 ± 0.09	0.1 ± 0.05	0.4	0.12 ± 0.09	0.12 ± 0.07	0.9
TBGL-IgA (U/mL)†	4.36 ± 8.4	1.28 ± 1.21	0.041*	—	—	
TBGL-IgG (U/mL)†	—	—		7.5 ± 11.6	2.5 ± 1.5	0.042*
Serum IgG (mg/dL)†	1439 ± 277	1515 ± 677	0.5	1615 ± 404	1355 ± 135	0.46
Serum IgA (mg/dL)†	277 ± 95	279 ± 74	0.37	410 ± 165	313 ± 138	0.015*
OPN (ng/mL)†	176.3 ± 199.9	136 ± 172.5	0.67	280 ± 275	115 ± 109.7	0.03*
Leptin (ng/mL)†	7.33 ± 6.16	7.18 ± 4.12	0.68	7.33 ± 6.16	7.18 ± 4.12	0.07

Abbreviations: HIV-AC, newly diagnosed cases of asymptomatic human immune-deficiency virus infection; OPN, osteopontin.
†mean ± SD; IFN-γ-nc: levels of IFN-γ, measured in the nonstimulated QFT-plasma samples; P for statistical differences between QFT-positive and QTF-negative groups; *significant differences ($P < 0.05$).

Table 4: Comparison between HIV-AC with high§ and low‡ CD4+ T-cell count.

Variables	CD4+ high§ (n = 32)	CD4+ low‡ (n = 24)	P value¶
Age; mean (range)	25.5 (19~45)	25 (22~48)	0.018*
Gender: male; n (%)	31 (97)	23(98)	1.0
CD4+ count (/μL); median (range)	618 (356~1466)	201 (13~349)	<0.001*
QFT-positive; n (%)	10(31)	3(12.5)	0.12
TBGL-IgG positive; n (%)	16 (50)	16 (67)	0.27
TBGL-IgA positive; n (%)	5(16)	11(46)	0.018*
TBGL-IgG+ IgA positive; n (%)	5(16)	7(29)	0.32
IFN-γ-nc (IU/mL)	0.14 ± 0.12	0.13 ± 0.09	0.9
TBGL-IgG (U/mL)†	4.6 ± 8.4	3 ± 2.8	0.59
TBGL-IgA (U/mL)†	1.55 ± 2	5.16 ± 9.6	0.048*
Serum IgG (mg/dL)†	1352 ± 185	1549 ± 380	0.5
Serum IgA (mg/dL)†	265 ± 89	423 ± 149	<0.001*
OPN (ng/mL)†	119 ± 126	214 ± 246	0.19
Leptin (ng/mL)†	7.7 ± 6	6.6 ± 4.9	0.5

Abbreviation: HIV-AC, newly diagnosed cases of asymptomatic human immune-deficiency virus infection; OPN: osteopontin.
§High: CD4+ T cell count ≥350/μL; ‡low: CD4+ T-cell count <350/μL; †mean±SD; IFN-γ-nc: levels of IFN-γ, measured in the non-stimulated QFT-plasma samples; P values for statistical differences between QFT-positive and QTF-negative groups; *significant differences ($P < 0.05$).

association between the serum IgA levels and the TBGL-IgA titers in HIV-AC (P = 0.02) (data not shown).

3.5. Comparison of Biomarkers between HCW and HIV-AC. The levels of IFN-γ-nc ($P < 0.001$) were significantly higher in HCWs than in HIV-AC. However, the titers of TBGL-IgA (P = 0.012), but not -IgG, were significantly higher in HIV-AC than in HCWs. Similarly, the serum IgA levels were also higher (P = 0.058). The OPN levels were significantly higher ($P < 0.0001$), and the leptin levels were considerably lower ($P < 0.001$) in the HIV-AC compared to the HCWs (Table 1).

