Current Research in
Tuberculosis

Academic Journals Inc.

Evaluating the Indeterminate Results of the QuantiFERON-TB Gold in-Tube Test

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ABSTRACT

QuantiFERON-TB Gold In-Tube Test is an *in vitro* laboratory diagnostic test which is used as an aid in diagnosing *Mycobacterium tuberculosis* infection or disease. Sometimes it is associated with indeterminate results which make the diagnosis difficult for the patients. Here we sought to understand the possible reasons for indeterminate results obtained in QuantiFERON-TB Gold In-Tube Tests conducted in a small setting at Super Religare Laboratories, Gurgaon. In present study, we found that most of the indeterminate results obtained in QuantiFERON-TB Gold In-Tube Tests were due to possible immunosuppression in the patients and not due to any laboratory technical error. The reasons for immunosuppression should be investigated in the patients before concluding the indeterminate results.

Key words: *Mycobacterium tuberculosis*, latent and active tuberculosis, QuantiFERON-TB gold, indeterminate results, immunosuppression

INTRODUCTION

The QuantiFERON-TB Gold In-Tube Test was implemented as an aid for diagnosing tuberculosis infection or disease. The indeterminate results are uncommon but their importance cannot be neglected during diagnosis of tuberculosis infection. The use of mitogen tube as a positive control was introduced to assess the underlying cause of indeterminate results i.e., whether the indeterminate results were due to the immunosuppression or the results of some possible technical errors.

In 2005, the QuantiFERON-TB Gold (QFT-G) and QuantiFERON-TB Gold In-Tube Test (QFT-G IT) tests (Cellestes) were approved by US Food and Drug Administration for the diagnosis of latent tuberculosis infection (LTBI). According to CDC guidelines, the QFT-G test can be used in lieu of the Tuberculin Skin Test (TST) for tuberculosis screening and control programs (Aabye *et al.*, 2009; Jensen *et al.*, 2005). QuantiFERON-TB Gold In-Tube Test detects interferon-γ (IFN-γ) to peptide antigens simulating *Mycobacterium tuberculosis* antigens (ESAT-6, CFP-10 and TB7.7). These antigens are absent from BCG strains and in most strains of non-tuberculous mycobacteria (except *M. kansasii, M. marinum, M. szulgai*). The specificity of the test for the low risk group people is around 98% and the sensitivity for patients with *Mycobacterium tuberculosis* infection is 89% (Aabye *et al.*, 2009; Kariminia *et al.*, 2009). The sensitivity is higher in HIV negative than in HIV positive patients and the test performance is impaired by low CD4 cell count

(Kobashi *et al.*, 2009). However, the QFT-GIT assay may be a sensitive tool for the detection and prediction of active tuberculosis in HIV-1-infected individuals. Besides high specificity, other advantages of QFT-G are logistical convenience, avoidance of poorly reproducible measurements such as skin induration, need for fewer patient visits and the ability to perform serial testing without inducing the boosting phenomenon (Lalvani, 2007). Sex, younger age, transitional changes during four year seasons, microbiological findings and radiological findings including extension of lesions do not affect the indeterminate results (Mazurek *et al.*, 2005). Current screening methods for diagnosing LTBI rely solely on TST. However, the TST is limited by lack of specificity in BCG vaccinated population, the need for a return visit to interpret the test result and the subjectivity involved in interpreting the results (Mori *et al.*, 2004; Tissot *et al.*, 2005). For low tuberculosis risk, health care workers etc. the rate of indeterminate results in QFT-G assay is very low (0-0.3%). In renal dialysis patients who are commonly suffering from immunosuppression valid results are obtained in more than 93% of cases. Indeterminate results are uncommon in patients undergoing therapy with corticosteroids and other anti-rheumatic drugs. Patients undergoing treatment with anti-TNFα therapies are more likely to be QFT-G indeterminate. However, the test works well in these patients prior to use of anti-TNF α therapy when a test for tuberculosis is most needed.

MATERIALS AND METHODS

The phlebotomists at the collection centre of Super Religare Laboratories and its clients draw blood from voluntary test seekers for QFT-G assay and place it directly into three 1 mL tubes each containing either ESAT-6, CFP-10 and TB7.7 or PHA (a mitogen used as a control with a positive assay result) or saline (used as a nil sample to measure the background level of IFN-γ). Samples are mixed vigorously by hand after collection and delivered to the laboratory within 16 h of collection by a courier where, they are accessioned and incubated at 37°C for 16-18 h. After overnight incubation, the samples are processed according to recommendations of manufacturer (Cellestes Ltd. Carnegie, Australia). The viability of lymphocytes in the blood sample was determined using vital dye trypan blue (0.4%). The dead cells take up the blue stain while the living cells appear as white. The concentration of living and dead cells was determined using Neuber haemocytometer. Lymphocyte count was performed using Leishman stain. The viable cell density and the total leukocyte count in the original sample were calculated using the formula:

