

# Interferon-gamma release assay for detection of latent tuberculosis infection in casual and close contacts of tuberculosis cases

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مقايضة انطلاق الغاما-إنترفيرون للكشف عن عدوى السل الخافية بين المخالطين لحالات السل مخالطةً عارضةً أو مخالطةً لصيقة  
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الخلاصة: لاختبار التوبركولين الجلدي العديد من أوجه القصور في تشخيص عدوى السل الخافية. وتهدف هذه الدراسة المصرية إلى تقدير فائدة مقايضة انطلاق الغاما-إنترفيرون في الكشف عن العدوى الخافية بين المخالطين لحالات السل الناشطة. وقد تم إدراج 116 مشاركاً في الدراسة، وجرى تقسيمهم إلى ثلاث مجموعات: مجموعة الشواهد من المجتمع، ومجموعة المخالطين مخالطةً عارضةً (في المختبرات والعيادات)، ومجموعة المخالطين مخالطةً لصيقة عن قرب (ضمن الأسرة). وقد بلغت نسبة من جرى تشخيص إصابتهم بعدوى السل الخافية باختبار التوبركولين الجلدي 11.5% من مجموعة الشواهد، و71.1% من المخالطين مخالطةً عارضةً، و29.6% من المخالطين مخالطةً لصيقة. وبلغت نسبة من جرى تشخيص إصابتهم بعدوى السل الخافية عن طريق مقايضة انطلاق الغاما-إنترفيرون (QuantIFERON®-TB Gold In-Tube) 5.9% بين الشواهد، و31.0% بين المخالطين مخالطةً عارضةً، و33.3% بين المخالطين مخالطةً لصيقة. وكان مجمل التوافق بين اختبار التوبركولين الجلدي وقياس إطلاق الغاما-إنترفيرون 66.7% ( $\kappa=0.28$ ). مما يدل على أن مقايضة انطلاق الغاما-إنترفيرون قد تكون أكثر فائدة من اختبار التوبركولين الجلدي في الكشف عن العدوى الخافية للسل بين المخالطين.

ABSTRACT The tuberculin skin test (TST) has many limitations for the diagnosis of latent tuberculosis (TB) infection. The aim of this study in Egypt was to estimate the usefulness of an interferon-gamma release assay (IFN- $\gamma$ ) assay for the detection of latent TB infection in contacts of active TB cases. A total of 116 participants were enrolled and divided into 3 groups: community controls, casual (laboratory and clinic) contacts and close (household) contacts. Subjects diagnosed with latent TB infection by TST were 11.5% of controls, 71.1% of casual contacts and 29.6% of close contacts. Subjects diagnosed as latent TB infection by IFN- $\gamma$  assay (QuantIFERON®-TB Gold In-Tube) were 5.9% of controls, 31.0% of casual contacts and 33.3% of close contacts. The overall agreement between TST and IFN- $\gamma$  was 66.7% ( $\kappa = 0.28$ ). The IFN- $\gamma$  method could be more helpful than TST for detection of latent TB infection in contacts.

## Test de quantification de la libération d'interféron gamma pour le dépistage des infections tuberculeuses latentes chez les contacts occasionnels et rapprochés des patients atteints de tuberculose

RÉSUMÉ Le test tuberculique cutané comporte de nombreuses limitations pour le diagnostic d'une infection tuberculeuse latente. L'objectif de la présente étude conduite en Égypte était d'évaluer l'utilité d'un test de quantification de la libération d'interféron gamma (IFN- $\gamma$ ) pour le dépistage des infections tuberculeuses latentes chez les contacts de patients atteints de tuberculose active. Au total, 116 participants ont été recrutés et répartis en trois groupes : les témoins communautaires, les contacts occasionnels (laboratoires et établissements de soins) et les contacts rapprochés (vivant sous le même toit). Les sujets ayant reçu le diagnostic d'infection tuberculeuse latente aux moyens du test tuberculique cutané représentaient 11,5 % du groupe témoin, 71,1 % du groupe des contacts occasionnels et 29,6 % du groupe des contacts rapprochés. Les sujets ayant reçu le diagnostic d'infection tuberculeuse latente aux moyens du test IFN- $\gamma$  (QuantIFERON®-TB Gold In-Tube®) représentaient 5,9 % du groupe témoin, 31,0 % du groupe des contacts occasionnels et 33,3 % du groupe des contacts rapprochés. La concordance globale entre les deux tests s'élevait à 66,7 % ( $\kappa = 0,28$ ). La méthode du test IFN- $\gamma$  pourrait être plus utile que le test tuberculique cutané pour le dépistage des infections tuberculeuses latentes chez les sujets contacts.

