Evaluation of QuantiFERON -TB Gold in Diagnosis of Tuberculous Pericardial Effusion

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ABSTRACT

Background: Prompt treatment of tuberculous pericarditis can save lives, but definite diagnosis requires detection and isolation of the tubercle bacilli from pericardial fluid and/or biopsy which is often delayed and difficult. Objectives: To evaluate the diagnostic role of QuantiFERON -TB Gold test (QFT-G) as a rapid non invasive immunological assay in diagnosis of tuberculous pericarditis with pericardial effusion in comparison to Adenosine deaminase enzyme (ADA) activity and Polymerase chain reaction (PCR) either individually or in combination. Subjects and methods: 27 patients suffering of pericarditis accompanied with pericardial effusions highly suspicious to be tuberculous (clinically and radiographically) were subjected to pericardial fluid aspiration and biopsy, Ziehl-Neelsen stain, culture and histopathological examination(biopsy) for tuberculosis were done for each sample. Pericardial fluid was submitted to Polymerase chain reaction and measurement of adenosine deaminase activity and finally QuantiFERON -TB Gold test in blood was done. Results: Out of 27 probable tuberculous pericarditis patients with pericardial effusion, 19 were definite positive cases. Considering the value of 40 U/L ADA activity, 16/19 cases were positive so the sensitivity was 84.2% which was the same as the results of QuantiFERON -TB Gold test while there were 10 PCR positive cases, so PCR sensitivity was 52.6%. There were 2 positive ADA and one QuantiFERON -TB Gold positive results among the 8 culture negative cases, while none was detected by PCR, so its specificity was 100% while (QFT-G) specificity was 87.5% and ADA 75%. The sensitivity, specificity, PPV and NPV of combined QFT-G and PCR results were 89.5%, 87.5%, 94.4% and 77.8% respectively. While the sensitivity, specificity, PPV and NPV combined QFT-G with ADA results were 84.2%, 75%, 88.9% and 66.7% respectively. Conclusion: QuantiFERON -TB Gold test have good sensitivity and specificity for diagnosis of tuberculous pericardial effusion. The best combined sensitivity and specificity in the current study were reported between PCR and QuantiFERON test results but it is not much greater than that of QuantiFERON test alone. So, QuantiFERON -TB Gold test can be used as an adjunct rapid immunological non invasive test for diagnosis of tuberculous pericardial effusion. However negative QuantiFERON -TB Gold assay does not exclude the disease because of the low NPV of this assay.

INTRODUCTION

Tuberculosis (TB) is a major health problem in South Africa, with an annual incidence rate of 350 per 100,000 population(1). In Egypt, tuberculosis is the second most important health problem after Bilharziasis(2). Approximately 1 to 2% of these cases are complicated by tuberculous pericarditis (TP)(3). Tubercle bacilli reach the pericardium from the adjacent tracheobronchial lymph nodes either directly or via lymphatic channels. Less commonly seedling of the pericardium occurs with miliary tuberculosis and rarely mycobacteria spread directly from pleura or adjacent rib(4). Tuberculous pericarditis is a rare cause of pericardial effusion in developed countries, while it is the cause of up to 70% of cases in developing countries(5). Tuberculous pericardial effusion usually presents as a slowly progressive febrile illness. When it presents as an acute pericarditis, which is uncommon, or as cardiac tamponade(6), which is frequent, the diagnosis is more likely to be delayed or missed. The delay from hospital admission to diagnosis was 5.2 weeks in a report from Spain(7) and in another from the USA the diagnosis was first made only at necropsy in 17% of patients(8). The probability of obtaining a definitive diagnosis is greatest when pericardial fluid and a pericardial biopsy specimen are examined early in the effusive stage(7,8). Considering the mortality from tuberculous pericarditis (17% to 40%)(9) and survival time without treatment is approximately four months even when the patients receive treatment, the mortality rate ranges from 3 up to 40% (10), rapid and accurate diagnosis (which is often difficult) and treatment are crucial to reduce mortality and morbidity from this pericardial disease(11,12). Ziehl-Neelsen (ZN) stained smears of pericardial fluid have poor sensitivity for
detecting *Mycobacterium tuberculosis*, while culture is both slow and insensitive\(^{13-15}\). Pericardial biopsy is invasive, requires technical skills and is often not diagnostic\(^{7,13,16}\). Clinicians thus have to rely heavily on the clinical features of pericardial tuberculosis to initiate therapy\(^{17-19}\).

