

Diagnostic Value of Interferon-Gamma Assay in Tuberculosis Pericardial Effusions: Study on A Cohort of Iranian Patients

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Abstract- Tuberculosis pericarditis as a potentially fatal complication of tuberculosis requires effective diagnosis and treatment. We evaluated the efficacy of interferon-gamma (IFN-gamma) and adenosine deaminase (ADA) for diagnosing tuberculosis pericarditis in a cohort of Iranian patients presenting with pericarditis. We enrolled 38 patients with presentation of pericarditis. All patients underwent diagnostic and therapeutic pericardiostomy with drainage and biopsy. Adenosine deaminase and interferon-gamma levels were determined in pericardial fluid samples of all patients. Pericardial tissue samples were submitted for histopathologic and microbiologic studies. Polymerase chain reaction (PCR) was performed on all pericardial fluid samples to detect *Mycobacterium tuberculosis*. From 38 patients with pericarditis, 7 cases were diagnosed as having tuberculosis pericarditis (18.4%). Mean concentration of interferon-gamma in tuberculosis group was significantly higher compared to non-tuberculosis group (69257 pg/l [range: 26600-148000] vs. 329 pg/l [range: 0-2200], $P < 0.000$). Receiver operating characteristic (ROC) curve showed a value of 14400 pg/l as the cutoff point with a sensitivity of 100% and specificity of 100% for diagnosing tuberculosis pericardial effusion. Adenosine deaminase was not found to be significantly higher in tuberculosis group in comparison with non-tuberculosis causes of pericardial effusion (35.7 [range: 9-69] vs. 36.03 [range: 8-420], $P = 0.28$). In this study interferon-gamma showed to be a valuable diagnostic test for detection of tuberculosis pericarditis among a cohort of Iranian patients. We suggest using interferon-gamma to diagnose tuberculosis pericarditis to make diagnose in case of suspicion. While in this study, adenosine deaminase measurement did not prove to have the characteristics of an accurate diagnostic test for tuberculosis pericarditis.

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Introduction

Tuberculosis (TB) is an important public health problem in developing countries and the incidence of the disease has increased steadily during the past decade (1). Iran is considered an endemic area in Middle East and faces serious health problems regarding tuberculosis (2,3).

Tuberculosis pericarditis, as one of TB complications is responsible for 4% and 69.5% of cases referred for pericardiocentesis in developed countries

and Africa, respectively (4). Despite efficient therapeutic regimens, TB pericarditis could be a fatal complication and early diagnosis and treatment of tuberculosis pericarditis is of utmost importance (5). However, it is usually difficult to establish a definitive diagnosis (6). Ziehl-Neelsen (ZN) staining of the pericardial fluid smear lacks sensitivity for detecting *Mycobacterium tuberculosis* and cultures, despite being conclusive, are time consuming (6-8). Pericardial biopsy, as an alternative, is an invasive procedure that

makes it less convenient for clinicians and patients (9). These limitations, sometimes force physicians to make the diagnosis of TB pericarditis and initiate therapy solely based on its clinical characteristics (10) while missing the diagnosis in a considerable number of patients (11). This problem has raised interest in developing other methods in diagnosis tuberculosis pericarditis. Adenosine deaminase (ADA), an indicator of cellular immunity, has been studied extensively as a diagnostic tool in TB pericardial effusions and has been suggested as a part of initial diagnostic work-up (12). However, study results are not consistent regarding ADA efficacy for diagnosing TB pericarditis (5,6,9,13-15).

Measurement of interferon-gamma levels has proven useful in the diagnosis of TB pleuritis and peritonitis (16-20), as well as TB pericarditis, showing high sensitivity and specificity (5,12,21). Available literature suggests measurement of interferon-gamma levels in pericardial fluid as an efficient tool to help establishing a definitive diagnosis (5,12,21). While TB is considered endemic in Iran (22), efficacy of interferon-gamma for diagnosing TB pericarditis has not been yet studied in this country. In this study we aim to evaluate the efficacy of interferon-gamma and ADA for diagnosing tuberculosis pericarditis among a cohort of Iranian patients presenting with pericarditis.

