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Comparison of Different Laboratory Routine Methods with Adenosine Deaminase Test for Diagnosis of Tuberculous Pericarditis in Two Iranian Teaching Hospitals, Tehran, Iran

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Abstract: In this study, laboratory routine tests were compared with adenosine deaminase (ADA) test for diagnosis of tuberculous pericarditis. Sampling was performed among 30 patients admitted to two Iranian teaching hospitals (Imam and Modarres). For each patient two specimens were obtained from the pericardial fluid and biopsy. Pericardial fluid was used for staining, culture and measuring of ADA activity. The biopsy specimens were homogenized and cultured on Lowenstein-jensen media too and examined by H & E stain for presence of caseous granulomas and tubercule bacilli. In this survey, the ADA activity level of less than $45 \mu\text{L}^{-1}$ in pericardial fluid were determined as negative, while more than $45 \mu\text{L}^{-1}$ were considered as positive reaction indicating of tuberculous pericarditis. In this study, from a total of 30 subjects, tuberculous pericarditis was diagnosed by routine laboratory tests in 13 patients who all had clinical symptoms of the disease. Tuberculous pericarditis was diagnosed by positive results of pericardial fluid cultures in 6 of 13 patients (46.2%), by pericardial biopsy cultures in 6 patients (46.2%), by pericardial fluid staining in 3 patients (23.1%) and by tuberculin skin test in 10 patients (76.9%). Finally we observed that all of 13 patients with tuberculous pericarditis had ADA levels of more than $45 \mu\text{L}^{-1}$ in their pericardial fluids. The results of the present study confirmed that high levels of ADA have a prognostic value and due to its high specificity and sensitivity and also being faster and easier than laboratory routine tests, so this test can utilized as effective diagnostic method for diagnosis of tuberculous pericarditis.

Key words: Adenosine Deaminase (ADA), tuberculous pericarditis

INTRODUCTION

Bacterial pericarditis occurs by direct infection during trauma, thoracic surgery, or catheter drainage, by spread from an intrathoracic, myocardial, or subdiaphragmatic focus and by hematogenous dissemination. The frequent causes are *Staphylococcus* and *Streptococcus* (rheumatic pancarditis), *Haemophilus* and especially *Mycobacterium tuberculosis* (Bernhard *et al.*, 2004). For example in South Africa, with an annual incidence rate of 350 cases per 100000 population of tuberculosis (TB), approximately 1 to 2% of these cases are complicated by tuberculous pericarditis (Burgess *et al.*, 1995). In the era before antituberculous therapy, tuberculous pericarditis was rapidly fatal, with an early mortality rate of 80%. Since the introduction of chemotherapy in 1945, mortality from acute tuberculous pericarditis has decreased significantly (Lesley *et al.*, 2002).

In the last decade in the developed countries, tuberculous pericarditis has been primarily seen in immunocompromised subjects especially in AIDS patients. The mortality rate in untreated acute effusive tuberculous pericarditis approaches 85% (Bernhard *et al.*, 2004). The predominant symptoms of tuberculous pericarditis are cough, dyspnea and chest pain. Night sweats, orthopnea, weight loss and ankle edema are also common. As for signs, the most frequent are cardiomegaly, pericardial rub, fever and Tachycardia (Telenti *et al.*, 1991). Indeed tuberculosis infection of the pericardial membrane (pericardium) covering the heart is becoming more common. The infection can result in fluid around the heart or fibrosis of the pericardium, which can be fatal (Mayosi and Ntsekhe, 2005).

There is considerable urgency in establishing the correct diagnosis so that appropriate treatment can be started; however, it is often difficult to establish a

definitive bacteriologic diagnosis of tuberculous pericarditis. The diagnosis is made by the identification of mycobacterium tuberculosis in the pericardial fluid or tissue by culture, PCR and or the presence of caseous granulomas in the pericardium (Trautner and Darouiche, 2001).

