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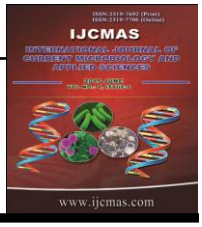

Original Research Article

Diagnostic Performance of Gamma Interferon in Rapid Diagnosis of Tuberculosis Peritonitis

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A B S T R A C T

Peritoneal tuberculosis (TB) is a considerable problem in certain developing countries. Current diagnostic tests for diagnosis of peritoneal TB are difficult and time-consuming. This study aimed to investigate the performance and diagnostic accuracy of IFN-gamma for TBP both in serum and peritoneal fluid and to investigate the validity of the specific immunoglobulins for rapid detection of TBP. The study included twenty eight patients diagnosed as TBP by ZN, culture, PCR and histopathology, eighteen control non TBP patients were included. IFN-gamma and TB IgG, IgM, IgA were detected in serum and peritoneal fluid. TB was detected in 10.7% samples by ZN and in 85.7% by culture and/ or PCR. Serum IgM had statistically higher prevalence among TBP patients (64.3%, P= 0.0001) followed by serum IgG (57.1%, P= 0.03). PCR was the most sensitive and accurate method for diagnosis of TBP (95.8%, 92.9%). Serum IgM was the most sensitive and accurate serological marker (70.8%, 71.4%) followed by serum IgG (62.5%, 64.3%). Both serum and peritoneal fluid IFN-gamma levels in TBP (mean SD= 50.3± 75.2 pg/ml, 27.7± 48.7 pg/ml) had statistically significant higher levels (P=0.009, P=0.023) compared to the levels in non TBP (mean ± SD 1.87±5.6, 0.52± 0.5 pg/ml). Cutoff value of serum IFN-gamma of 1.1 pg/ml had sensitivity 72% and specificity 81% while in peritoneal fluid the same cutoff value had sensitivity 72% and specificity 76%. PCR has the highest sensitivity and accuracy. IFN-gamma for TBP either in serum or peritoneal fluid is a new promising, rapid and ease test with good sensitivity and specificity. The validity of the specific immunoglobulins in our research gave us the best one was S. IgM followed by serum IgG. It is essential to recognize that a combination of different diagnostic tests is used in order to arrive at the detection of tuberculosis peritonitis. There is no optimum laboratory test that accurately diagnoses all cases of TBP.

Keywords

TB peritonitis, Serology, PCR, Culture

Introduction

Tuberculosis (TB) was a prevalent infection in ancient culture like Egypt. The disease was put under control only after the advent of antimicrobial therapy in 1946 (Mimidis et al., 2005). After a few decades of decreasing incidence of TB, there has been a worldwide reemergence of the disease. This may be due to several reasons such as the widespread of

HIV, high numbers of immigrants and the primary resistance to first –line drugs. One-third of the world population is in risk of acquiring TB according to WHO and more than 30 million deaths due to TB were expected, especially in Africa and Asia (WHO, 1992).

Tuberculous peritonitis (TBP) is a manifestation of TB, which constitutes about 3% of extra pulmonary tuberculosis (EPTB) cases. The later constitutes about 15%-20% of all cases of TB in immunocompetent patients and accounts for more than 50% of cases in human immunodeficiency virus-positive individuals (Sharma and Mohan, 2004). Most often, EPTB is the result of reactivation of latent disease established by hematogenous spread during primary pulmonary infection. Cases of TBP are expected to increase with the increasing incidence of TB worldwide (Sharma and Mohan, 2004; Riquelme *et al.,* 2006). Peritoneal TB is manifested clinically as ascites of insidious onset, abdominal pain and fever (Chow *et al.,* 2002). Although rare disease, associated with vague symptoms of TB peritonitis and its challenging clinical course can interfere with a definitive diagnosis, and it is often misdiagnosed with other abdominal diseases. Late diagnosis may lead to higher morbidity and mortality of TB peritonitis (Akın *et al.,* 2000, Sanai and Bzeizi, 2005).