$$\%\text{Viability} = \frac{\text{Total No. of viable cells}}{\text{Total No. of viable and non-viable cells}} \times 100$$

$$\text{Viable cells mL}^{-1} = \frac{\text{Total No. of viable cells counted in 4 squares}}{4} \times 10000$$

$$*\text{TLC} = \text{Total No. of cells counted in four squares} \times 50$$

$$\left(\text{i.e.,} \frac{\text{Total No. of cells counted in four squares} \times \text{dilution}}{\text{Area} \times \text{depth}} = \frac{\text{N} \times 20}{4 \times 0.1} \right)$$

$$*\text{Absolute lymphocyte count} = \frac{\text{TLC} \times \%\text{lymphocyte}}{10}$$

RESULTS

We screened a total of 546 whole blood samples using QFT-G In-Tube tests for diagnosing tuberculosis during five months period (i.e., from October 2008 to February 2009). There were 252 (46.15%) negative, 219 (40.10%) positive and 75 (13.73%) indeterminate test results. Of the 75 indeterminate test results, 73 (97.33%) were due to low mitogen (i.e., Phytohaemagglutinin) response, with IFN-γ less than 0.5 IU mL^{-1}. Two samples with indeterminate test results were due to high nil value (above 10 IU mL^{-1}). A separate study on 10 specimens was done to gain an insight into the possible reason for the low mitogen response observed in the indeterminate results. A total leukocyte count was performed in another set of 10 patients from samples received (TLC) and the average TLC was found to be 7555 μL^{-1}.

DISCUSSION

An indeterminate test result in QFT-G In-Tube test indicates that the real disease status of the person could not be ascertained. An indeterminate result is meaningful and it does not indicate a failed test. The indeterminate test results may be due to possible immunosuppression or due to some technical errors and is indicated by low mitogen response. The advanced age and underlying disease in patients receiving immunosuppressive treatment comprise the risk factors that can promote indeterminate results on QFT-G test. Lymphocytopenia including CD4 lymphocytopenia showing an immunosuppressive state or hypoproteinemia showing a poor nutritional state could significantly influence the risk of indeterminate results. Immunosuppressive drugs such as corticosteroid drugs or tumor necrotic factor alpha (TNF-α) inhibitor directly reduce the production of inflammatory cytokines, such as IFN-γ, interleukin-1 and TNF-α from T-lymphocytes. Other causes of indeterminate test results include improper specimen transport (delayed transport can affect lymphocyte viability) and/or improper specimen handling or storage, as well as other technical factors (e.g., incomplete washing of ELISA plate).

In our laboratory, there was unexpectedly high rate (13.73%) of indeterminate test results in the QFT-G assay. This prompted a review of the QFT-G In-Tube testing process. The optimal follow-up of persons with the indeterminate results has not been determined. A follow up of the specimen transport and specimen processing was done. We found that the specimens were transported within the specified time interval and also a proper procedure was followed during the specimen storage and processing. The viability of the lymphocytes and the cell counts were determined. The average viability of cells on receiving the samples was 96.7% (n = 10). The blood samples were stored at 37°C, RT and 2-8°C and were monitored on three successive days. After 24 h, the average %viability of cells stored at 37°C and RT was nearly similar and only 2-3.3% decline in cell viability, from day zero, was observed. There was 5% decline in the cell viability of the samples stored at 2-8°C. After 48 h, there was 12.9, 13.9 and 21.6% decrease in cell viability for the samples stored at 37°C, RT and 2-8°C, respectively. After 72 h, 39.7, 40.9 and 49.2%, respectively, decline in cell viability was observed for samples stored at 37°C, RT and 2-8°C. In present findings, the viability of cells was appropriate to perform a valid QFT-G In-Tube test and was not associated with the indeterminate results obtained during our tests. The cells were most viable at 37°C and the viability at room temperature was nearly similar to that at 37°C while, the viability at 2-8°C was least. The lymphocyte count (n = 10) for the samples was within range (20-40%) and the average lymphocyte count was 26.6%.The mean, total leukocyte count, was found to be within range (4000-11000 μL^{-1}) i.e.,7555 μL^{-1}. These findings suggest that the indeterminate

results during our tests were due to a reduced T-lymphocyte activity or the inability of T-lymphocytes to produce IFN-γ which may be associated with functional immunosuppression and not due to any technical error.

Based on present results, we suggest that in the northern regions of India, most of the indeterminate results are due to immunosuppression and not due to any technical error. This may require detailed investigation to establish the reason for functional immunosuppression.

ACKNOWLEDGMENT

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