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VALUE OF POLYMERASE CHAIN REACTION IN PATIENTS WITH PRESUMPTIVELY DIAGNOSED AND TREATED AS TUBERCULOUS PERICARDIAL EFFUSION

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ABSTRACT

Objective: To know the sensitivity of polymerase chain reaction (PCR) in pericardial fluid and response to antituberculous treatment (ATT) in PCR positive patients who were presumptively diagnosed and treated as tuberculous pericardial effusion.

Methodology: This was a descriptive cross sectional study carried out from1st June 2009 to 31th May 2010 at Cardiology Department, Lady Reading Hospital, Peshawar. Patients with presumptive diagnosis and receiving treatment for tuberculous pericardial effusion were included. Pericardial fluid sample was aspirated under fluoroscopy for the routine work up. The specimens were subjected to PCR detection of mycobacterium tuberculous DNA.

Results: During 12 month study period, a total of 54 patients with large pericardial effusion presented to Cardiology department, Lady Reading Hospital, Peshawar. Of them, 46 patients fulfilled the criteria for presumptive diagnosis of tuberculous pericardial effusion. PCR for mycobacterium tuberculous DNA in pericardial fluid was positive in 45.7%(21).Patients were followed for three months. In PCR positive group, 01 patient while in PCR negative group 3 patients were lost to follow up. Among PCR positive patients 17(85%) while in PCR negative group 11(47.82%) patient responded to ATT both clinically and echo-cardiographically. We found that patients who were PCR positive responded better to therapy than those who were PCR negative and this finding was statistically significant (p=0.035).

Conclusion: PCR, with all its limitations, is potentially a useful diagnostic test in patients with presumptively diagnosed tuberculous pericardial effusion. A PCR positive patient responds better to therapy as compared to PCR negative patient.

Key Words: Pericardial effusion, Tuberculosis, Mycobacterium tuberculosis, Pericardiocentesis, Polymerase Chain Reaction.

INTRODUCTION

Tuberculosis (TB) is an important public health problem in the world. There is pandemic of TB in the developing countries and its incidence is increasing due to co-infection with HIV infection.[1,2] According to WHO the global incidence of tuberculosis is 136 cases per100,000 population.[3] Pakistan ranks the eight among high TB burden countries in the world, with an estimated prevalence of 177/100,000.[4]

Tuberculous pericarditis (TP) is a rare form of extra-pulmonary tuberculosis frequently associated with high mortality, short and long term complications.[5] The main complications of TP are: constrictive pericarditis with or without restrictive heart failure; cardiac tamponade a most frequent cause of death if not detected and properly managed.[6] Pericardial effusion (PE) in TP is characterized by a lymphocytic exudate, but occasionally it can be either neutrophilic or mixed.[7] Prompt treatment of tuberculous pericarditis can save lives.[8] Effective treatment requires a rapid and accurate diagnosis, but this is often difficult.[9] Ziehl-Neelsen (ZN)-stained smears of pericardial fluid have poor sensitivity for detecting Mycobacterium tuberculosis, while culture is both slow and insensitive.[8-10] Pericardial biopsy is invasive, requires technical skills and is often not diagnostic.[6,11,12]

Clinicians thus have to rely heavily on the clinical features of pericardial tuberculosis (TB) to initiate therapy[13-15] but in view of the potential toxic effects and the duration of anti-tuberculous chemotherapy, it is important to identify which clinical and basic laboratory features should be used. Nowadays new diagnostic tools are used for rapid diagnoses of suspected cases of tuberculous pericarditis such as polymerase chain reaction (PCR) analysis, adenosine deaminase (ADA) activity and pericardial interferon gamma (IFN-g) levels.[16-18]

There is no gold standard investigation for the diagnosis of tuberculous pericardial effusion (TPE) in routine clinical practice. Patients are diagnosed and treated on the basis of history and supportive evidence from fluid analysis and echocardiography. No study has been done so far in our country on PCR for diagnosis of tuberculous pericardial effusion. We conducted this study to know the sensitivity of polymerase chain reaction (PCR) in pericardial fluid and response to antituberculous treatment (ATT) in PCR positive patients who were presumptively diagnosed and treated as tuberculous pericardial effusion.