The current clinical diagnostic techniques for TBP are time consuming and inefficient. The efficient diagnosis requires histological confirmation of caseous granulomas. Bacteriologic confirmation can be performed using ascitic fluid derived acid-fast bacilli smears as well as cultures for *Mycobacterium tuberculosis* (*M. tuberculosis*). The diagnostic gold standard for TB is the detection of *Mycobacterium tuberculosis* (MTB) by culture or molecular methods. However, four weeks are required for culture of *M. tuberculosis*, smears are too insensitive to meet the current diagnostic demand (Inadomi *et al.,* 2001) and the cost of molecular methods is major issue to consider particularly in poor countries where TB is endemic (Sanai and Bzeizi, 2005). Despite the limitation of detecting dead bacilli, PCR is rapid, precise and can be used on various clinical samples (Gopinath and Singh, 2009). The search for reliable, precise and specific serological tests for diagnosis of TBP has been an area of active research for many years (Sanai and Bzeizi, 2005). Laparoscopy-guided biopsy is advantageous for rapid TBP diagnosis, but also there is side effects related to anesthesia and potential injury and bleeding (Vogel *et al.,* 2008). An evaluation of existing techniques is urgently required as is the development of new methods with high sensitivity and specificity for early and accurate TBP diagnosis.

Interferon-gamma (IFN-γ) is an important cytokine following infection with *M. tuberculosis* (Barnes *et al.,* 1990, Jalapathy *et al.,* 2004). Several studies from different areas of the world have demonstrated the efficacy of IFN-γ for the diagnosis of TB pleural and pericardial effusions (Poyraz *et al.,* 2004; Burgess *et al.,* 2002), and its diagnostic efficacy has been compared with that of adenosine deaminase (ADA) in terms of cost-effectiveness (Sharma and Banga, 2005). Some studies have also evaluated the role of IFN-γ in the diagnosis of TB ascites (Ribera *et al.,* 1991; Soliman *et al.,* 1994; Sathar *et al.,* 2004). However, whether IFN-γ detection contributes to accurate TBP diagnosis remains controversial. Our aim is to 1) Investigate the performance and diagnostic accuracy of IFN-γ for TBP both in serum and peritoneal fluid 2) Investigate the validity of specific immunoglobulins for rapid detection of TBP compared to culture.

Material and Method

The study included twenty eight (28) patients clinically presented with symptoms suggesting of tuberculosis peritonitis, admitted to Mansoura University hospital from March 2013 till March 2014. The diagnosis of TBP was based on (1) Detection of mycobacteria on smear stained by ZN stain from ascitic fluid or peritoneal biopsy (2) *Mycobacterium tuberculosis* culture on Lowenstein-Jensen media or Mycobacterial growth indicator tubes (MGIT) (Becton Dickinson Diagnostic) of ascitic fluid and/or peritoneal biopsy specimen (3) Positive polymerase chain reaction for TB (4) Positive biopsy which taken through laparoscopic procedure. Eighteen patients were diagnosed non tuberculous peritonitis based on their clinical diagnosis eg. SLE, hepatic, cancerous and confirmed with laboratory diagnosis by TB ZN, culture, PCR and biopsy were included as a control group. The study was approved by our ethical committee of Mansoura Faculty of Medicine and a signed written consent was obtained from each patient.

Blood samples were withdrawn from each participating and sera were separated and kept frozen at -20^{0}C for further detection of specific immunoglobulins G, M and A (IgG,IgM,IgA) for TB.

Peritoneal biopsies and fluid were obtained from patients under laparoscopy. Laparoscopy was done under general anesthesia in all patients. The first trocar (10mm) was introduced by open method in all patient in the subumblical region to avoid bowel injures due to adhesions. A second 5 mm trocar introduced in the right sub costal region in the mid clavicular line. 50 ml of ascetic fluid aspirated for Ziehl Nelson (ZN) staining, culture and cytology.3 or 4 peritoneal biopsies taken by sharp biopsy forceps different sites of the peritoneum and sent for histology. Trocars sites were closed with sutures.

