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Original Research Article

Diagnosing genital tuberculosis in female infertility by clinical, histopathological, culture and polymerase chain reaction techniques: an evaluative study

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ABSTRACT

Background: In developing countries, the genital tract tuberculosis is one of the common causes of tubal damage leading to infertility. Objective of present study was to evaluate the efficacy of Histopathological examination (HPE), culture and Polymerase Chain Reaction Technique in diagnosing genital tuberculosis.
Methods: It was a prospective evaluative study. 173 women were subjected to investigations for tuberculosis. AFB smear, culture and HPE examination and PCR testing were carried out on 173 endometrial samples, 81 POD fluid and 52 urine samples. Based on the clinical profile and laparoscopic findings a diagnostic criterion was derived to suspect GTB and the specific diagnostic tests were evaluated against this diagnostic criterion.
Results: Based on the diagnostic criteria, tuberculosis was suspected in 61 of the 153 cases. AFB smear was positive in 4.6%, culture was positive in 3.5%, HPE positive in 4.0% and PCR was positive in 28.1% of cases. On evaluating against the diagnostic criteria, the sensitivity of PCR, HPE, culture and AFB smear were; 44.3%, 8.2%, 6.6% and 6.7% respectively. PCR was positive in 18 of the 92 cases in whom GTB was not suspected. The PCR results were negative in 34 of the 61 clinically suspected cases.
Conclusions: This study has shown that HPE, AFB smear and culture have low pick up rates. PCR is found to be useful in confirming diagnosis in clinically suspected cases. False negative PCR was an important limitation in this study.

Keywords: Diagnostic methods, Female infertility, Genital tuberculosis

INTRODUCTION

In developing countries, the genital tract tuberculosis is one of the common causes of tubal damage leading to infertility. When tuberculosis affects the genital organs of young females, the disease often remains silent, or may present with non specific symptoms. As a result, the disease is either not diagnosed at all or diagnosed at an advanced stage, when permanent damage has already occurred. At this stage, in spite of treatment with medical and surgical methods, the prognosis for fertility is poor.[1,2]

In developing countries like India, genital tuberculosis (GTB) is the major causative factor for severe tubal disease requiring assisted reproduction techniques.[3] The incidence of GTB varies greatly from country to country, being highest in India and South African countries compared to Western countries. Various Indian studies have shown that tuberculous endometritis and salpingitis account for 4-9% of all infertility cases.[4,5]

The justification for this study was based on the fact that, the prevalence of GTB is high in developing countries

and is one of the major causes of tubal infertility, but the diagnosis of the condition is difficult and challenging. Unlike the tuberculosis affecting the other parts of the body, especially of the lungs, GTB eludes diagnosis and remains undiagnosed. A high degree of suspicion is required to initiate investigations to diagnose the disease.

Clinical parameters such as Mantoux (Mx) and ESR are not specific to diagnose the disease. Although characteristic features have been described, an absolute diagnosis of GTB cannot be made from hysterosalpingogram (HSG) or from the characteristic findings at laparoscopy.[6] Therefore, specific diagnostic tests are needed to diagnose GTB. But, the culture and histo-pathological examination (HPE) have low pick up rates. In recent years, PCR technique has evolved as a rapid technique for the diagnosis of pulmonary and extra-pulmonary tuberculosis. In a country like India, there is a need to diagnose GTB early, so that treatment with medical methods may improve the prospects of cure, thereby preserving fertility. The aim of this study was to analyse the clinical parameters to arrive at some criteria to suspect GTB, based on which specific investigations can be initiated and to evaluate the efficacy of AFB smear, culture, HPE, PCR in diagnosing GTB in female infertility.

METHODS

This prospective study was conducted at the Institute of Obstetrics and Gynecology, Chennai from January 2008 to December 2012. The patients were followed up from 2013 to 2015. The study consisted of 173 subjects who met the inclusion criteria. Ethical committee approval was obtained, and informed consent was obtained from each patient. Sampling was done by convenience sampling technique. Women in whom tubal damage was suspected or proved by HSG and / or by laparoscopy, and those with unexplained infertility were included in the study. Women in whom infertility was due male factors, ovulation disorders, endometriosis, sexual dysfunction, and peritoneal adhesions were excluded from the study.