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## Introduction

Globally, about 2 billion people are infected with *Mycobacterium tuberculosis*; 8 to 10 million develop active disease and 2 million die from tuberculosis (TB) every year [1]. In Egypt, the incidence of TB is estimated at 21 per 100 000 population per year [2]. One of the first priorities of TB control programmes is the identification of people with latent TB infection [3] in order to prevent development of active disease [4]. Accurate diagnosis of latent TB infection is scientifically challenging, however, because of the low burden of dormant tubercle bacilli, which are not directly detectable or quantifiable. Besides, latent TB infection is asymptomatic, with patients having no clinical or radiographic signs of active TB yet carrying a small but significant risk of progression to active TB [5].

Until recently, the tuberculin skin test (TST), which uses purified-protein derivative (PPD), was the only method available for the diagnosis of latent TB infection [5]. The TST has many limitations, including the need for at least 2 patient visits, problems in administration, problems in interpretation and difficulty in separating true infection from the effects of prior bacille Calmette-Guérin (BCG) vaccination and infection by non-tuberculous *Mycobacteria* spp. [6]. Despite these drawbacks, TST remains in widespread use because of its advantages, being inexpensive, simple and practical in many settings [7].

Advances in genomics and immunology have led to a promising alternative to TST: *in vitro* interferon-gamma (IFN- $\gamma$ ) release assays. These tests are based on the principle that T-cells of individuals infected with *M. tuberculosis* bacilli release IFN- $\gamma$  when they re-encounter TB-specific antigens [8]. The QuantiFERON®-TB assay is of these tests. The latest generation of these tests—the QuantiFERON-TB® Gold In-Tube method (QFT Gold-IT)—has increased specificity for detection

of *M. tuberculosis* infection due to the use of 3 specific antigens. It requires a single patient visit, the interpretation is less subjective than the TST and the test is not affected by prior BCG vaccination or infection by most non-TB *Mycobacteria* [9].

The aim of this study was to estimate the usefulness of an IFN- $\gamma$  assay for the detection of latent TB infection in contacts of active TB cases.

## Methods

This was a comparative cross-sectional study conducted in the Department of Medical Microbiology and Immunology, Faculty of Medicine, University of Zagazig, Egypt. The study was conducted over 7 months from August 2008 to February 2009.

### Participants

Clinically suspected TB patients were selected by systemic random sampling from those attending outpatient clinics in Zagazig Chest Hospital and Chest Department in Zagazig University Hospitals during the study period. All those with bacteriological confirmation of the diagnosis were used as the index cases. Three groups of people were selected for the study according to their degree of exposure to the index cases (on the assumption that contact either directly with TB patients or with specimens is a risk factor [10,11]).

- Close contacts: healthy individuals with a history of close contact with a TB patient for more than 8 hours per day, i.e. household contacts. We selected 2 contacts by systemic random sampling from all household contacts of confirmed TB cases.
- Casual contacts: health care workers with a history of casual contact with TB cases or specimens. They were recruited from among medical staff members and laboratory technicians who were on duty during

the study period. Employees with a history of TB were excluded.

- Community controls: healthy individuals without an identified risk for *M. tuberculosis* exposure. These were recruited from employees in other departments of the Faculty of Medicine.

By using data from the records, people were excluded if they had symptoms suggestive of active TB, showed abnormal chest radiographs or had history of active TB. All participants were selected by simple random sampling.

The sample size was determined assuming a prevalence of TB infection in Egypt of 27 per 100 000 per year (based on World Health Organization data [2]) and non-response rate of 20% (based on previous studies in the same locality and the same population [12]) and using  $\kappa$  coefficient > 0.75;  $\alpha$ -error = 0.05 and  $\beta$ -error = 0.20. Using these parameters and assumptions, and *Epi-info*, version 9 software, the minimum sample size for each group was estimated to be 25.

### Data collection

After giving their informed written consent, participants were interviewed and asked about their age, sex, history of prior TB disease and history of exposure to TB cases.

All groups were subjected to TST and INF- $\gamma$  assay as follows. TST was performed by using 0.1 mL of 5 tuberculin units PPD (Vacsera), and interpretation of the results was done according to American Thoracic Society guidelines [6]. For the INF- $\gamma$  assay a blood sample was collected into 3 evacuated tubes, the first tube coated with TB-specific antigens (ESAT-6, CFP10 and TB7.7), the second tube coated with heparin-negative control and the third tube coated with mitogen (phytohaemagglutinin) as positive control. Blood was incubated overnight at 37 °C. Plasma was then separated to measure the concentration of IFN- $\gamma$  by enzyme-linked immunosorbent assay using the QFT-

Gold IT assay (Cellestis) following the manufacturer's instructions. The cut-off value for positive findings was  $\geq 0.35$  IU/mL [13].