From non tuberculous patients ascitic fluid was obtained and subjected for ZN, culture and PCR for TB. Moreover, detection of specific IgG, IgM and IgA for TB were detected in peritoneal fluid.
Gamma interferon was measured both in blood and peritoneal fluid samples for each participating.

Culture of TB

Specimen processing

The sample of peritoneal fluid was first concentrated by using a 50 ml Falcon tube and centrifuged at 3000 *g* for about 10 min then the sediment was decontaminated by the standard N-acetyl-L-cysteine and sodium hydroxide method, with a concentration of 3% NaOH (BBL MycoPrep). After half an hour, the suspension was centrifuged and the sediment suspended in 1.5 ml sterile phosphate buffer (pH 6.8). The tube was centrifuged for 20 min at 3000 × g. Supernatant was discarded and the pellet was reconstituted with 1 mL of sterile phosphate buffer

Acid-fast bacilli smears

Smears were prepared using the dissolved sediment then stained by the ZN method, and checked for presence of AFB with a light microscope. Smears were recorded as positive if at least 10 AFB per 100 high power field (HPF) were observed. Smears that were initially recorded as scanty by the laboratory (IUATLD/WHO scale b10 AFB/100 HPF) were recorded as positive for analysis. Acid-fast bacilli may be seen on microscopy of centrifuged peritoneal fluid.

Culture of the specimen

The suspension remaining after AFB smear preparation was inoculated in parallel into both liquid (MGIT) and solid (LJ) media.

Löwenstein-Jensen Media (LJ) culture

Two LJ slants were processed for each specimen with 0.1 mL of the specimen suspension inoculated onto each LJ media. LJ slants were incubated at 37 °C for 8 weeks in an incubator and observed for growth everyday for the 1st 3 days, and then once a week until 8 weeks. Time to detection was calculated as the time from the date of culture inoculation to the earliest date of visible colonies.

Mycobacterial Growth Indicator Tube (MGIT) liquid media

The MGIT is a high volume, non radiometric instrument that offers continuous monitoring of culture growth. We inoculated 0.5 mL of specimen suspension into each 7-mL mycobacterial growth indicator tube (MGIT) culture tube, which contained Middlebrook 7H9 broth base enriched with oleic acid, albumin, dextrose, and catalase (BBL MGIT OADC) and an antibiotic mixture of amphotericin B, polymyxin B, trimethoprim, nalidixic acid and azlocillin (BBL MGIT PANTA). Tubes were incubated at 37 °C for up to 42 days. The MGIT monitors specimens hourly for an increase in fluorescence, at which time; the operator is audibly and visibly alerted to the location of tubes sensing the presence of mycobacteria. All specimens identified as positive by MGIT were confirmed using subsequent AFB smears. If no AFB were observed, the MGIT tube was returned to the instrument and incubated for a total of 42 days**.** Time to detection was calculated as the time between the date of culture inoculation and the earliest date that recorded positive growth.

Identification of mycobacteria (Srisuwanvilai *et al.*, 2008)

All specimens found to be positive from any culture were examined by AFB staining (ZN) on the day of detection to confirm the presence of AFB. AFB-positive cultures were subcultured onto LJ slants for identification. Mycobacteria were differentiated as MTB and NTM based on slow growth rate on solid media, colony characteristics and appearance, absence of pigmentation, absence of growth on LJ using para-nitrobenzoid acid, the niacin production test, and the nitrate reduction test. Contaminants were not identified.

Interferon gamma (IFN-γ) assay

IFN-γ was measured by RayBio® Human ELISA kit. It is an in vitro ELISA for the quantitative measurement of human IFN-γ cell lysate and tissue lysate. This test had antibody specific for human IFN-γ coated on a 96-well plate. We had pipetted the standards and samples into the wells and IFN-γ present in a sample is bound to the wells by the immobilized antibody. Then we had washed the wells and added the botinylated anti-human IFN-γ antibody. After washing we had put HRP-conjugated streptavidin.