Materials and Methods

Patients and measurement

This study was carried out in Tehran Heart Center between April 2006 and March 2007. Written informed consents for participation in the study were obtained from all patients. The study protocol was approved by the Ethics Committee of Tehran University of Medical Sciences. A total of 38 patients admitted for pericardial effusions were enrolled (inclusion criteria). Patients with a positive test result for HIV and those taking immunosuppressive drugs or corticosteroids, with a dose of > 15 mg/kg for at least 3 weeks, were excluded from the study. Demographic data were recorded and complete physical examination was done for each patient. Echocardiography, ECG and chest X-rays were obtained in all patients and the results were recorded. All patients underwent diagnostic and therapeutic pericardiostomy with drainage and biopsy. All pericardial fluid samples were studied for a complete cell count with differentiation, biochemical tests, total protein,

lactate dehydrogenase (LDH) level, carcinoembryonic antigen (CEA) level, interferon-gamma level, ADA level, cytologic examination and culture. Pericardial tissue samples were submitted for histopathologic and microbiologic studies to hospital laboratory. Polymerase chain reaction (PCR) was performed on all pericardial fluid samples to detect *Mycobacterium tuberculosis*.

Definitions and analysis

A definite diagnosis of TB pericardial effusion was defined as a positive result for culture and/or stain for acid fast bacilli, a positive TB-PCR test, or identifying typical caseating granuloma in pericardial tissue samples. Patients were then divided into two groups due to their final diagnosis; TB group and non-TB group (i.e. other etiologies). Exudative effusions were diagnosed according to Light's criteria (23).

SPSS software was used for data analysis (SPSS version 14.0, SPSS Inc., Chicago, USA). Since using Kolmogorov-Smirnov test, variables failed to show normal distribution, interferon-gamma and ADA level were compared between groups using non-parametric Mann Whitney test. Receiver operating characteristic (ROC) curves were used to determine optimal diagnostic cut-off values. A *P*-values less than 0.05 were considered statistically significant.

Results

A total of thirty eight patients presenting with pericardial effusion during a two year period were enrolled in the study (16 male, 22 female). Age ranged between 22 and 80 years with a mean of 60 (\pm 15.3). Of these, 7 cases were diagnosed as having TB pericarditis (18.4%) and 31 as having pericardial effusions due to other etiologies, including purulent (n=2), malignancy (n=2), hypothyroidism (n=4), rheumatoid arthritis (n=2), trauma (n=1), end stage renal disease (n=1), Dressler syndrome (n=1) and effusions of unknown origin (n=18). Table 1 shows the characteristics of pericardial effusion in TB and non-TB groups. Among the seven patients with the definite diagnosis of TB as the cause of pericardial effusion, five had a positive PCR test. ZN stain was positive in one patient and TB culture was positive in four patients. In four patients, typical caseating granulomas were identified in histopathologic study of the pericardial tissue (Table 2).

Table 1. Pericardial effusion characteristics.

Variable	Tuberculosis pericardial effusion (n=7)	Non-tuberculosis pericardial effusion (n=31)	P-value
Age (years)	72.43	60.68	0.670
Sex M/F	5/2	11/20	0.108
PPD (mm)	8	1.26	0.002
Pericardial fluid protein concentration (g/dl)	10.35	4.18	0.017
Pericardial fluid glucose concentration (g/dl)	67.29	85.81	0.235
Pericardial fluid LDH (IU/l)	2199.57	610.65	0.014
Pericardial fluid WBC	3806	1719	0.249
Pericardial fluid RBC	219786	303092	0.572
Pericardial fluid pH	7.68	7.88	0.397
Pericardial fluid CEA (ng/mL)	5.81	2.27	0.016
Pericardial fluid ADA (U/l)	35.7	36.03	0.283
Pericardial fluid interferon-gamma (pg/l)	69257	329	0.000

