ORIGINAL PROF-2141

# DIAGNOSIS OF TUBERCULOUS ASCITIES;

## Role of polymerase chain reaction and adenosine deaminase activity

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**ABSTRACT…..**Diagnosing Tuberculous ascites is a challenge. Polymerase chain reaction (PCR) and adenosine deaminase activity (ADA) have come up as promising modalities to aid diagnosis of tuberculosis in body fluids. **Objective:** To find and compare the usefulness of ADA and PCR in ascitic fluid in diagnosis of peritoneal tuberculosis. **Study Design:** A cross-sectional study. **Place of Study:** Medical Unit-I, Ghulam Mohammad Mahar Medical College Hospital, Sukkur. **Duration of Study:** From January 2010 to July 2011. **Methods:** Fifty five patients of exudative ascites, were diagnosed as peritoneal tuberculosis by following criteria- clinical suspicion, PPD (Skin Tuberculin test) positive, suggestive bacteriological and/or imaging findings and ascitic fluid showing lymphocytosis with decreased glucose and SAAG (Serum- Ascites albumin gradient) of <1.1 gm/ dL. PCR and ADA were performed in the ascitic fluid and positivity rates determined and compared. **Results:** Out of 55 study subjects, 50 patients (90.9%) were PCR positive and 48 (87.3%) were ADA positive; both were equiefficacious (p=0.54). High agreement between PCR and ADA tests was noted. **Conclusions:** ADA and PCR are comparable as diagnostic modality for tuberculous peritonitis, however ADA scores over PCR because of easy availability, low cost, less infrastructure requirement and less-time consuming.

**Key words:** Ascites; peritoneal tuberculosis; ADA; PCR.

**Article Citation**
Phulpoto JA. Diagnosis Of Tuberculous Ascities; Role of polymerase chain reaction and adenosine deaminase activity . Professional Med J 2013;20(3): 381-384.

## INTRODUCTION

Tuberculous peritonitis constitutes 4-10% of all patients with extra pulmonary tuberculosis[1]. There is no gold standard for the diagnosis of extra-pulmonary tuberculosis in the absence of demonstrable acid-fast bacilli. Conventional histo-pathological and microbiological methods are often inadequate for diagnosing abdominal tuberculosis[2]. The result of various serological tests is variable due to uncertainty of antibody response to Mycobacterium tuberculosis, poor reproducibility and lack of specificity[2,3].

The best confirmation is by laparoscopic peritoneal biopsy followed by histo-pathological and culture studies[2]. Polymerase chain reaction (PCR) for tuberculosis and adenosine deaminase activity (ADA) for tuberculosis are non-invasive techniques that have shown significantly high sensitivity and specificity in clinical trials[4-7]. Both these tests are being increasingly utilized in clinical practice for diagnosis of tuberculosis in body fluids like pleural fluid, pericardial fluid and cerebrospinal fluid. Studies on ascitic fluid estimation by these methods have been done on small number of patients but have shown promising results. Various reports have suggested 100% sensitivity for diagnosis of peritoneal tuberculosis with specificities in the range of 92-100%[5,6]. Polymerase chain reaction (PCR) amplification of the IS6110 sequence of Mycobacterium in ascitic fluid is a useful tool when peritoneal tuberculosis is suspected[5,12]. However its validity still needs to be evaluated.

The need of the hour is to evaluate these new emerging modalities for the diagnosis of tuberculous ascites. The present study aims to find out and compare the usefulness of polymerase chain reaction and adenosine deaminase activity (ADA) in the diagnosis of peritoneal tuberculosis.

## MATERIAL & METHODS

Fifty five adult patients (>18 years) who were admitted in the Medical Unit I of Ghulam Mohammad Mahar Medical College & Hospital, Sukkur (Pakistan) with exudative ascites were included in the study after they fulfilled two or more of the following five criteria;

1. Suggestive history (including family history of contact),
2. Clinical evidence of tuberculosis elsewhere in the body,
3. Imaging[8,9] and bacteriological features suggestive of peritoneal tuberculosis,
4. Cyto-biochemical analysis of ascitic fluid showing lymphocytic predominant leucocytosis with decreased glucose level and SAAG (Serum to Ascites albumin gradient) of less than 1.1[10],
5. PPD test positive[9].

Only newly diagnosed cases were included for the study and cases of malignant ascites, acute abdomen, and ascitic fluid culture coming positive for aerobic bacilli; were excluded.

## Collection of Specimen

With the patient in supine position, using aseptic technique, under local anaesthesia, a large bore needle was introduced in the flank midway between the anterior superior iliac crest and umbilicus and ascitic fluid was drained slowly (10 to 20 mL) in a plain sterile tube. Cyto-biochemical examinations of the aspirated fluid was performed with the following points in consideration- physical appearance, total protein and albumin, glucose, ADA and PCR, total and differential cell count and malignant cells. Gram stain and AFB stain of the smear were also performed.

Routine blood investigations and chest X-ray (PA view), and ultrasound of abdomen was done in all patients; and if patient had cough, sputum was subjected for AFB staining on smear.