The wells had been washed again, a TMB substrate solution was added to the wells and colour develops in proportion to the amount of IFN-γ bound. The stop solution changes the colour from blue to yellow, and the colour intensity was measured at 450 nm. For the assay calibration curves were blotted on semi log papers and values of OD of samples calculated from the standard curve.

Enzyme immunoassay for detection of TB IgG, IgM and IgA

These antibodies were measured by specific ELISA kits (IBL-international). It is solid phase enzyme-linked immunosorbent assay (ELISA) based on the sandwich principle. The wells were coated with antigen. The specific antibodies in the sample were fixed to the antigen coated wells and were detected by a secondary enzyme conjugated antibody specific for human IgG, IgM and IgA. After the substrate reaction, the intensity of the color developed was proportional to the amount of specific antibodies detected.

PCR of peritoneal biopsy specimen

PCR was used for the detection of DNA (IS986) specific for the mycobacterium complex. The size of the amplification product was 123 base pairs (bp). A PCR reaction was performed using an *M tuberculosis* kit (code H.02; Experteam; Venice, Italy) according to the manufacturer's instructions.

DNA Extraction: Peritoneal biopsy specimens (2 mm in diameter, and 2 to 3 punches per patient) were homogenized in normal saline solution using a sterile wide-gauge needle for each patient sample to avoid cross-contamination. Peritoneal biopsy homogenates were centrifuged at 5,000g for 10 min, and the pellet was resuspended in 162 µL lysis buffer. Eighteen microliters each lysozyme and mutanolysin were added to the pellet, which then was incubated at 37°C for 1 h. Then 20 µL proteinase K and 200 µL buffer alkaline lysate were added to the pellet and incubated at 56°C for 30 min, and for a further 30 min at 95°C. Later, DNA was extracted from the pellet with mini spin columns (QIAamp; Qiagen; Valencia, CA). To monitor for cross-contamination, one water-containing negative control tube was used per five sample tubes.

PCR: A typical PCR reaction mixture contained 2.5 µL buffer, 2.5 µL deoxynucleoside triphosphates, and 1 µL each primers BMT002 (CCTGCGA GCGTAGGCGTCGG) and BMT003 (CTCGTCC AGCGCCGCTTCGG), to which 0.3 µL Taq DNA polymerase, 7.7 µL distilled water, 10 µL DNA solution extracted with QIAamp columns from each Peritoneal biopsy sample, and 25 µL mineral oil were added. Each set of the PCR reaction contained a positive control containing DNA extracted from TB bacilli that had been isolated in our laboratory and two negative controls, one containing the same amount of DNA extracted from the lymphocytes of healthy individuals and the other containing the same amount of distilled water. The thermal cycle was programmed for 5 min at 94°C for 30 cycles, denaturation at 94°C for 2 min, annealing at 68°C for 2 min, and extension at 72°C for 2 min. The amplified products were detected by gel electrophoresis using 2% agarose gel with ethidium bromide, and a 123-bp amplified band was visualized on an ultraviolet transilluminator.

Statistical analysis

Data were analyzed using SPSS (Statistical package for Social Sciences) version 16; Qualitative data was presented as number and percentage. Quantitative data was presented as mean ±SD. Chi – square test was used for comparison of proportion. Independent sample t-test was used for comparison of quantitative data. P value of < 0.05 considered to be significant. Validity tests were done using sensitivity, specificity, Positive predictive value (PPV), Negative predictive value (NPV) and accuracy.

Receiver operative characteristic (ROC) curve for serum and peritoneal fluid INF-gamma was plot of the sensitivity versus specificity.