A detailed history was taken regarding demographic details, gynecological symptoms, and past history of TB. Details of previous investigations and treatment were noted to rule out other causes of infertility. After physical and gynecological examination all subjects underwent the following investigations: Hb%, TC, DC, ESR, Mx test, chest x-ray, and HIV I and II tests. All patients had USG and laparoscopy and HSG was carried out in 131 cases. In order to rule out other organisms such as Chlamydia trachomatis and Neisseria gonorrhoea which can also cause tubal damage, the AMPLICOR CT/NG PCR Test (Roche Diagnostic Systems, Inc.,) was performed on the endocervical swabs taken from 173 women.[7]

For specific investigations, the material for the study was collected from the Pre-menstrual endometrium, fluid from pouch of douglas and urine. The collected samples were subjected to AFB smear examination, culture, HPE and PCR studies. For HPE, a portion of the endometrial tissue was fixed in 10% formalin; routine processing was done and stained with haematoxylin and Eosin. For microscopic examination of AFB, biopsy material was ground well and the concentrated mix was taken for smear and was stained with Ziehl Neelsen stain. For culture, the tissue sample was centrifuged, and the deposit was decontaminated by 5% H_2SO_4 and added to culture media namely: Lowenstein Jensen Medium (L.J.), L-J enriched with sodium pyruvate (L.J.P.) and Selective Kirchner's medium (SK). At the end of 8 weeks, mycobacterium culture (MTB) was identified in positive cultures and confirmed by biochemical reaction.

PCR test was carried out using IS6110 and TRC4 probes. The test involved processing of samples, amplification and isolation of DNA. PCR test was performed using Gene amplification 9700 Thermal cycler with standard 25□l working volume. (Gene Amplification PCR System 9700- Applied Biosystems, USA). Precautions were taken to avoid false positivity. In order to avoid carry over contamination, preparation of PCR reagents, addition of template DNA and analysis of amplified products were done in three different rooms. Reagents were aliquoted and each aliquot was used only once. By adding wax beads non-specific amplification was avoided. DNAs from the samples were amplified using the following primers: IS6110a and IS6110b primers (PRIMER DESIGNER – Version 2.0 – copy right 90,91 Scientific and Educational Software), and TRC4 primers 1 and TRC4 primer 2 (TRC4 nucleotide sequence has been assigned Gen Bank Accession GenBank Accession No. µ 84405). The IS6110 primers amplify a fragment with a length of 123bp, while the 18-mer TRC4 primers amplify a fragment with a length of 173bp. DNA extraction chemicals and PCR chemicals were obtained from USB, Amersham Bioscience. Detection of amplified products was done by agarose gel electrophoresis (2%) at 80 volts for 45 minutes. Gel was stained with ethidium bromide and viewed under UV transilluminator. (VILBER-LOURMAT, France, TCP- 20.M) The technician performing the PCR technique was blinded to the clinical impression of tuberculosis and the results of other investigations.

Evaluation of specific diagnostics

Cultivation of MTB is considered the Gold standard for the diagnosis of tuberculosis. In GTB one cannot use culture as gold standard as the detection rate of culture is very low. In conditions, where there is no gold standard technique available to evaluate a diagnostic test, one may have to develop and justify a combination of clinical profile and criteria against which the new test has to be assessed.[8] Therefore, based on the results of clinical profile and laparoscopic evaluation, a group of clinical parameters were identified and using these parameters a diagnostic criterion was derived to suspect GTB. In this study, a woman was said to be suspected of having GTB

if she has had findings suggestive of tuberculosis by laparoscopy with one or more of the following findings: past history of tuberculosis, a positive Mantoux test, an elevated ESR, and characteristic findings suggesting tuberculosis on HSG. This newly derived diagnostic criteria were used to evaluate the efficacy of AFB smear, culture, HPE and PCR in diagnosing GTB.

Follow-up of patients

Patients who were positive for MTB by HPE, culture or PCR were treated with standard Anti tuberculosis treatment (ATT) and were followed up.

Statistical analysis

Data entry was done using Microsoft Excel in Windows^{xp}. Analysis was performed in OPEN EPI VERSION 2.2.1 software.

Inferential Statistics the specific diagnostic tests

AFB smear, culture, HPE and PCR were evaluated against the newly derived criteria by using bivariate two by two tables. Sensitivity, specificity, Positive Predictive Value (PPV) and Negative Predictive Value (NPV) were calculated with 95% confidence interval. Also, chance corrected agreement was evaluated using Kappa statistics for PCR results of endometrium sample and POD aspirate.