Any cases of latent TB diagnosed were referred to the corresponding clinician for further management.

### Statistical analysis

Concordance between the test results from the TST and the QFT-Gold IT assay was assessed using kappa coefficients ( $\kappa = 0.75$  excellent agreement;  $\kappa = 0.40$  poor agreement; and  $\kappa = 0.40-0.75$  fair to good agreement). We measured the correlation of the 2 tests with the risk of latent TB infection by estimating the odds ratio (OR) and 95% confidence intervals (CI) relating the test results to the likelihood of TB infection. Data were analysed using SPSS, version 11 software package.

### Results

A total of 116 participants were included in this study: 28 household contacts of TB cases (12 males and 16 females); 48 health care workers (16 males and 32 females) comprising 30 medical staff members and 18 laboratory technicians; and 40 controls (13 males and 27 females). The median and range of ages of the groups are shown in Table 1.

A documented TST result was obtained in 91 (78.5%) of the 116 participants; 8 did not attend the follow-up reading of the test, 5 refused to perform it fearing pain and 12 refused to be

injected by foreign material. There were no significant differences in the personal and medical characteristics of the refusers and active participants. Of the 91 participants tested, 38 (41.8%) were positive for TST. In the close contacts group there were 8/27 (29.6%) positive cases (4 males and 4 females, age range 15–60 years). In the casual contacts group there were 27/38 (71.1%) positive cases (11 males and 16 females, age range 2–55 years). In the control group there were 3/26 (11.5%) positive cases (1 male and 2 females, age range 28–70 years) (Table 2).

A valid QFT-Gold IT assay result was obtained in 100 of the 116 participants; 16 readings were indeterminate and were excluded from further analysis. Among the 100 valid results, there were 23 (23.0%) positive cases. In the close contacts group there were 8/24 (33.3%) positive cases (4 males and 4 females, age range 2–55 years) and in the casual contacts group there were 13/42 (31.0%) positive cases (4 males and 9 females, age range 20–55 years). In the control group there were 2/34 (5.9%) positive cases (1 male and 1 female, age range 33–70 years) (Table 2).

There were 75 subjects with valid (i.e. determinate) results for both TST and QFT-Gold IT. They were distributed as follows: 20 controls, 32 casual contacts and 23 close contacts. The concordance between both tests within different subgroups is shown in Table 3. Using the TST, the risk of infection

appeared to be greatly increased in the casual contacts group (OR = 18.8, 95% CI: 4.1–99.5,  $P < 0.001$ ) compared with the close contacts group (OR = 3.2, 95% CI: 0.6–8.1,  $P = 0.1$ ). With the QFT-Gold IT assay, the increased the risk of infection with exposure was similar in the casual contacts group (OR = 7.2, 95% CI: 1.4–50.5,  $P = 0.006$ ) and close contacts group (OR = 8.0, 95% CI: 1.3–62.4,  $P = 0.01$ ) (Table 2).

### Discussion

In high-burden countries, the diagnosis and treatment of active TB receives greater priority; testing for latent TB infection is usually done only in selected high-risk groups. Traditionally, latent TB infection has been diagnosed with a TST. However, the TST has limitations. The antigens present in PPD and used for the TST are also present in the BCG vaccine strain and therefore false positive tests are common. This has led to the development of novel tests of latent TB infection that use *M. tuberculosis*-specific antigens [14]. So in the present study we aimed to estimate the usefulness of an IFN- $\gamma$  assay for the rapid detection of mycobacterial TB infection in individuals in contact with TB cases or specimens.

In the control group, participants diagnosed as latent TB infection were 11.5% by TST and 5.9% by IFN- $\gamma$ . Our findings agree with those of Porsa et al. in the United States, who found latent

**Table 1 Demographic characteristics of the 3 groups of participants**

Variable	Community controls (n = 40)	Casual contacts (n = 48)	Close contacts (n = 28)	P-value
<b>Age (years)</b>				
Mean (SD)	41.9 (16.2)	36.4 (9.2)	22.5 (16.7)	
Median (range)	33.5 (25–76)	36.0 (18–55)	19.5 (2–55)	< 0.001
<b>Sex [no. (%)]</b>				
Male	13 (32.5)	16 (33.3)	12 (42.9)	> 0.5
Female	27 (67.5)	32 (66.7)	16 (57.1)	

SD = standard deviation.