Result and Discussion

Mycobacteria tuberculosis was detected in 10.7% by ZN and in 85.7% by culture and or PCR. Serologically, serum IgM had statistically higher prevalence among patients with tuberculous peritonitis (64.3%, P=0.0001) followed by serum IgG (57.1%, P=0.03) .While peritoneal fluid IgG assay had significant higher prevalence among tuberculous patients (42.9%, P=0.007) (Table 1).

Positive and negative cases detected by either ZN, PCR or serological markers in relation to culture as a gold standard are shown in table 2. PCR was the most sensitive and accurate method for diagnosis of TB peritonitis (95.8%, 92.9% respectively) while ZN showed the least sensitivity and accuracy (12.5%, 25% respectively). Serologically, serum IgM was the most sensitive and accurate serological markers in diagnosis of tuberculous peritonitis (70.8%, 71.4% respectively) followed by serum IgG (62.5%, 64.3%). On the other hand, lower sensitivity and accuracy was found for the serological markers of peritoneal fluid that revealed 50%, 57.1%, sensitivity and accuracy for IgG respectively (Table 3).

Both serum and peritoneal fluid interferon gamma levels in patients with tuberculous peritonitis (mean SD=50.3$\pm$ 75.2 pg/ml, 27.7$\pm$ 48.7 pg/ml respectively) had statistically significantly (P=0.009, P=0.023) higher levels compared to the levels in non tuberculous peritonitis patients (mean$\pm$ SD 1.87$\pm$ 5.6, 0.52$\pm$ 0.5 pg/ml respectively) (Table 4)

Cut off value for serum IFN gamma of 1.1pg/ml had sensitivity 72% and specificity 81% while in peritoneal fluid the same cut off value had sensitivity 72% and specificity 76% (Figure1)

Peritoneal TB is an important and serious type of extra pulmonary TB, which can be fatal, if left untreated. It usually arises from the reactivation of latent TB foci in the peritoneum, acquired as a result of hematogenous spread from a primary lung focus. Other postulated mechanisms for acquiring the disease include hematogenous spread from contagious tissue infected by *Mycobacterium TB* (Barnes *et al.,* 1990). Early diagnosis of TBP is beneficial for treatment of TB, the prevention of complications and minimizing of mortality rate (Lingenfelser *et al.,* 1993).

The diagnosis of TBP is often delayed because the non specific clinical symptoms associated with this disease resemble those of other diseases. The development of rapid, less invasive techniques and that have high sensitivities, specificities, PPV and NPV would aid in the diagnosis of TBP and the rapid initiation of treatment (Sathar *et al.,* 2004, Shawar *et al.,* 1993).

To allow for the detection of mycobacteria in stained smears, presence of at least 5000 bacilli/ml of specimen is required (Dineeen *et al.,* 1976). In the present work, AFB smears were positive for only 3/28 of positive TBP patients (10.7%) with the least sensitivity and accuracy (12.5%, 25% respectively) and high specificity (100%). In a study for Saleh *et al.* (2012) AFB smears were positive for only 3/14 (21.4%) with 21.4% and 100% sensitivity and specificity respectively. Another study reported that the ascitic fluid smear of peritoneal TB in Qatar showed AFB in 2% (1/53) (Khan *et al.,* 2012).

Table.1 Demographic and laboratory findings in patients with TB peritonitis compared with non TB peritonitis

	TB peritonitis (n=28)	**Non-TB peritonitis (n=18)**	**P = value**
Age	45.2 ±1.5	45.2± 2.3	
Sex Male Female	 19 (67.9%) 9 (32.1%)	 8 (44.4%) 10 (55.6%)	
Ziehl-Neelsen	3 (10.7%)	0 (0%)	
Culture for TB	24 (85.7%)	0 (0%)	
PCR for TB	24 (85.7%)	0	
Combined culture , PCR & histopathology	28 (100%)	0	
Serology in serum IgA IgM IgG	 8(28.6%) 18 (64.3%) 16 (57.1%)	 2(11.1%) 1(5.6%) 4 (22.2%)	 0.3 0.0001 0.03
Serology in peritoneal fluid IgA IgM IgG	 0 4 (14.3%) 12 (42.9%)	 1 (5.6%) 1 (5.6%) (5.6%)	 0.4 0.6 0.007