RESULTS

The patients were aged between 20 and 37 years. The mean age of presentation was 27.35 years. 161 (93.1%) patients were investigated for primary infertility and 56.6% of women did not have any Gynaecological symptoms other than infertility. Menstrual disturbances such as secondary amenorrhoea, oligomenorrloea and menorrhagia were seen in 48 (27.7%) cases. Dysmenorrhoea and dyspareunia were seen in 15.6% and 1.7% of cases, respectively. Vaginal discharge not responding to treatment for more than two years was seen in five cases. Other symptoms such as abdominal bloating, bowel disturbance was seen in two cases each. Urinary symptom with haematuria was seen in one case. Chronic pelvic pain not responding to treatment was seen in 9 (5.2%) cases. A definite past history of tuberculosis was available in 11 (6.4%) cases affecting the lungs, axillary nodes, cervical nodes and gastro – intestinal tract, 2-15 years earlier and were treated with ATT for 9 months to 2 years. In seven cases (4%) there was a history of close contact with family members who were treated for tuberculosis in the past.

ESR was elevated more than 15 mm in one hour in 27 (15.6%) cases. On X-ray chest, only four patients (2.3%) showed evidence of old healed lesions. HIV testing was negative in all women. A positive Mx test with an induration of >10 mm was seen in 37 (21.4%) cases. The USG findings were suggestive of tuberculosis in 23 (13.3%) cases with adnexal masses, loculated ascites, and calcification.

Table 1: Investigation results and laparoscopy findings.

Diagnostic parameter	No. of cases with abnormal results	Percentage
ESR	27	15.6
X-ray chest	4	2.3
Mantoux	37	21.4
USG	23	13.3
HSG	57/131	47.5
Laparoscopy findings		
Normal	93	53.8
Definite evidence of TB	20	11.6
Probable evidence of TB	18	10.4
Suspicion of TB	42	24.2
Total	173	100

Results of tubal evaluation by HSG was available in 131 cases and 57 (43.5%) of them showed abnormal findings. Among them, characteristic features of tuberculosis such as calcification, beaded appearance of tube, distorted uterine cavity, intravasation of dye, and fimbrial block with large hydrosalpinx, were seen in 22 cases. At laparoscopy, the findings were suggestive of tuberculosis in 80 (46.2%) of them. In 20 of these 80 cases, there was a definite evidence of tuberculosis with findings such as granulomas, caseation, calcification and tubercles. In another 18 cases (10.4%), there was probable evidence of tuberculosis with hydrosalpinx, dilated retort shaped tubes, tubes covered with white plaques and exudates, dense adhesions and loculated ascites. In the remaining 42 cases (24.2%), there was suspicion of tuberculosis because of minimal adhesions, and cornual block (Table 1).

Table 2: Results of specific diagnostic tests for GTB.

Test	No.	Positive result	Percentage
Endometrial samples			
AFB Smear	173	8	4.6
Culture	173	6	3.5
HPE	173	7	4.0
PCR	160	45	28.1
POD fluid			
AFB Smear	81	5	6.2
Culture	81	0	0
PCR	81	16	19.8
Urine			
Culture	52	0	0
PCR	52		7.7

Table 2 shows the results of specific diagnostic tests in the endometrial samples, POD aspirate and urine. In the

endometrial samples, the PCR positivity was higher compared to HPE and culture. In the POD aspirate, though the AFB smear was positive in 6.2% of cases, culture was negative in all cases and the PCR positivity was seen in 19.8% of cases. Similarly. all urine samples were negative by culture but the PCR was positive in 7.7% cases.

Among the 173 women studied, 13 women were positive for Chlamydia trachomatis and 3 were positive for gonococcal infection. For the evaluation of specific diagnostic tests, only cases in whom all four specific test results were available and those who were negative for gonococci and Chlamydia were included. 153 cases fulfilled the above criteria.

Based on the clinical profile and laparoscopic findings an attempt was made to identify a group of clinical parameters to derive at criteria to suspect GTB.