METHODOLOGY

This was a descriptive cross sectional study carried out from 1st June 2009 to 31th May 2010 at Cardiology Department, Lady Reading Hospital, Peshawar. All patients of any age and either gender with presumptive diagnosis of tuberculous pericardial effusion who were supposed to be started on anti-tuberculous treatment (ATT) on basis of clinical and laboratory work up were included. Written informed consent was givenby each patient. Hospital ethical committee approved the study. Presumptive diagnoses of tuberculous pericardial effusion was considered in patients having following features; 1) Large pericardial effusion $\geqslant$ 10mm epi-pericardial separation during diastole on echo-cardiography, 2) Hemorrhagic pericardial fluid 3) Patient who were started on anti-tuberculous treatment (ATT) based on clinical suspicion and laboratory workup. Patients with small to moderate effusion with an obvious cause like asymptomatic small pericardial effusion in patients with myocardial infarction, uremia and following first 3 months of cardiac surgery and pericardial effusion due to malignancy were excluded. Patients were followed for 03 months to look for clinical improvement (appetite, fever, weight, dyspnoea, and cough) and echocardiographic (Quantification of pericardial effusion) response to therapy. Patients were divided in to two groups on the basis of PCR test result. Furthermore, for the purpose of this study response to therapy was taken as a yard stick to label patient as tuberculous pericardial effusion and vice versa.

Patient's demographics, clinical symptoms and signs, relevant investigations such as electrocardiography, chest X-ray and echocardiography were recorded. After informed consent and explanation of the procedure pericardial fluid sample was aspirated under fluoroscopy. Pericardial effusion samples were subjected to cell count, differential count and cytology, biochemistry (LDH, total protein) and tested for PCR detection of mycobacterium tuberculous DNA. M. tuberculosis DNA (if present) was extracted from the sample accessible to DNA amplification by polymerase chain reaction. Specific primers were used and conditions so as to amplify 541-base pair target sequence located with in IS987 gene. IS987 is a putative insertion sequence that is specific for the strains of M. tuberculosis complex only and occurs in multiple copies (1 to more than 19), in majority from 7 to 15 copies. The amplified products were subjected to agarose gel electrophoresis, ethedium bromide staining and UV visualization.

The collected data was recorded on Statistical Package for Social Sciences version 16.0 software (SPSS Inc., Chicago Illinois). Continuous variables like age were presented as Mean $\pm$ SD. Categorical variables like dyspnea, fever, chest pain, and tachycardia. Electrocardiographic, Radiological and Echocardiographic finding such as sinus tachycardia, low voltage, electrical alternans, cardiomegaly, pleural effusion, right atrial (RA) collapse, right ventricular (RV) collapse and positivity of PCR were presented as percentages. Comparison was performed by using student-t test for numerical variables and Chi-Square test for categorical variables. P value $\leqslant$ 0.05 was considered significant.

RESULTS

During 12 month study period, a total of 54 patients with large pericardial effusion presented to Cardiology Department, Lady Reading Hospital, Peshawar the referral centre for the province. In this, 46 patients fulfilled the criteria for presumptive diagnosis of tuberculous pericardial effusion and were started on treatment (ATT) as per local protocol. Among these patients, males were 18 (39.1%) and females were 28 (60.9%). The mean age was 51.54 ± 17.87 (95% CI 56.85 - 46.24).Base line characteristics are shown in Table 1.

All pericardial fluid samples were hemorrhagic, lymphocytic and exudative. PCR for mycobacterium tuberculous DNA in pericardial fluid was positive in 21(45.7%) of patients with presumptive diagnosis of tuberculous pericardial effusion.

Table 1: Baseline Characteristics of Presumptively Diagnosed and Treated as Tuberculous Pericardial Effusion

Variables	Study Population N (%)
Gender	
Male	18(39.1%)
Female	28(60.9%)
Age	51.54 ± 17.87 years
Symptoms	
Dyspnea (NYHA III-IV)	39(84.8%)
Fever	39(84.8%)
Night sweat	25(54.3%)
Chest pain	14(30.4%)
Cough	39 (84.8%)
Weight loss	30(65.2%)
Signs	
Tachycardia	33(71.7%)
Elevated JVP	38(82.6%)
Hypotension(SBP < 90mmHg)	06(13.0%)
Pericardial Rub	02(04.3%)
Electrocardiographic Findings	
Sinus Tachycardia	34(73.9%)
Low Voltage	16(34.8%)
Electrical Alternans	04(08.7%)
Radiological Findings	
Cardiomegaly	16(34.8%)
Pleural Effusion	23(50.0%)
Echocardiographic Findings	
Right Atrial Collapse	29(63.0%)
Right Ventricular Collapse	17(37.0%)