Table.2 Number of positive and negative cases detected by either ZN, PCR or serology in relation to culture. Values are number (%)

	Culture/Histopathology (n=28)		**Total**	**P-value**
	Positive	**Negative**		
ZN : Positive: Negative: Total:	 3 (12.5) 21 (87.5) 24 (100)	 0 4 (100) 4 (100)	 3 (10.7) 25 (89.3) 28 (100)	 0.61
PCR : Positive: Negative: Total:	 23 (95.8) 1 (4.2) 24 (100)	 1 (25) 3 (75) 4 (100)	 24 (85.7) 4 (14.3) 28 (100)	 0.005
Serum IgA: Positive: Negative: Total:	 8 (33.3) 16 (66.7) 24 (100)	 0 4 (100) 4 (100)	 8 (28.6) 20 (71.4) 28 (100)	 0.23
Serum IgM : Positive: Negative: Total:	 17 (70.8) 7 (29.2) 24 (100)	 1 (25) 3 (75) 4 (100)	 18 (64.3) 10 (35.7) 28 (100)	 0.11

Serum IgG : Positive: Negative: Total:	 15 (62.5) 9 (37.5) 24 (100)	 1 (25) 3 (75) 4 (100)	 16 (57.1) 12 (42.9) 28 (100)	 0.19
Fluid IgA: Positive: Negative: Total:	 0 24 (100) 24 (100)	 0 4(100) 4 (100)	 0 28 (100) 28 (100)	 -
Fluid IgM: Positive : Negative: Total:	 4 (16.7) 20 (83.3) 24 (100)	 0 4 (100) 4 (100)	 4 (14.3) 24 (85.7) 28 (100)	 0.51
Fluid IgG: Positive : Negative: Total:	 12 (50) 12 (50) 24 (100)	 0 4 (100) 4 (100)	 12 (42.9) 16 (57.1) 28 (100)	 0.08

ZN= Ziehl-Neelsen stain
PCR= Polymerase Chain Reaction

Table.3 Diagnostic value of ZN, PCR, and Serological markers used for detection of TB peritonitis compared to culture as a gold standard. Values are percentages

	Sensitivity	**Specificity**	**PPV**	**NPV**	**Accuracy**
ZN	12.5	100	100	16.7	25
PCR	95.8	75	95.8	75	92.9
Serum IgA	33.3	100	100	20	42.9
Serum IgM	70.8	75	94.4	30	71.4
Serum IgG	62.5	75	93.8	25	64.3
Fluid IgA	0	100	0	14.3	14.3
Fluid IgM	16.7	100	100	16.7	28.6
Fluid IgG	50	100	100	25	57.1