A positive finding at laparoscopy in the absence of gonococcal or Chlamydia infection was taken as a major criteria to suspect GTB with one or more of the following findings: A definite past history of tuberculosis, characteristic features on HSG, elevated ESR, and positive Mantoux test. In 11 women with past history of tuberculosis, in 10 of them there was evidence of tuberculosis by laparoscopy, Mantoux test was positive in 9 cases, ESR was elevated in 7 cases, and PCR was positive in 8 cases. Culture and HPE were positive in 2 cases. As in 10 of the 11 cases, one or more of the other diagnostic parameters were positive a past history of tuberculosis was taken as one of the parameters to arrive at a diagnostic criterion to suspect GTB.

Among the 27 cases with elevated ESR, in 21 of them the other diagnostic parameters were also positive. Therefore, elevated ESR has been included as one of the components to suspect tuberculosis.

In 29 of the 37 cases with positive tuberculin test, other diagnostic parameters were also positive. Therefore, Mx test has been taken as one of the components of the diagnostic criteria to suspect GTB. Characteristic features seen on HSG was also taken as a criteria to suspect GTB.

Based on the above criterion cases were divided into two groups.

- Group A: Those with suspicion of tuberculosis 61/153
- Group B: Those in whom tuberculosis was not Suspected 92/153.

On evaluating the specific diagnostic tests: AFB smear, culture, HPE and PCR against the newly derived diagnostic criteria the HPE and culture were highly specific in predicting the disease with 100% PPV. But, the sensitivity was very low. The sensitivity of PCR was 44.3 % and the specificity was 80.4% (Table 3).

Table 3: Results of comparative evaluation of various diagnostic tests in endometrial samples.

Test	Sensitivity	Specificity	PPV	NPV
PCR	44.3% (95% CI 32.51,56.7)	80.4% (95% CI 71.18, 87.25)	60% (95% CI 45.45, 72.98)	68.5% (95% CI 59.25, 76.51)
HPE	8.2% (95% CI 13.55, 17.79)	100% (95% CI 95.99, 100)	100% (95% CI 56.55, 100)	62.1% (95% CI 54.13, 69.57)
Culture	6.6% (95% CI 12.57, 15.68)	100% (95% CI 95.99,100)	100% (95% CI 51.01, 100)	61.7% (95% CI 53.74, 70.01)
AFB smear	6.7% (95% CI 2.57, 15.68)	98.9% (95% CI 94.1, 99.81)	80% (95% CI 37.55,96.38)	61.5% (95% CI 53.45, 68.94)

Table 4: Results of PCR evaluated against the clinical criteria.

PCR	Clinical criteria		Total
	Positive	Negative	
Positive	27	18	45
Negative	34	74	108
Total	61	92	153

The PCR results were further evaluated for false positive and false negative results. Out of the 153 cases, in 34 patients the clinical criteria were positive, but the PCR results were negative. This could indicate the possibility of false negative PCR results. Of the 153 women, in 18 of them PCR was positive, but the clinical profile was negative. This raises the possibility of false positive PCR results. In 13 of these 18 cases, PCR was positive both in the endometrium and POD aspirate and 2 urine samples were also positive for PCR (Table 4).

In 81 women, PCR results were available both in endometrium and POD aspirate. Out of 16 cases that showed positivity in POD aspirate, 13 of them were positive in the endometrial samples also. In 66 (81.5%) of the 81 samples there was (crude) agreement in the results and the Kappa statistics of chance corrected agreement was 52% which indicates good agreement (Table 5).

Table 5: PCR results of 81 endometrium and POD aspirate samples.

POD aspirate	Endometrium sample		Total
	Positive	Negative	
Positive	13	3	16
Negative	12	53	65
Total	25	56	81

Kappa = 52% (good agreement)

Reliability of PCR in diagnosing GTB was checked by repeat sampling on patients and re-testing of saved samples. On repeat PCR testing, in 9 of the 10 endometrial samples, there was agreement in the results of PCR between the first visit and second visit (Table 6).

Table 6: Results of PCR on repeat sampling.

Case No.	First visit		Repeat visit		Clinical criteria
	IS 6110	TRC_4	IS 6110	TRC_4	
16	Neg.	Pos.	Neg.	Pos.	Pos.
36	Neg.	Neg.	Neg.	Neg.	Pos.
81	End-Pos POD-Pos Uri.-Pos	Neg.	Pos.	Neg.	Neg.
96	Pos.	Neg.	Pos.	Neg.	Neg.
100	Neg.	Neg.	Neg.	Neg.	Neg.
108	Neg.	Pos.	Neg.	Neg.	Neg.
111	Neg.	Neg.	Neg.	Neg.	Pos.
114	Neg.	Neg.	Neg.	Neg.	Pos.
120	Neg.	Neg.	Neg.	Neg.	Pos.
128	Neg.	Neg.	Neg.	Neg.	Neg.