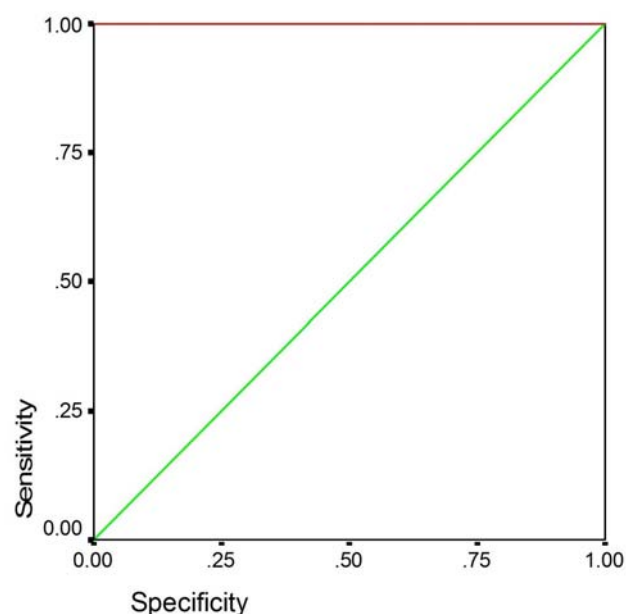
Table 2. TB pericarditis cases according to previous bold standard.

Pathology	TB-PCR	TB-culture	ZN staining	Patient number
Granulomatous pericarditis	-	-	-	1
Granulomatous pericarditis	+	-	-	2
Pericardial fibrosis	-	+	-	3
Granulomatous pericarditis	+	+	giant cell	4
Fibrinous pericarditis	+	-	-	5
Pericardial fibrosis	+	+	-	6
Granulomatous pericarditis	+	+	-	7

The most common clinical symptom at the time of admission in all patients with pericardial effusion, regardless of etiology, was dyspnea (present in 81.6%). Chest pain was the second most common symptom among patients. Of the seven samples with the final diagnosis of TB, five showed neutrophil dominance in the cell count and two were lymphocyte dominant. In all of them, cardiomegaly was found in chest X-rays.

Mean concentration of interferon-gamma in TB group was significantly higher compared to non-TB group (69257 pg/l [range: 26600-148000] vs. 329 pg/l [range: 0-2200], $P < 0.000$). Highest concentration of interferon-gamma among non-TB diagnoses was found in a malignancy pericardial effusion patient (2200 pg/l). ROC showed a value of 14400 pg/l as cutoff point for interferon-gamma with a sensitivity of 100% and specificity of 100% for diagnosing TB pericardial effusion (Figure 1). ADA was not found to be significantly higher in TB group in comparison with non-TB causes of pericardial effusion (35.7 U/l [range: 9-69] vs. 36.03 U/l [range: 8-420], $P = 0.28$). Considering a cutoff point of 32.5 U/l for ADA

according to ROC curve, a sensitivity of 57% and specificity of 80.6% was calculated for it (Figure 2).

**Figure 1.** Receiver operator characteristic curve for interferon-gamma.

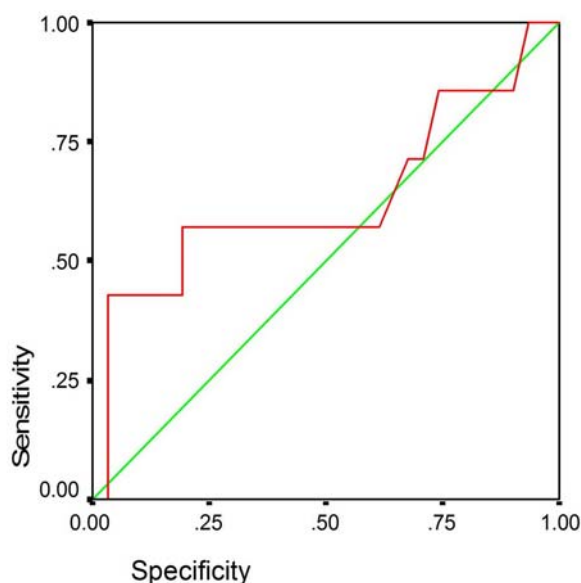


Figure 2. Receiver operator characteristic curve for ADA.