Due to this difficulty of establishing the diagnosis of tuberculous pericarditis using clinical, radiological, cytological or even microbiologic evaluation, attempts to correlate tuberculous pericarditis diagnosis with other markers have been pursued\(^{20}\). Adenosine deaminase enzyme (ADA) activity in tuberculous pericarditis\(^{21,22}\) and Polymerase chain reaction (PCR) for diagnosis of TB in pericardial fluid and tissue were documented\(^{12,23}\).

The recently developed interferon-γ (IFN-γ) release assay (IGRA) measures TB-specific T cells responding to *Mycobacterium tuberculosis* derived antigens, including early secreted antigenic target 6 (ESAT-6) and culture filtrate protein 10 (CFP-10)\(^{24}\). Among these tests, the QuantiFERON -TB Gold (QFT-G) is approved and recommended for diagnosing latent and active TB\(^{25}\). This study was done to evaluate the diagnostic role of QuantiFERON- TB Gold test as a rapid non invasive immunological assay for diagnosis of tuberculous pericarditis with pericardial effusion in comparison to ADA activity and PCR either individually or in combination.

**SUBJECTS & METHODS**

Twenty-seven patients suffering of pericarditis accompanied with pericardial effusions clinically and radiographically (ages, 23 to 62 years; 17 males and 10 females) were included in this study. They were admitted to Zagazig University hospitals from April 2008 to January 2010. Informed written consent was obtained from the patients. Pericardial fluid and biopsy specimens were obtained from patients by pericardiocentesis subxiphoid pericardiostomy as described by Cegielski et al\(^{17}\). For definite diagnosis\(^{12,23}\), each sample was submitted for:

1. Ziehl-Neelsen staining .
2. culture and identification of *Mycobacterium tuberculosis* using Bactec 460TB(Becton Dickinson).
3. Histopathological examination of the pericardial biopsy.

Pericardial fluid was further submitted to measurement of ADA activity, PCR and finally QuantiFERON- TB Gold test was done in blood.

**1. ADA activity** (U/L) was determined in every pericardial fluid specimen according to the method described by GIUSTI\(^{26}\). The cut-off value for ADA activity that considered adequate to presume the diagnosis of tuberculous pericardial effusion was 40 U/L\(^{22,27}\).

**2. Polymerase Chain Reaction** using COBAS AMPLICOR (Roche Diagnostics) for every sample. The test was done in four steps: specimen preparation; Polymerase Chain Reaction nucleic acid amplification of target DNA using biotinylated primers; hybridization of amplified products to oligonucleotide probes specific to the target and detection of the probe-bound amplified products by color formation.


- Five ml blood was collected from each subject in heparinized tube, incubation with stimulation antigens as soon as possible (within 12 hours) after collection must be done.
- One ml aliquots of heparinized whole blood from each sample was dispensed into 4 wells of 24 well tissue culture plate.
- The TB-specific antigens, nil and mitogen controls were thoroughly mixed, then the dropper bottle was hold vertically and added 3 drops of ESAT-6 TB specific antigen, CFP-10 TB specific antigen, mitogen control and nil control to the appropriate wells containing blood and the plates were incubated for 16-24h at 37°C.
- 200-300 µl of plasma was removed carefully from above the sedimented red cells using a variable micropipette and was transferred into separate tubes. Plasma tubes can be stored for up to 3 months at -20 C.
- Fifty ul of freshly prepared conjugate were pipetted to microwells of ELISA plate then fifty µl of plasma samples were pipetted to appropriate wells containing conjugate and fifty µl of each of the standards were pipetted to appropriate wells containing conjugate.
- Microwell strips were covered and incubated for 2 hours at room temperature (18-25°C) on a microplate shaker then microwell strips were
was washed at least 4 times with wash buffer.
- One hundred µl of enzyme substrate solution was pipetted to all wells and mixed well using a microplate shaker then the microwell strips were covered and incubated at room temperature for about 30 minutes on a microplate shaker set at 100 rpm.
- Fifty µl enzyme stopping solution was pipetted to all wells.
- Color intensity was measured at 450 nm and 620 nm as a reference wave length within 5 minutes.
- The IFN-γ concentration (IU/ml) for each of the test plasma samples were determined by using the standard curve to read off the IFN-γ concentration. The manufacturer's cutoff for a positive test, indicating likely M. tuberculosis infection, is ≥0.35 IU/ml of IFN-γ for the TB-specific antigen stimulated plasma sample above the amount of IFN-γ in the negative control sample.