ROC curve analysis was used to discriminate HIV from HCW groups using the net IFN-γ, leptin, and plasma levels of OPN (log) as biomarkers. As shown in Figure 3, the plasma levels of OPN (log) exhibited the greatest ability to discriminate HIV from HCWs based on the AUC (0.883), followed by leptin (0.763) and net IFN-γ (0.648). However,

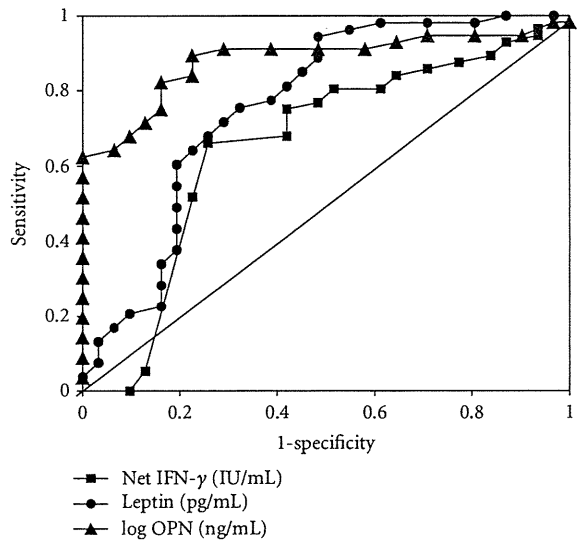

FIGURE 3: Receiver operating characteristic analysis for comparison of biomarkers between healthcare workers and asymptomatic HIV carriers. The result showed that the OPN plasma levels of OPN (log) exhibited the greatest ability to discriminate HIV from HCW based on the AUC (0.883), followed by leptin (0.763) and net IFN-γ (0.648).

QFT assay as well as TBGL-IgA and -IgG did not show such profiles (data not shown).

4. Discussion

In our data, the application of QFT assay to HCWs in the Philippines demonstrated a high incidence (48%) of LTBI, which was comparable to other already published data in HCWs in TB-endemic developing countries [4]. The increased risk of LTBI among HCWs was confirmed by the recent introduction of IGRA [20, 21]. In our country, a higher incidence of LTBI in HCWs was reported in high-risk groups for TB, such as homeless areas [22], compared to other areas [23].

We aimed to clarify the relationship between the TBGL-IgG and -IgA responses and that of IFN-γ in the QFT assay in LTBI. The rate of TBGL-IgG positivity was significantly higher in the QFT-positive than QTF-negative group of HCWs. The significant association between the two assay systems indicated by the κ value in HCWs demonstrated the TBGL-IgG in LTBI. However, about 30% of QFT-positive populations from among the HCWs lacked TBGL-IgG, and 30% of those of the QFT-negative group have elevated TBGL IgG antibody, and the discordant cases were higher in TBGL-IgA. However, the reasons for such discordances between the two systems in HCWs are not clear. It is possible that the generation of antibody requires larger amounts of antigens than does the generation of T-cell responses. Although associated immunosuppressive conditions were found as risk factors for false-negative QFT responses [24], such cases were excluded from HCWs in our study.

The mechanism of the synthesis of anti-TDM antibody is not clear, though TDM is known to bind to Mincle (macrophage-inducible C-type lectin) that is present on macrophages [25], and upon the activation, on T cells [26]. It was found that Mincle is specific for the ester linkage of a fatty acid to the trehalose, which explains the strong binding of TDM, but not trehalase-treated TDM, soluble trehalose, or purified mycolate [26]. The conversion of TDM into glucose monomycolate (GMM) upon mycobacterial infection might be the mechanism by which mycobacteria escape from the Mincle-mediated immunity. However, the immune system possesses other tools to monitor and eliminate live mycobacteria through CD1 molecules expressed on the activated macrophages and dendritic cells, which are different from MHC I, II molecules. Recently, GMM but not TDM was demonstrated to interact with CD1b and may induce adaptive immunity [27]. Although it is not known whether the adaptive immune system leads to antibody synthesis, the generated antibody may recognize both TDM and GMM because the two molecules are structurally very similar.

Interestingly, the IFN-γ-nc levels that were observed to have a significant association with the TBGL-IgA titers in LTBI of HCWs. IgA is a typical marker of the mucosal immune response. An elevated serum IgA has been proposed to have a protective role in IFN-γ-positive immunocompetent LTBI individuals [28]. Frequent exposure to tubercle bacilli can possibly stimulate the mucosal immune system in TB-endemic countries. It is also known that commensal bacteria on the mucosal surface induce IgA in an NO-dependent manner [29], although it is not known whether MTB in LTBI has a similar effect in lung mucosa. Circulating glycolipid immune complexes might lead to nonspecific stimulation of T cells, but a component of TBGL, TDM, could also enhance the in vivo production of IL-12p40 and IFN-γ in mouse model [30]. IgA antibody and IFN-γ induce TNF-α and NO production, which mediated the inhibitory mechanism for *M. tuberculosis* infection in mouse model [28]. Furthermore, there is strong evidence of a synergic effect between IgA and IFN-γ in bactericidal activities against MTB infection [31]. Therefore, the association between anti-TBGL-IgA and IFN-γ may indicate protective, mucosal immune activities in LTBI in HCWs.