Figure 1: Summary of Patient Follow up and their Response to Therapy

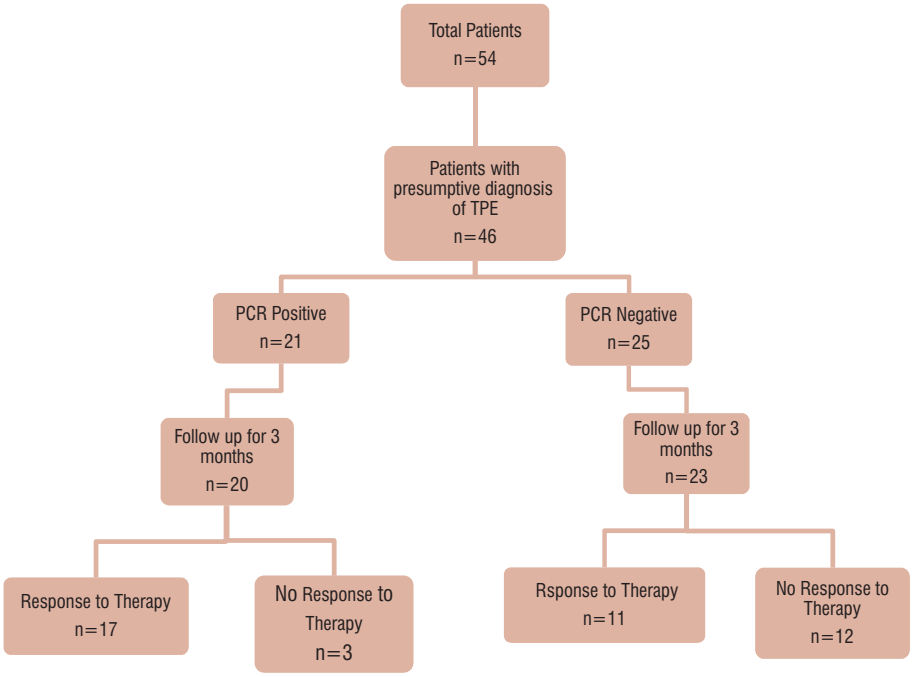

Table 2: Sensitivity and Specificity of PCR for Diagnosis of TB Pericardial Effusion on Basis of Response to Therapy

	PCR Positive	PCR negative	Sensitivity & Specificity
Response to Therapy	17	11	Sensitivity 60.71%
No-Response to Therapy	3	12	Specificity 80%

Patients were followed for three months. In PCR positive group, 01 patient was lost. In remaining 20 patients, 17 (85 %) who were PCR positive responded to therapy both clinically and echocardiographically, while in PCR negative group, 02 patients were lost to follow up and the rest i.e.23(92%) were followed, out of which 11(47.82%) responded to therapy both clinically and echo-cardiographically as shown in Figure1. It was observed that patients who were PCR positive responded better to therapy than those who were PCR negative and this finding was statistically significant ($p= 0.035$).

The sensitivity and specificity of PCR for diagnosing of TB pericardial effusion, after taking response to therapy as a yard stick at three month follow up was 60.71% and 80%, respectively as shown in Table 2.

Fever, cough, dyspnoea (NYHA III-IV), elevated JVP and electrical alternans on ECG were clinical features of patients associated with PCR positive patients ($p< 0.05$).

The frequencies of PCR positive patients were more common in female (28.3%) though it was not statistically significant ($p> 0.05$).

DISCUSSION

Rapid diagnosis and treatment is essential for reducing the mortality and morbidity from tuberculous pericardial disease. As various etiologies contribute to effusive pericarditis, thus prompt diagnosis of tuberculous pericarditis would greatly facilitate the management of many patients with pericarditis. The accurate diagnosis of

tuberculous pericarditis is important, because without specific treatment, the mean survival rate is 3.7 months, with a mortality rate approaching 85% at 6 months.[19]

In recent years, protocols based on the use of polymerase chain reaction (PCR) raised hopes for a reliable and rapid diagnosis of extrapulmonary tuberculosis.[20-22]The polymerase chain reaction (PCR) has also been suggested for detecting M tuberculosis DNA in pericardial fluid.[23,24]

We conducted this study to see the sensitivity of polymerase chain reaction (PCR) in pericardial fluid and response to antituberculous treatment (ATT) in PCR positive patients who were presumptively diagnosed and treated as tuberculous pericardial effusion in our local population. This study is first of its kind as previously none of the study has taken presumptive diagnosis of TB pericardial effusion in consideration as well as follow-up of patients to see response to therapy in PCR positive patients.