Re-testing was also carried out in 10 of the 34 reported false negative samples and 9 of the 18 reported false positive samples. On repeat testing, all the 10 negative samples were consistently negative and 8 of the 9 positive samples were consistently positive. Seven women positive by conventional methods, and 45 women positive by PCR were treated with ATT for 9 months; initial two months with isoniazid (600 mg.), rifampicin (450 mg.) and ethambutol (1200mg.) thrice weekly followed by isoniazid and rifampicin thrice weekly for the next 7 months. At 3 years follow-up 6 pregnancies were reported in those patients who were positive by PCR and had negative clinical criteria.

DISCUSSION

GTB is a difficult disease to diagnose even with intense investigations. In countries with high prevalence of TB, there is always dilemma as to whether all infertile women should be investigated for GTB with specific investigations. One of the aims of our study was to arrive at a diagnostic criterion to suspect GTB, based on which specific investigations can be initiated. Though definite evidence of TB was seen only in 20 cases, we have taken positive findings at laparoscopy as the main criteria to suspect GTB, as we have already ruled out gonococcal and chlamydial infections. Other parameters such as past history of TB, positive Mx, raised ESR and abnormal findings at HSG were taken as additional criteria, as there were corroborative evidences from other test results to suspect GTB. Similar attempts have been made in the past using simplified algorithm looking for a presumptive evidence of GTB.[9]

For a smear test to be positive there should be 10,000 organisms per milliliter of specimen.[10] Due to the extra-pulmonary site, AFB positivity is low in endometrial samples. In present study, only 4.6% of the endometrial samples and 6.2% of the POD aspirates were positive for AFB smear and our results are similar to that of Rozati et al study.[11] Absence of AFB does not exclude the diagnosis of GTB and positive smears must be followed by culture to confirm MTB.[12] MTB culture remains the gold standard technique for the diagnosis of tuberculosis. However, in spite of inoculation into multiple media, in our study only six samples (3.5%) were positive for MTB. Other studies have also shown such low detection rates by culture.[13,14] This low pick up rates may be due to the paucibacillary nature of the endometrial site or to the presence of a bacteriostatic substance which inhibits the growth of the bacilli.[15] The detection rate by HPE was also low, as only 7 (4%) samples were positive for tuberculosis and similar observations were made by other authors.[13,16,17] The possible reasons could be that, during sampling the infected site can be easily missed and in 50% of cases, the infection may be limited to the fallopian tubes.[18] Moreover, because of the cyclical shedding, the endometrium may not show evidence of tuberculosis in all the cycles.[19]

In recent years, PCR technique has been found to be useful in confirming the diagnosis in a substantial number of GTB.[20,21] In present study PCR was positive in 28.1% of endometrial samples. The PCR positivity varies from 22.5% to 57.3% in various studies.[13,14,22,23] One of the advantages of PCR is that, it can be applied to sterile fluids like peritoneal fluid where the culture is difficult due to low bacterial load.[24] In present study, PCR was positive in 19.8% of POD aspirate samples. Similar results were reported by Bhanu et al, where 16% of POD aspirate were positive for PCR.[13] In this study 4 of the 52 urine samples were also positive for PCR reaction.

In the past authors have suspected GTB clinically and evaluated various specific diagnostic tests against the clinically suspected cases.[11] Bhanu et al have used laparoscopic findings to suspect GTB and to evaluate the diagnostic tests.[13] Jindal et al, has used a simplified algorithm looking for a presumptive evidence of GTB.[9] Therefore, in order to evaluate the specific diagnostic tests, a diagnostic criterion was derived to suspect GTB and the specific diagnostic tests were evaluated against the newly derived diagnostic criteria. Though, culture and HPE are specific tests, the sensitivity of HPE and culture were low 8.2% and 6.6% respectively, compared to PCR showing a sensitivity of 44.2%. Other authors have shown a higher sensitivity of PCR in endometrial