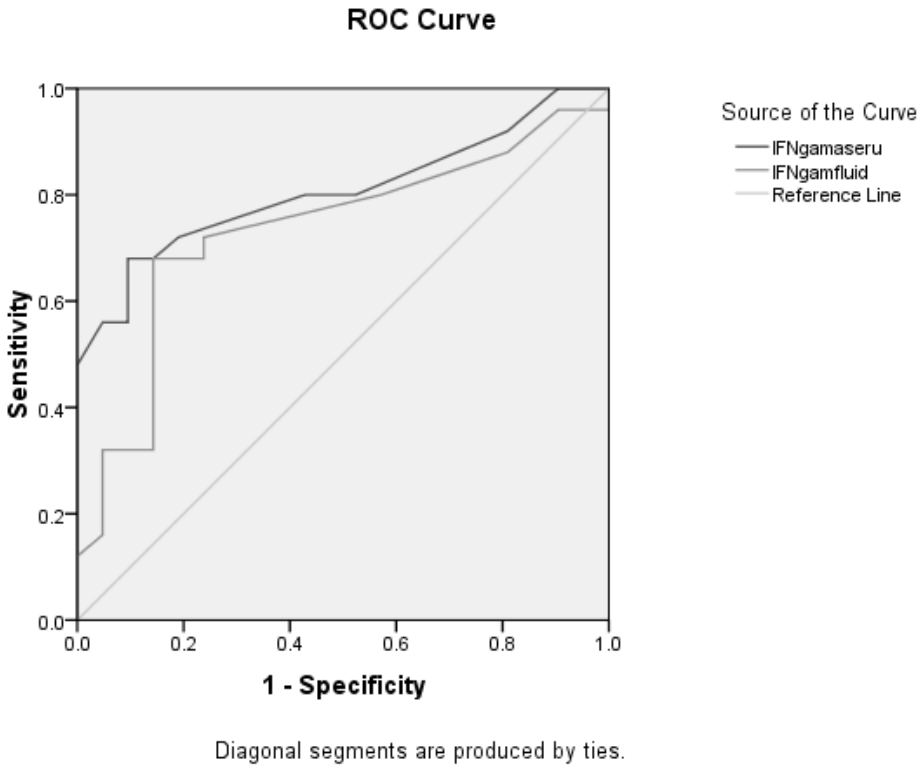
PPV= Positive predictive value
NPV= Negative predictive value
ZN= Ziehl-Neelsen stain
PCR= Polymerase Chain Reaction

Table.4 Interferon gamma values of peritoneal fluid and serum in the studied groups. Values are mean ± SD

	TB peritonitis (n=28)	Non-TB peritonitis (n=18)	95% confidence interval	P-value
Peritoneal fluid IFN gamma	50.3 ± 75.2	1.87 ± 5.6	12.5 – 84.4	0.009
Serum IFN gamma	27.7 ± 48.7	0.52 ± 0.5	3.98 – 50.39	0.023

IFN gamma= Interferon gamma

Figure.1 Receiver operative characteristic curve for serum and peritoneal fluid IFN gamma



Sanai and Bzeizi (2005) reported that ZN staining of ascetic fluid for mycobacterial detection is positive in only about 3% of proven TBP cases. AFB smears are too insensitive to meet the current needs (Inadomi *et al.*, 2001).

The gold standard for diagnosis of TB is either isolation of *mycobacteria tuberclulosis* (MTB) by culture or detection of MTB-specific nucleic acid by molecular methods (Diagnostic Standards and Classification of Tuberculosis in adults and children (2000). Updated guidelines for the use of nucleic acid amplification tests in the diagnosis of tuberculosis. We detected TB either by culture after centrifugation and/or PCR in 24/28 of TBP cases (85.7%). Previous studies showed significantly improvement in TB culture of TBP patients

by centrifugation of large volume of ascitic fluid from 35% (culture of fluid by regular method) to 66-83% (after centrifugation) (Chow *et al.,* 2002, Menzies *et al.,* 1986). However in addition to requiring laboratory infrastructure, culture methods have a long turnaround-time, taking weeks to months.

PCR has the highest specificity and accuracy in our results (95.8%, 92.9%) respectively. Previous reports suggested that the performance of various PCR tests is reasonably good with sensitivity reaching up to 95%. Molecular methods have high cost and technology requirements. Also, the issue of false-positives as a result of cross-contamination of samples has raised concerns of over-diagnosing TB in areas of endemicity. Samples from sites with a possible latent infection focus or DNA from dead bacilli may also give a positive reaction (Chow *et al.,* 2002).

TB serology offers an alternative method for TB diagnosis and does not require a specimen from the site of the disease and can be considered as a rapid, inexpensive format requiring little laboratory equipments. So, it was considered option for resource-limited settings and could ultimately serve as a point-of-care test (Achkar *et al.,* 2011).