In HIV carriers, the QFT-positive responses were significantly lower than in HCWs and were greatly dependent on the high CD4+ T-cell counts in the present study. Much evidence suggests that the baseline CD4+ T-cell count is a determining factor for a positive QFT response in HIV infection [32]. Since HIV infection is a disease of immune deficiency, immune deprivation may be less prominent in relatively young QFT-positive cases because IFN-γ could be synthesized properly by stimulation with the appropriate signals. In contrast, the response could be altered in advance immune-deficiency state, as indicated by low CD4+ T-cell counts. Therefore, it is expected that significant numbers of false-negative reactions are present in QFT-negative HIV carriers. The relatively low IFN-γ levels by mitogen stimulation in some of the QFT-negative responders also support this possibility. Therefore, for TB diagnosis

in advanced immunosuppression, the ratio of the IFN-γ response/CD4+ T-cell count Elispot assay was suggested to improve the sensitivity of the assay [33].

It is not clear why HIV infection does not diminish the TBGL antibody titers. It is known that the CD-1 presentation pathway persists in patients with HIV, but antiglycolipid antibodies were found to have no relationship with the TST results [34] or bacillary yield [35]. Similarly, we did not find any correlation between the QFT result and anti-TBGL antibodies. It is also possible that concomitant non-TB mycobacterium infection may stimulate the TBGL antibody synthesis in HIV-AC [7]. Significant numbers of HIV carriers have antibodies to TBGL, but we could not confirm if they indicate LTBI or not.

The increases of serum IgA in advanced HIV infection and of IgG in the early stage were already reported [36]. Although specific antibody titers in HIV infection are decreased by some infectious agents including hepatitis B virus but not in hepatitis A virus, probably because of alterations in the immune systems in advanced HIV infection [37], it is not known whether nonfunctional or functional IgA was synthesized in our cases. The main limitation of the current study is the small number of study subjects and the lack of a follow-up study for estimating the risk of developing active tuberculosis.

Finally, to determine the correlations between biomarkers in infected states, we evaluated data by ROC curve analysis (Figure 3). In this study, the plasma levels of OPN were most specific to HIV and the levels were not elevated in LTBI HCWs (Figure 3, Table 2). Therefore the levels can be a good marker for active TB in non-HIV individuals, because the OPN is known as a marker of active TB [17]. In HIV-AC, the OPN plasma levels are already elevated as described here, and it was already reported that the levels further increase when they developed active TB [38, 39]. It is also known that interferon-inducible protein-10 (IP-10) and IL-18 were elevated in HIV/TB patients than in HIV patients and suggested to be helpful in monitoring the treatment for patients [38]. All these biomarkers were mainly produced by macrophages, and it was also reported that OPN is synthesized by macrophages as well as CD4+ T cells in HTLV-1-induced lymphoma [40, 41].

In this study we noted elevations of anti-TBGL antibody in LTBI in HCWs, but no link between the elevations with LTBI in HIV-AC was confirmed, probably due to the inflammatory conditions in HIV.

5. Conclusion

We have found the elevation of TBGL-IgG titers in LTBI in HCWs. In addition, the association between TBGL-IgA and IFN-γ in HCWs was found, and it was hypothesized that the mucosal immunity is involved in LTBI in HCWs. We could not find any relationships between QFT and TBGL in HIV-AC. Low CD4+ cell count was associated with inflammatory conditions as represented by high OPN in HIV-AC, which may be the reason for ambiguous results.

Conflict of Interests

The authors declare that they have no conflict of interests.

Authors' Contribution

US and PL did experiments using the samples and contributed equally as the first author. HCY and BS contributed to the experimental system and statistical analysis. HS and YA and YS contributed to the planning and proposal of the work. TH and ET coordinated the work.

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