samples.[11,13] The major concern in using PCR in the diagnosis of GTB is its false negative and false positive results. In 34 patients there was a possibility of false negative PCR results. When comparing PCR results with the conventional methods of diagnosis, in the seven cases that were positive by either culture or HPE, PCR was positive only in three cases. Similar observation was also made by Rozati et al where in 65 clinically suspected GTB, four of the seven histology positives were not supported by PCR.[11] Bhanu et al study also reported that, 63.9% of their samples were negative for the mpt 64 gene in spite of the positivity based on the laparoscopy.[13] The possible explanation for these false negative results of PCR could be due to the presence of inhibitors of PCR. Blood containing specimens are characterized by the presence of numerous PCR inhibitors. Restrepo et al have shown that mycobacterial DNA amplification was compromised when the human: bacterial genome ratio was at least 190:1.[25] As endometrial samples are always mixed with blood, this could possibly explain the false negative results in this study.

In 18 patients PCR showed false positive results. During this study in order to avoid contamination, precautions were taken in collection, processing of the specimen and handing of the reagents. In order to avoid carry over contamination, three separate rooms were used for preparing the of PCR reagents, for adding template DNA and for the analysis of amplified products. The prepared reagents were used only once. Wax beads were added to minimize non-specific amplification. Also, each PCR run was controlled by adding concurrent negative control (without templates). No positive bands were noted in these controls and all were PCR negative Therefore, it is unlikely that positive PCR reaction and a negative clinical profile is a case of false positivity. But PCR has probably detected the early disease with low number of bacilli or with latent infection when women are still asymptomatic and before structural damage to the tube has taken place. During early stages of infection, subtle clinical manifestation of GTB may be overlooked at laparoscopy. The pelvic structures and the covering peritoneum may appear normal; however, there may be an outpouring of bacilli from the tube into the uterine and peritoneal cavity. Vynck et al from South Africa (1990) reported that, in proved cases of GTB by microbiological studies on menstrual fluid, in 44.5%, the pelvis was clear at laparoscopy.[26] Similar findings were shown by Deshmukh et al from India, where 3 of the 45 histologically proved GTB did not show evidence of TB at laparoscopy.[5] The problem of false positivity can also be minimized by way of multiple areas of sampling and repetitive sampling. In present study, in 13 of the 18 cases, PCR was positive both in the endometrium and POD aspirate and 2 urine samples were also positive for PCR. Our study has shown that, false positive results are probably true positives. In repeat sampling also, there is concordance in the results of PCR between the first and second visit in 90% of cases. Bhanu et al study also suggested that multiple sampling and repeat sampling from the patient will enhance the sensitivity of PCR as a diagnostic tool.[13] The PCR test was also re-validated by re-testing of samples. Six pregnancies were reported following treatment with ATT in PCR positive patients. As in our study, Puri et al have reported six pregnancies following ATT treatment in PCR positive cases.[27]

CONCLUSION

In areas where TB is endemic, the possibility of GTB should always be considered in infertile women. Though non-specific investigations, HSG, and laparoscopy findings would suggest the possibility of tuberculosis, they are not sufficient criteria to diagnose and treat GTB. However, they indicate the need for further investigations. Given the low sensitivity of AFB smear, culture and HPE, PCR with its comparatively higher sensitivity may be a useful tool in diagnosing GTB in clinically suspected cases. Based on the results of PCR, our study has shown that a positive PCR in a patient with reasonably high pre-test possibility is fairly confirmatory of tuberculosis. Therefore, in clinically suspected cases, in the presence of positive PCR results, an infertile woman should be considered as having GTB and should be treated. Our study has also shown that PCR is able to pick up early/ latent GTB. Therefore, in women with unexplained infertility, even in the absence of positive clinical criteria, PCR of the endometrium should be carried out and if positive, should be treated. The strength of this study is that, in a large series of 173 patients, clinical, histology, microbiology and molecular biological features have been evaluated and a clinical criteria has been derived to suspect GTB. This study has also evaluated the results of diagnostic tests on largest number of samples from multiple sources. And in a proportion of patients, false positive results were ruled out by multiple specimens processing from the same patient. This study is one of the first ones where re sampling and repeat testing was done for PCR results. The limitation of this study is that the diagnostic parameters have been evaluated against the assumed diagnosis of GTB. However, we are probably justified in this action as there is no ‘gold standard technique available’ to evaluate the diagnostic tests for GTB. Also, the high false negative result is an important limitation in this study. A negative PCR may result in missing the diagnosis in a few cases. Therefore, when GTB is suspected clinically, but the PCR results are negative, it indicates the need for further evaluation.

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