Statistical analysis
Analysis of the collected data was done using SPSS version 10.0. Data were expressed as number and percentage for quantitative and qualitative variables. The usefulness of determining ADA activity, PCR and QuantiFERON -TB Gold test as a diagnostic tool for tuberculous pericarditis with effusion was evaluated by calculating Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV).

RESULTS
Among Twenty-seven patients with pericarditis and pericardial effusions. Only 19 were confirmed to have definite tuberculous pericarditis. Among these patients there were 16 positive cases by measuring (40 U/L) ADA activity and also, 16 cases showed positive QuantiFERON -TB Gold results, so the sensitivity for both tests was 84.2%. While there were 10 PCR positive cases, PCR sensitivity was of 52.6%. There were 2 positive ADA and one QuantiFERON -TB Gold positive results among the 8 culture negative cases, while no positive case was detected by PCR. Its specificity was 100% while (QFT-G) specificity was 87.5% and ADA 75% (table 1). The sensitivity, specificity, PPV and NPV of ADA and PCR tests in combination with QFT-G were assessed. QFT-G test results when combined with PCR results, the sensitivity, specificity, PPV and NPV were 89.5%, 87.5%, 94.4% and 77.8% respectively (table 2). When QFT-G results was combined to ADA results the sensitivity, specificity, PPV and NPV were 84.2%, 75%, 88.9% and 66.7% respectively (table 3).

Table (1): Diagnostic performance of different methods individually in diagnosis of tuberculous pericarditis.

<table>
<thead>
<tr>
<th>Presumed cases=27</th>
<th>ADA activity</th>
<th>PCR</th>
<th>QFT-G</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Definite +ve (no:19)</td>
<td>Definite -ve (no:8)</td>
<td>sensitivity</td>
</tr>
<tr>
<td>ADA activity</td>
<td>+ve</td>
<td>16</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>-ve</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>PCR</td>
<td>+ve</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>-ve</td>
<td>9</td>
<td>8</td>
</tr>
<tr>
<td>QFT-G</td>
<td>+ve</td>
<td>16</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>-ve</td>
<td>3</td>
<td>7</td>
</tr>
</tbody>
</table>

Table (2): Diagnostic performance of PCR in combination with QFT-G for diagnosis of tuberculous pericarditis.

<table>
<thead>
<tr>
<th>Presumed cases=27</th>
<th>QFT-G+ PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Definite +ve (no:19)</td>
</tr>
<tr>
<td>QFT-G+ PCR</td>
<td>+ve</td>
</tr>
<tr>
<td></td>
<td>-ve</td>
</tr>
</tbody>
</table>
Table (3): Diagnostic performance of ADA in combination with QFT-G for diagnosis of tuberculous pericarditis.

<table>
<thead>
<tr>
<th>Presumed cases=27</th>
<th>Definite +ve (no:19)</th>
<th>Definite -ve (no:8)</th>
<th>sensitivity</th>
<th>specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>QFT-G+ADA</td>
<td>+ve</td>
<td>16</td>
<td>2</td>
<td>84.2%</td>
<td>75%</td>
<td>88.9%</td>
</tr>
<tr>
<td></td>
<td>-ve</td>
<td>3</td>
<td>6</td>
<td></td>
<td></td>
<td>66.7%</td>
</tr>
</tbody>
</table>

DISCUSSION

Effective treatment of tuberculous pericarditis requires a rapid and accurate diagnosis, but this is often difficult(13). Acid fast bacilli stains of pericardial effusion sediment are usually negative and culture sensitivity is not greater than 50%(12). The diagnostic sensitivity for TB by pericardial biopsy ranges from 10% to 64%(13,28). Due to the difficulty of establishing the diagnosis of tuberculous pericarditis using clinical, radiological, cytological or even microbiologic evaluation, attempts to correlate tuberculous pericarditis diagnosis with other markers have been pursued(20). Reuter et al. (23) mentioned that where the diagnostic infrastructure and resources are available, suspected cases of tuberculous pericarditis may be diagnosed using polymerase chain reaction (PCR) analysis, adenosine deaminase (ADA) activity and pericardial interferon gamma (IFN- γ) levels.