There is controversy about the use of serological tests for diagnosis of TB. The sensitivity of TB serology estimates range from 0% to 90% and specificity estimates range from 47% to 100% (Steingart *et al.,* 2007). In our study, serological results revealed that serum IgM had a statistically significant higher prevalence among TBP patients (64.3%, P=0.0001) followed by serum IgG (57.1%, P=0.03) while peritoneal fluid IgG had a significant higher prevalence among TB patients (42.9%, P=0.007). So, serum IgM was the most sensitive and accurate serological marker in diagnosis of TBP patients (70.8%, 71.4% respectively) followed by serum IgG (62.5%, 64.3%).

In a South African study dealing with active disease, an ELISA to detect IgG found it to be highly sensitive (approaching 100%) and 97% specific for pleural and ascetic fluid (Wadee *et al.,* 1990). In another report from India, the seropositivity of the IgA and IgG was 88.4% in active gastrointestinal TB (Gupta *et al.,* 1995).

Pottumarthy *et al.* (2000) evaluated seven commercially available serological tests and found that the diagnostic sensitivities of these tests in patients with active TB ranged from as low as 16% and maximum up to 57%.

Steingart *et al.* (2011) found that ELISA has pooled sensitivity of 60%. WHO made a policy statement that commercial serological tests provide inconsistent and imprecise findings resulting in highly variable sensitivity and specificity adversely impacting patient safety. Overall data quality was graded as very low and it is strongly recommended that these tests should not be used for the diagnosis of both pulmonary and extra-pulmonary TB (Singh and Katoch, 2011). The basic promise of serological tests was ease, rapidity and ever increasing demand in TB endemic countries.

M. tuberculosis infection initiates an immunologic cascade involving the secretion of various cytokines and recruitment of Th1 lymphocytes with abundant cell recruitment at the morbid site, the levels of various cytokines are markedly elevated, IFN- gamma is an important cytokine following infection with *M. tuberculosis* (Jalapathy *et al.,* 2004).

In our study, both serum and peritoneal fluid interferon gamma levels in TBP patients (mean SD=50.3±75.2 pg/ml, 27.7± 48.7

pg/ml respectively) had significantly (P=0.009, P=0.023) higher levels compared to the levels in non TBP patients (mean ±SD 1.87±0.56, 0.52±0.5 pg/ml respectively). In addition, ROC characteristic for both serum and peritoneal IFN- gamma showed equal sensitivity (72%) and differ marginally in specificity (81% vs. 76%).

Sathar *et al.* (2004) had showed that ascetic IFN- gamma concentration were significantly higher (P<0.0001) in TBP patients (mean 6.70 U/ml than malignant and cirrhotic groups with 93% sensitivity and 98% specificity. Sharma *et al.* (Sharma *et al.,* 2006) observed higher levels of ascetic fluid IFN- gamma: 560 pg/ml vs. 4.85 pg/ml (P <0.001) were significantly different between TB and non-TB groups with higher sensitivity and specificity (97%, 97%).

Saleh *et al.* (2012) had used QFT-G assay using blood samples that gave sensitivity 92.9% and specificity 100%.

Kobashi *et al.* (2006) found that the use of QFT-G assay for detection of TB in patients with active pulmonary TB yielded sensitivity 86% and specificity 94%. Also, Mori *et al.* (2004) reported a sensitivity of 89% in a selected population of patients with clinical signs suggestive of TB. Another blinded prospective study of 82 patients with clinical signs of active TB showed that QFT-G assay was 85% sensitive for the detection of TB (Ravan *et al.,* 2005).

We concluded that PCR has the highest sensitivity and accuracy but their limitations are the high cost and the issue of false-positives. IFN-gamma for TBP either in serum or peritoneal fluid is a new promising, rapid and easy test with good sensitivity and specificity. The diagnostic performance of the specific immunoglobulins in our research gave us the best result was serum IgM followed by serum IgG. We recommend a combination of different diagnostic tests in order to confirm the diagnosis of TBP. Up till now, there is no single test that can consistently yield a diagnostic tool by itself.

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