As part of PPD testing, intra-dermal injection of 10 TU of PPD was given on the ventral aspect of forearm. Measurement of induration was done after 72 hours. Induration of more than 10mm was considered positive[9].

**Polymerase chain reaction** was performed using phenol chloroform method of DNA extraction from ascitic fluid sample. PCR amplification of primer sequence IS6110 specific for M. tuberculosis was done. Amplified mycobacterial DNA was identified using 2% agarose gel electrophoresis. A band of 123 bp is obtained in positive amplified samples, which can be compared with simultaneously run DNA ladder and known M. tuberculosis positive control.

**Adenosine Deaminase Activity** was measured using ADA-MTB kit comprising of five reagents. Adenosine deaminase hydrolyses adenosine to ammonia inosine. The ammonia formed further reacts with a phenol and hypochlorite in an alkaline medium to form blue indophenols complex. Intensity of the blue colored indophenols complex formed is directly proportional to the amount of ADA present in the sample. Values more than 40 U/L were taken as positive[3].

## STATISTICAL ANALYSIS

The data obtained was analyzed by SPSS 15 software. P-value $<0.05$ was taken as significant. Fischer's exact test was used for comparing usefulness of PCR and ADA.

## RESULTS

The age of the study subjects varied from 18-62 years, 52.7% were males. Maximum patients (41.8%) were in 20-30 years age group followed by 31-40 years (30.9%) and 41-50 years (12.7%). Out of the 55 patients, 18 (32.7%) suffered from pulmonary tuberculosis. Eight of these subjects were detected on the basis of a suggestive chest X-ray, whereas 10 had respiratory symptoms in addition to x-ray chest findings. Four patients were sputum positive for acid fast bacilli. No patient was positive on Ziehl-Neilsen stained smear examination of peritoneal fluid. One patient had culture positive for Mycobacterium tuberculosis and also was ADA and PCR positive. Twelve of the 26 female patients had symptoms suggestive of pelvic tuberculosis. All 12 were PCR

positive whereas 10 patients were ADA positive. The main clinical symptoms were pain in abdomen in 13 patients (23.64%), followed by fever, loose stools and vomiting in 11 patients (20.0%) and constipation in 10 patients (18.18%).

Among the study subjects, 50 patients (90.9%) were PCR positive and 48 (87.3%) were ADA positive. Both PCR and ADA were negative in 3 patients, PCR and ADA both were positive in 46 patients. Two patients had ADA positive but PCR negative, while four patients had PCR positive but ADA negative.

The accuracy of PCR was similar to that of ADA (90.9% vs 87.2% respectively; p=0.5; z= 0.61). Fisher's exact test shows high association (p=0.012) between PCR and ADA tests. The measure of agreement (Kappa) between the two tests is also highly significant (k=0.441, p=0.012).

## DISCUSSION

Diagnosing peritoneal tuberculosis remains a significant challenge, because of its insidious nature, the variability of its presentation and the limitations of available diagnostic tests. A high index of suspicion is required even in areas with high prevalence of tuberculosis, whenever confronted with chronic symptoms of fever, abdominal pain and unexplained ascites. The condition carries good prognosis if promptly diagnosed and treated. Based on a systematic review of the literature, it is recommended that peritoneal tuberculosis should be considered in the differential diagnosis of all patients presenting with unexplained lymphocytic ascites and those with a serum-ascites albumin gradient (SAAG) of <11 g/L[10] in this part of the world. Culture growth of Mycobacterium tuberculosis of the ascitic fluid or peritoneal biopsy is the gold standard test[3]; however, as the yield of mycobacterium in ascitic fluid is very low and conventional cultural techniques take a long period of time[3,5], biopsy being invasive procedure is often denied by the patients. Therefore, ADA and PCR could not be tested against a gold standard to determine the sensitivity and specificity of these modalities[13-16]. However, this study took the most popular conventional method of diagnosis employed in clinical practice i-e. after excluding other causes of exudative (low SAAG) ascites, if there is a high degree of clinical suspicion, PPD is positive and sonographic findings are suggestive then the patient can be diagnosed as tuberculous peritonitis.

The results of this study indicate that PCR and ADA estimation are equieficacious as diagnostic tests in detecting tuberculous ascites. However, PCR determination requires good infra-structure, costly gadgets and high cost per test. PCR may also show false positivity. In contrast, ADA can be done with simple colorimetric machine and it can be done in routine laboratories. Therefore, ADA estimation appears to be a more convenient and promising tools for diagnosis of peritoneal tuberculosis. Most of the studies done in this regard have taken small number of patients and have been done on other body fluids like pleural fluid. Further work for standardization of cut off values for ADA in ascitic fluid is strongly recommended. For it, we need much larger sample size of proven negative and positive cases of tuberculosis.

We conclude that, all the patients clinically suspected to be having tuberculous peritonitis can be subjected to ADA estimation vis-à-vis PCR estimation, since the former is more convenient, less-time consuming, cheaper and easily available.



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Article received on: 29/11/2012
Accepted for Publication: 10/02/2013
Received after proof reading: 25/03/2013