The analysis of ADA activity is one of the most studied possibilities and highlighted as an accurate measurement method for values greater than 40 U/L(22,27). The ADA activity sensitivity for tuberculous pericarditis detection in our study was 84.2% which was similar to that found in other studies(22,29). A meta-analysis evaluating ADA activity levels in tuberculous pericarditis revealed a mean sensitivity of 88%(30). Therefore, measuring ADA activity in pericardial effusion is, indeed, useful as a screening investigation for tuberculous pericarditis.

The same conclusion cannot be reached when analyzing the specificity value (75%) that we verified and was not helping in the exclusion of the diagnosis when patients present with normal values of ADA activity. This specificity value was in agreement with results reported in several studies(31,33).

The difficulty to establish the etiologic diagnosis of tuberculous pericarditis fosters the search of a reliable, quick and affordable diagnostic test for this illness. Several studies have demonstrated the importance of the nucleic acid amplification(21). In this study, PCR for M. tuberculosis was performed in pericardial fluid samples for all patients. Positive PCR results were obtained for ten ‘definite’ tuberculous effusions and was negative for nine of them. This resulted in sensitivity, specificity, PPV and NPV of 52.6%, 100%, 100% and 47%, respectively. Reuter et al. (23) reported that The use of PCR for the detection of M. tuberculosis provides a test with high specificity for the diagnosis of pericardial TB; however, sensitivity was low at 32%. This surprisingly poor sensitivity has also been reported in different pericardial effusion studies with specificities between 96–100%,(12,22). Explanations for the poor sensitivity of PCR in tuberculous exudates include the presence of inhibitors such as fibrin and hemoglobin, as well as low numbers of tubercle bacilli or their DNA in the specimens(31,32).

In the current study the QuantiFERON -TB Gold test results were positive in 16 cases out of 19 definite positive cases by ZN and/or culture and/or histopathological examination, while there were one QuantiFERON positive result among the definite negative cases, so its sensitivity was 84.2% and the specificity was 87.5%. These results were in agreement with that of Raven et al(33) and Nishimura et al (34) who reported that QuantiFERON test sensitivity were 80% and 85% respectively and the specificity were 87% and 91.2% respectively. Also nearby results were reported by Lee et al (35) as sensitivity of the test was 78% and specificity was 79%. But in another study of Kim et al (36) lower sensitivity and specificity (70.1% and 64.3% respectively) of QuantiFERON test were reported and attributed to the difference in radiographic extent of the disease and its location. Also immunological state and other biological factors may interfere and lead to different results(34).

In the present study, three definite positive cases were negative by QuantiFERON, these cases were diagnosed clinically as severe pericarditis with huge pericardial effusion that may be associated with weak T-cell response, which was also suggested by previous studies(33,34).
Considering the good diagnostic performance of QuantiFERON-TB Gold test among the studied tests and in order to reach maximum diagnosis of tuberculous pericardial effusion, the current study evaluated whether combination of ADA or PCR results to QuantiFERON test results could lead to better diagnostic performance than that of each test alone.

The best combined sensitivity and specificity in our study was reported between PCR and QuantiFERON results (89.5% and 87.5% respectively) but it is not much greater than that of QuantiFERON test alone, while combined sensitivity and specificity of ADA and QuantiFERON test results were (84.2% and 75% respectively) and did not add more.

Pericardiocentesis is not always feasible, and non-invasive tests that are indicative of tuberculous aetiology of pericardial effusion are thus highly desirable. Major advantage of QuantiFERON test include non-invasiveness, availability, cost-effectiveness and rapidity. However, none of the immune based tests can replace conventional tests such as ZN stain for acid fast bacilli, culture and histopathological examination for the diagnosis of tuberculous pericardial effusion especially in immune-compromised patients.