Table 2 Comparison of tuberculin skin test (TST) and QuantiFERON-TB® Gold In-Tube (QFT-Gold IT) assay results

Group	TST				QFT-Gold IT assay				
	Tested <sup>a</sup> No.	+ve No.	%	OR (95% CI)	Tested <sup>a</sup> No.	+ve No.	%	OR (95% CI)	P-value for trend
Community controls	26	3	11.5	1.0	34	2	5.9	1.0	-
Casual contacts	38	27	71.1	18.8 (4.1-99.5)	42	13	31.0	7.2 (1.4-50.5)	0.006
Close contacts	27	8	29.6	3.2 (0.6-8.1)	24	8	33.3	8.0 (1.3-62.4)	0.01

<sup>a</sup>Number of participants with valid results for the corresponding test.  
OR = odds ratio; CI = confidence interval.

TB in 9.0% and 5.4% of subjects by TST and IFN- $\gamma$  respectively [15].

In our casual contacts group, 71.1% of subjects were diagnosed as latent TB infection by TST and 31.0% by IFN- $\gamma$  assay. This discrepancy might be explained by a high rate of false positives for the TST. Health care workers in our faculty receive repeated BCG vaccination and also repeat TSTs during their employment. These 2 factors may further boost their reaction to the TST and thus lead to a false positive TST result [16]. This hypothesis is supported by the results of many previous studies [17].

In our close contacts group, 29.6% of subjects were diagnosed as latent TB infection by TST and 33.3% by IFN- $\gamma$  assay. This disagrees with the Korean study of Kang et al., in which 71% of subjects were diagnosed as latent TB infection by TST and 44% by IFN- $\gamma$  assay. This difference might also be explained by a higher rate of false positive results in their study for TST than in ours due to different vaccination schedules. In Korea, BCG vaccination is given at birth and again at age 12 or 13 years if the child proves to be a TST non-responder [18]. In Egypt, on the other hand, BCG vaccination is given in infancy, which is unlikely to affect TST reactions in adolescence or adult life [19].

Our study showed a poor correlation between TST and IFN- $\gamma$  among the investigated groups. The overall agreement was 66.7% ( $\kappa = 0.28$ ), which is similar to the findings of Mahomed et al. in South Africa, who found agreement of 69% ( $\kappa = 0.32$ ) [9].

As there is no gold standard for the diagnosis of latent TB infection the measurement of correlation with the risk of infection could be a more sensitive method to compare the utility of the TST and IFN- $\gamma$  than a detailed determination of the specificity and sensitivity of tests. In our study, the TST results showed that TB exposure increased the risk of infection 18.8-fold in the casual contacts group and 3.2-fold in the close contacts group. The IFN- $\gamma$  results showed that exposure increased the risk of infection 7.2-fold in the casual contacts group and 8-fold in the close contacts group and this was in agreement of the findings of Kang et al. [18].

Although our study showed better results with the IFN- $\gamma$  assay, we should consider the relatively higher costs, practical inconvenience and the presence of indeterminate test results. By conducting a preliminary estimation of the cost of QFT-Gold IT to process a sample from 1 patient, the cost would be between 250 and 300 EGP (Egyptian pounds) while the cost of the TST is as low as 15 and 20 EGP (US\$ 1 = 6 EGP). In addition, for the QFT-Gold IT, at least 22 patients should be analysed per run so as not to waste wells and therefore the minimum sample costs will only be achieved when all wells are used.

In conclusion, the IFN- $\gamma$  assay showed better performance than the TST in subjects with latent TB infection and it correlated better with exposure to *M. tuberculosis*. An IFN- $\gamma$  assay might help to avoid the limitations of

Table 3 Concordance between tuberculin skin test and QuantiFERON-TB® Gold In-Tube assay results

Group	Observed agreement	Coefficient $\kappa$ -value (SE)	P-value
	%		
Community controls	95.0	0.77 (0.21)	< 0.001
Casual contacts	40.6	0.02 (0.12)	0.56
Close contacts	78.2	0.50 (0.20)	0.007
All	66.7	0.28 (0.10)	0.004

SE = standard error.



the TST in BCG-vaccinated populations as it uses antigens more specific to *M. tuberculosis* than the PPD used in the TST, and prior BCG vaccination does not affect the results. We recommend that the QFT-Gold IT could be more helpful than the TST for the detection of latent TB infection

despite the risk of underestimation of cases.

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