Tuberculosis skin test (PPD test) in TB group (mean: 8, range: 0-25) was significantly higher than the non-TB group (mean: 1.26, range: 0-8) ($P=0.002$), although PPD test has been shown to be of limited value in diagnosis of TB (24,25).

Concentrations of LDH ($P=0.014$) and total protein ($P=0.017$) in TB pericardial fluid samples were significantly higher than non-TB group. Mean CEA level was also found to be significantly higher in TB group in comparison with non-TB group ($P=0.016$). The difference of ADA or interferon-gamma levels between different diagnostic classes, other than TB, was not statistically significant ($P>0.05$).

Discussion

Results of this study provide evidence for the interferon-gamma to be a diagnostic test for detection of TB pericarditis among a cohort of Iranian patients. ADA measurement did not have necessary characteristics to accurate diagnose and differentiate TB pericarditis from other etiologies in this study.

In this study, using a cutoff value of 14400 pg/l for interferon-gamma, we found a sensitivity and specificity of 100% for detection of TB pericarditis, which suggests it as a reliable diagnostic test (no false results). This is in line with a previous study by Burgess *et al.* that reports a sensitivity and specificity of 100% for interferon-gamma in diagnosis of TB pericardial effusions using a cutoff value of 200 pg/l (5). In another study, Reuter *et al.* showed a sensitivity of 92% and specificity of 100% for

the test (cutoff: 50000 pg/l) (9). These reports confirm our results, and present interferon-gamma measurement as a highly informative test, that could be used to accurately establish the diagnosis of TB pericarditis. Burgess *et al.* in their study on cytokine production in TB pericarditis showed that interferon-gamma was significantly higher in TB pericardial effusions compared with other diagnostic classes while the comparison for other cytokines including IL-2, IL-4, IL-6, IL-10 and TNF-alpha was not significant (21).

With a cutoff point of 32.5 U/l, ADA showed a sensitivity of 57% and specificity of 80.6% for detection of TB pericarditis in our study. This is in accordance with various previous studies, which considering different cutoff values, have reported sensitivities of 50-100% and specificities of 72-97% for ADA in diagnosing TB pericardial effusions (5,6,9,13-15). However, we did not find a significant difference in absolute ADA levels of pericardial effusion between TB and non-TB groups. This may be due to the fact that ADA, being an enzyme with higher activity in lymphoid tissues, may have higher levels in lymphocytic effusions. However, most of our patients with TB pericardial effusions had neutrophil dominant pericardial fluid samples. It might be possible that if sampling was delayed until the lymphocyte dominant phase of the inflammation, ADA level would have increased to significantly higher levels among patients with TB pericarditis. Moreover, ADA enzyme is only stable for 24 hours in room temperature (12). So any delay in storage of samples or ADA measurement may have caused falsely low readings.

Limitations of this study include: 1. the relatively small sample size because TB pericarditis has low prevalence. 2. The lack of diagnostic test for viral infections to confirm the diagnosis. 3. The study was conducted in a referral center; therefore the result may not be generalized to general population.

In conclusion, results of this study provide evidence for interferon-gamma measurement in pericardial effusion, to be a highly informative test, which could be able to efficiently diagnose and discriminate TB pericarditis, although further studies with larger number of patient should be conduct to confirm the result of this study. Its use, instead of more time consuming or less accurate methods such as culture, histopathologic examination or fluid sample PCR, may help clinicians to establish the proper diagnosis more rapidly and accurately, in order to initiate appropriate treatment.

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