Further studies are recommended on larger group of patients to fully ascertain the role of QuantiFERON assay in diagnosis of tuberculous pericardial effusion and for longer duration of follow up especially after treatment.

In conclusion QuantiFERON -TB Gold test have good sensitivity and specificity and can be used as an adjunct rapid immunological non invasive test in diagnosis of tuberculous pericardial effusion. However negative QuantiFERON -TB Gold assay does not exclude the disease because of the low NPV of this assay.

REFERENCES

تقييم اختبار كوانتيتيفرن تي بي جولد في تشخيص ارتشاح التامور الدرني

خلفية: العلاج الفوري لالتهاب التامور الدرني يؤدي لإنقاذ أرواح العديد من المرضى، ولكن التشخيص الواضح والأكيد يتطلب الكشف عن العصيات السلبية وعزلها من السائل التاموري، أو الخزيئة التي غالباً ما تكون من الصعبة بنظامداً وأخذ الكثير من الوقت.

الأهداف: هدف البحث هو تقديم الدور التشخيصي لاختبار كوانتيتيفرن (QFT-G) وامتداد كثافة (ADA) في تشخيص ارتشاح التامور في السائل التاموري، إضافة إلى دراسة وقتك تفاعل البرمجة (PCR) وتفاعل الماء المسال في المرضى مرتبطين بالترامية (PARR).

الأشخاص والطريقة: تم اختيار هذا البحث على 27 مريضاً يعانون من ارتشاح التامور في بعد الفحص في المرضى المصابين بالالتهاب التاموري المتكرر. تم جمع السائل السليم السحي في حالة عالية أن يكون السائل السليم السحي، وعمل مزرعة للسليم، وفحص الاستماع (الخزعة) لمرضى السليم. ناس، كما تم قياس نشاط أزمير الأدينوسان نازع الأمين وعمليات فحص الدم. نازع الأمين وعمل فحص الدم المستقبل للسليم التاموري، وفي أخير إجراء اختبار كوانتيتيفرن بدي جولد في الدم.

النتائج: نجحت 27 حالة متحدة لمرضى التامور الدرني مع ارتشاح التامور، كان هناك 19 حالة إيجابية أكيدة، وعين التقييم القائمة على نشاط أزمير الأدينوسان نازع الأمين هي 80% وحدة أثر، 11/11 حالة كانت إيجابية وبالتالي فإن 84% كانت نجمة الأكسنت كونتيتيفرن تي بدي جولد في الدم، بينما نظارت 10% حالات إيجابية فقط مع تفاعل الدم المستقبل. لذلك كانت نتائج مثبتة في تشخيص أزمير الأدينوسان 75% أيضًا كانت هناك حالات فاحظة في_formula_1 (%)، وأخذ أزمير الأدينوسان نازع الأمين وحالة واحدة باستخدام اختبار كوانتيتيفرن تي بدي جولد في الدم. نازع الأمين وحالة سلبية المرجعية في حين لم يتم الكشف عن أي منها باستخدام فحص الدم المستقبل لذلك كانت نتائج 100% بينما كانت نوعية اختبار كوانتيتيفرن تي بدي جولد 87% ونوعية قياس نشاط أزمير الأدينوسان نازع الأمين 75%.

المستقبل: مستقبل التامور الدرني في السائل التشخيصي الإيجابية، ونسبة التامور البترية الناضجة لاختبار كوانتيتيفرن تي بدي جولد في الدم مع تفاعل الدم المستقبل. ونسبة التامور التاموري أكيدة في تشخيص ارتشاح التامور الدرني في الدم مع تفاعل الدم المستقبل. ونسبة التامور الدفين، ولكنها ليست أكثر من ذلك بكثير مع استخدام اختبار كوانتيتيفرن تي بدي جولد في الدم. إذن، يمكن استخدام اختبار كوانتيتيفرن تي بدي جولد كعامل مساعد سريع ومباشر غير خطي في تشخيص ارتشاح التامور الدرني. ومع ذلك فإن سلبيات اختبار كوانتيتيفرن تي بدي جولد لا تستبعد وجود المرض وذلك لأنفاضة الفاقية التامورية الناسبة لل.