The probability of obtaining a definitive diagnosis is greatest when pericardial fluid and a pericardial biopsy specimen are examined early in the effusive stage. In most patients, this requires many weeks and extensive cultivation by multiple methods. Because of the difficulty in isolating the causative organism, pericardial tuberculosis often is missed. For this reason, other diagnostic tools, such as detection of pericardial adenosine deaminase (ADA) level, have been suggested (Lesley *et al.*, 2002; Cegielski *et al.*, 1997; Fowler, 1991). The adenosine deaminase (ADA) is an enzyme that contributes to the purine bases catabolism, promotes the deamination of adenosine and produces inosine and ammonia. The biological activity of this enzyme in the body is considered as indicator for the proliferation of lymphocytes in fluids such as pericardial fluid and also significant rise on ADA levels, indicates a marker for T cell stimulation against tuberculosis activity (Valde's *et al.*, 1993).

Rapid diagnosis and treatment are crucial to reducing mortality and morbidity from pericardial disease. Because the differential diagnosis of effusive pericarditis is broad, the ability to diagnose tuberculous pericarditis promptly would greatly facilitate the management of many patients with pericarditis (Cegielski *et al.*, 1997).

Establishing the diagnosis of tuberculous pericarditis in most of these patients required many weeks and extensive cultivation by multiple methods. Furthermore, examining for acid-fast bacilli are fast, but it is insensitive and do not distinguish between different Mycobacteria. Diagnosis by culture, is specific and relatively sensitive (Since the number of *Tubercle bacilli* in pericardial fluid was very low, the positive cultures only included 46.2%) but is slow and long culture periods often result in clinical and therapeutic decisions being made before these laboratory results become available (Cegielski *et al.*, 1997).

The tuberculosis infection is one of the most prevalent disease in Iran and thus to be of major medical concerns. Furthermore, tuberculous pericarditis is one of the most fatal complications of tuberculosis infection. So, in this study, laboratory routine methods were compared with Adenosine Deaminase (ADA) test for diagnosis of tuberculous pericarditis.

MATERIALS AND METHODS

Study population and setting : This study was conducted in two Iranian teaching hospitals (Imam and Modarres). In this study, the subjects were divided into two groups. The first group included the subjects were undergone a bypass cardiac surgery, but they had no symptoms of pericarditis. This group was analyzed for ADA activity as normal individuals. The second group included the patients who admitted to two teaching hospitals due to primary pericardial diagnosis. Sampling was performed in 50 patients as the first group and 30 patients with primary pericarditis diagnosis as the second group. In the first group, samples were collected from pericardial fluid and in the second group two samples were taken from each patient including pericardial fluid and biopsy.

Preparation of pericardial fluid: First, pericardial fluid was centrifuged at 5000 rpm/min for 10 min. The supernatant was used for ADA activity measurement and the pellet used for bacterial stain and culture (Barr, 1995).

Preparation of biopsy: The pericardial biopsy was homogenized with a 1 mL PBS within the grinder. Then the suspension was used for bacterial staining and culture (Barr, 1995).

Ziehl-neelsen staining: Smears from the pellet of pericardial fluid and biopsy suspensions were stained with ziehl-neelsen stain method and were examined microscopically for *Tuberculosis bacilli* (Barr, 1995; Cegielski *et al.*, 1997)

Culture: The pellets of the pericardial fluid and biopsy suspensions were selectively cultured on Lowenstein-jensen media. The plates were evenly inoculated with about 1 mL of each suspension. Excessive suspension was removed with a pipette. Then, the plates were inoculated in a CO₂ (5%)-enriched incubator for 4 days at 37°C. After that, the cultures were followed up every week for growth of *Mycobacterium tuberculosis*.