In present study the sensitivity of PCR for mycobacterium tuberculosis DNA in pericardial fluid was 45.7%.Cegielski et al, correctly diagnosed TPE by PCR in 13 patients (81%). Considering the individual specimens as the unit of analysis, Mycobacterium tuberculosis was identified by PCR (50%) from patients with tuberculous pericarditis. The sensitivity of PCR was higher with tissue specimens (80%) than with fluid specimens (15%).[25] Similarly Reuter et al, reported the sensitivity of PCR for mycobacterium tuberculosis DNA in pericardial fluid as 30%.[26] Previously majority of studies have shown low sensitivity of PCR for the diagnosis of tuberculous pericardial effusion. Go et al studied the role of PCR for early diagnosis of tuberculosis among patients with massive pericardial effusion reporting the sensitivity as low as 10%.[27] The reason for higher positivity of PCR in our study might be our selection criteria based on those patients who were with suspected diagnoses of TB pericardial effusion and not pericardial effusion of any etiology.

In this study patients were followed for three months to see response to therapy both clinically and echo-cardiographically. Among PCR positive patients, 17 (85%) while in PCR negative group, 11 (47.82%) patient responded to anti tuberculous treatment (ATT) both clinically and echocardiographically. It was observed that patients who were PCR positive responded better to therapy than those who were PCR negative and this finding was statistically significant ($p=0.035$).So far there is no data available to compare therapy response in PCR positive and negative patients as well as their follow-up.

In our study we utilized clinical improvement and reduction of pericardial effusion on follow-up echocardiography (response to therapy) as gold standard for labeling patient as tuberculous pericardial effusion. On the basis of above criterion, the sensitivity and specificity of PCR for diagnosis of tuberculous pericardial effusion was 60.71% and 80%, respectively. This high sensitivity and specificity is not in accordance with previous studies,[25, 26] the reason might be that no study till date has considered presumptive diagnoses as well as response to therapy as a yard stick for labeling of tuberculous pericardial effusion.

In this study the frequency of PCR positive patients were more in female (28.3%) and elderly (23.9%) patients, and they responded better to therapy, though it was not statistically significant ($p>0.05$). This group of patient is prone to develop chronic infections, probably due to malnourished and immunocompromised status.[10-12,19]

In present study we found that dyspnoea, fever, cough, weight loss, night sweat, tachycardia and elevated JVP were more common clinical features and their frequencies were almost similar to previous published data.[6, 9-11, 16] Fever, cough, dyspnea (NYHA III-IV), elevated JVP and electrical alternans on ECG were clinical features of patients associated with PCR positive patients ($p<0.05$). Patients with such clinical features have more chances of having positive PCR for M tuberculosis DNA in pericardial fluid.

The use of PCR for the detection of M. tuberculosis offers a useful assay with high specificity for the diagnosis of pericardial TB though with poor sensitivity. This lower sensitivity trend has also been reported in tuberculous pleural effusion[28] and pericardial effusion studies,[25] where sensitivities have ranged between 15-20%, and specificities between 96-100%.The reason for this poor sensitivity of PCR in tuberculous exudates may be poor specimen preparation, the presence of inhibitors such as fibrin and hemoglobin, as well as low numbers of tubercle bacilli or their DNA in the specimens.[28,16] Sensitivity improves when pericardial tissue is used for analysis by PCR.[24,27]

PCR has got few limitations. It can not differentiate between live and dead bacilli and its relevance must be judged in light of the overall clinical picture in cases where the patient has received anti-tubercular treatment recently, reactivation tuberculosis, or asymptomatic infection.[29-31] This sophisticated technique is limited by the need for a suitable infrastructure and the high cost of the test.

The present study has got limitations: Comparison was not done with tissue biopsy instead response to therapy was taken as yard stick for labeling patient as tuberculous pericardial effusion. Future studies will be needed for comparison of PCR with gold standard with large sample size.

CONCLUSION

PCR, with all its limitations, is potentially a useful diagnostic test in patients with presumptively diagnosed tuberculous pericardial effusion. A PCR positive patient responds well to therapy as compared to PCR negative patient.

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