Measurement of ADA: The supernatant of pericardial fluid was used for measuring of adenosine deaminase (ADA) activity. ADA activity was determined in all pericardial specimens according to the method described by Giusti (1974). This is a calorimetric method based on the measurement of the formation of ammonia by Berthelot reaction, which is produced when ADA acts on excess adenosine. One unit of ADA is defined as the amount of enzyme required to release 1 mol ammonia per

Table 1: Frequency of patients with tuberculous pericarditis and their ages in two Iranian teaching hospitals

Results/Ages	Positive		Negatives		Total	
	No.	%	No.	%	No.	%
0-15	0	0.0	1	100.0	1	3.3
16-30	1	33.3	2	66.7	3	10.0
31-45	4	44.4	5	55.6	9	30.0
46-60	3	42.9	4	57.1	7	23.3
61-75	4	44.4	5	55.6	9	30.0
>75	1	100.0	0	0.0	1	3.3
Total	13	43.3	17	56.7	30	100.0

min from adenosine under standard assay conditions. The enzyme is stable for at least 24 h at 25°C, for 7 days at 4°C and for 3 months at -20°C.1 (Lesley *et al.*, 2002; Komsuoglu *et al.*, 1995; Martinez-Vazquez *et al.*, 1986).

RESULTS

In this study, we examined 30 tuberculous pericardial patients admitted to two Iranian teaching hospitals. The patients were 19 male (63%) and 11 female (37%). The age of the patients ranged from 12-76 years with the average of 49 years. On the basis of clinical symptoms, physical and radiological findings and histopathologic characteristics, tuberculous pericarditis was confirmed in 13 patients including 9 male (69%) and 4 female (31%). Frequency of patients with tuberculous pericarditis and their ages is shown in Table 1. Comparison between the results of several laboratory methods which used in this study are presented in Table 2.

DISCUSSION

The aim of the present study was to develop a more available, faster, inexpensive and reliable prognostic method for detection of tuberculosis pericarditis in clinical and hospital laboratory. We measured ADA activity in pericardial fluid of pericarditis patients admitted in two teaching hospitals (Imam and Modarres). Then we compared it with other conventional diagnostic methods including Culture, histopathology, tuberculin skin test

and direct examination for diagnosis of tuberculous pericarditis. In this study, sampling was performed among 30 patients admitted in two teaching hospitals. The samples were collected from the pericardial fluids and biopsies. From a total of 30 subjects, tuberculous pericarditis was established in 13 patients who all had clinical symptoms of the disease by laboratory routine tests. The reports of other investigators in different countries have indicated that the adenosine deaminase activity is equal or less than 45 μL^{-1} in normal persons, while it is more than 45 μL^{-1} in tuberculous patients (Telenti *et al.*, 1991; Valde's *et al.* 1993). In this investigation, the ADA activity in all 13 tuberculous patients was more than 45 μL^{-1} .

Our findings agree with data reported from a study performed in south Africa (Hugo-Hamman *et al.*, 1994). In that study, the patients were consisted of 44 children with tuberculous pericarditis. They used culture, direct examination, tuberculin skin test, histopathology and ADA method for detection of tuberculous pericarditis. The investigators reported a positive response for histopathology and culture in 98% and also positive tuberculin skin test in 70% of the patients. They suggested ADA test can be used as an appropriate method for diagnosis of tuberculous pericarditis.

The efficacy of ADA test was also assessed in 13 patients with tuberculous pericarditis (Sagrasta *et al.*, 1998). In their study, tuberculous pericarditis was established by pericardial fluid culture in 3 patients, lymph nodes biopsy culture in 2 patients and pericardial biopsy culture in 3 patients. Finally, the ADA activity test performed in only 4 patients and results indicated that all 4 patients had ADA activity more than 45 μL^{-1} .

With regard to the advantages of ADA activity test including high specificity and sensitivity, rapidity (less than 2 h), simplicity and inexpensiveness, it is recommended that this test can be used efficiently for diagnosis of tuberculous pericarditis in hospital and clinical laboratories.

Table 2: Results of the applied methods for detection of tuberculous pericarditis

Lab methods	Positive Specimens		Negative Specimens		Total	
	No.	%	No.	%	No.	%
Culture	6	46.2	7	53.8	13	100
Staining	3	23.1	10	76.9	13	100
Histopathology	13	100.0	0	0.0	13	100
Skin Test (PPD)	10	76.9	3	23.1	13	100
Adenosine Deaminase Test	13	100.0	0	0.0	13	100

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