Original Article

A Study on Diagnostic Evaluation of Two Different Rapid DNA Polymerase Chain Reaction Techniques Namely Gene Xpert Mycobacterium Tuberculosis/Rifampin (MTB/RIF) and Mycoreal Polymerase Chain Reaction in the Diagnosis of Endometrial Tuberculosis Considering Culture as Gold Standard

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Abstract

Context: The study involves the evaluation of two polymerase chain reaction (PCR) techniques one of which has been endorsed by the WHO for their diagnostic capabilities. **Aims:** The aim of this study is to evaluate the diagnostic accuracy of GeneXpert mycobacterium tuberculosis/Rifampin (MTB/RIF) and mycoreal PCR techniques in the diagnosis of endometrial tuberculosis (TB) considering culture as the gold standard. **Settings and Design:** A retrospective study conducted at Gunasheela surgical and maternity hospital. Patients who attended the outpatient department between January 2013 and August 2016, satisfying the eligibility criteria, were included in the study. **Methodology:** Women included in the study underwent endometrial pipelle sampling premenstrually after ruling out pregnancy in that cycle. Endometrial samples were tested for TB by Mycoreal PCR, Gene Xpert and BACTEC culture. **Statistical Analysis Used:** Statistical analysis was done using the R software version 3.6.1. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and accuracy of test were calculated. **Results:** A total of 3229 samples were analyzed, of which 1754 were evaluated by Mycoreal TB PCR and 1475 were evaluated by Gene Xpert MTB/RIF assay. The sensitivity of mycoreal TB PCR technique was 34.78%, specificity was 99.08%, PPV was 33.33%, NPV was 99.13%, and accuracy was 98.23%. The sensitivity of GeneXpert MTB/RIF technique was 6.90%, specificity was 99.79%, PPV was 40.00%, NPV was 98.16%, and accuracy was 97.97%. **Conclusions:** MYCOREAL seemed to be more sensitive than Gene Xpert (MTB/RIF) considering culture as the gold standard in the diagnosis of endometrial TB.

Keywords: *Bactec culture, Gene Xpert (MTB/rifampin), mycoreal tuberculosis polymerase chain reaction*

Introduction

Incidence of female genital tuberculosis (FGTB), which is 3%–16%, is very high among women seeking infertility treatment in India.[1] Due to its varied clinical presentation and more often noninformative results on imaging and endoscopy, there is a lacuna in the ability to diagnose genital tuberculosis (TB).[2] There are also an array of tests available for diagnosis of TB such as acid-fast bacilli (AFB) staining, culture methods, immunological

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tests, molecular tests such as nucleic acid amplification tests with varying accuracy for detecting FGTB.[3]

Laparoscopy is a reliable modality for the detection of FGTB and abdominopelvic TB. Some of the laparoscopic features suggestive of TB include the presence of suprahepatic adhesions, adhesions in the abdominal cavity or pelvic cavity, presence of tuboovarian masses with caseous material, hydrosalphinges, pyosalphinges, beading of tubes, presence of tubercular nodules in the pelvic or peritoneal cavity. Hysteroscopic evaluation of the uterine cavity may reveal pale looking cavity, presence of tubercles or caseous nodules, and varying grades of intrauterine synechiae and Asherman's syndrome.[1]

For the definitive diagnosis of TB, the bacteria need to be isolated. Culture is considered as the gold standard for the confirmation of mycobacterium TB.[4] It also has an added advantage of drug-susceptibility testing and genotyping.[5] The major drawbacks of culture techniques are that it requires a higher bacillary concentration in the sample (100bacilli/ml), and it takes longer time for the results, that is, 4–6 weeks for solid cultures and 10–12 days for liquid cultures such as Bactec.[3,5] The reported sensitivity of Bactec culture in the diagnosis of FGTB is 40% and specificity is 90%.[6]

Mycoreal polymerase chain reaction (PCR) is a rapid DNA PCR nucleic acid amplification technique which allows the direct identification of mycobacterium TB on clinical specimens. PCR assays can detect <10 bacilli/ml in a specimen and are much faster than cultures as the reports are given out within 48 hours.[7] It has a reported sensitivity of 90%–94% and specificity of 70%–78%.[8]

The GeneXpert MTB/rifampin (RIF) assay is a cartridge based, semi-automated, rapid molecular assay, which permits rapid TB diagnosis through the detection of the DNA of mycobacterium TB and simultaneous identification of a majority of the mutations in the rpo B gene that confers resistance to rifampin. Since it is an automated closed system, the chances of DNA contamination are very low. The GeneXpert has a reported sensitivity of 46.6% and specificity of 100% for the diagnosis of endometrial TB.[9]

Gene Xpert (MTB/RIF) assay has been endorsed by the WHO for worldwide application in the diagnosis of extrapulmonary tuberculosis (EPTB) as it permits simultaneous detection of MTB and resistance to rifampicin.[4,10] The role of GeneXpert in EPTB has been studied extensively in samples from sites other than female genital tract such as lymph nodes, cerebrospinal fluid, bone, and urinary tract.[11] There are very limited studies which have evaluated the accuracy of GeneXpert in the diagnosis of FGTB. This study was conducted as an attempt to evaluate the accuracy of GeneXpert and Mycoreal PCR in the diagnosis of genital TB.

Objective

The objective of the study was to evaluate the diagnostic accuracy of GeneXpert and mycoreal PCR techniques in the diagnosis of endometrial TB considering culture as the gold standard in clinically suspected women with probable genital TB suffering from infertility.

Methodology

This was a retrospective study of data collected between January 2013 and August 2016. The sample size calculation was not done because this was a retrospective study. The statistical power was calculated rather than the sample size. This was calculated to estimate the sensitivity of the diagnostic method on a 2-tailed basis with an alpha error of 0.05 and found to be greater than 80%.[12] The data of infertile woman who visited Gunasheela surgical and maternity hospital, Bangalore for the evaluation of infertility were analyzed after obtaining approval from the institutional scientific advisory and human ethical committee (Study No. EC/OA/04/2019 approved on September 13, 2019).

Patient selection

The inclusion criteria for this study were women with either primary or secondary infertility with the diagnosis of unexplained infertility, recurrent implantation failure, recurrent pregnancy loss, damaged fallopian tubes, Asherman's syndrome, thin or pale endometrium. Women with ovulatory dysfunction, endometriosis, male factor and other uterine factors such as fibroid, polyp, adenomyosis, congenital uterine malformations, past history of TB were excluded from the study. Women satisfying the inclusion criteria visiting the center between January 2013 and December 2014 were tested by mycoreal PCR technique and those visiting between March 2015 and August 2016 were tested by Gene Xpert (MTB/RIF) TB PCR technique.

Collection of samples

Woman satisfying the eligibility criteria underwent endometrial sampling in the premenstrual phase (day 21–day 28) of the cycle on outpatient department basis. After making sure that there had been no sexual exposure in the present cycle, an informed consent for the procedure was taken. The vagina was washed with normal saline and endometrial biopsy samples were collected by curetting the endometrial cavity using the pipelle cannula without dilating the cervix and a no touch technique (cervix was not handled). The endometrial sampling was done using pipelle as it was safe, accurate, outpatient procedure, avoided general anesthesia because it did not require dilatation of cervix and was cost

effective.[13] The endometrial samples were transferred into sterile labeled wide mouth containers with normal saline and immediately sent to the laboratory.

Sample processing

BACTEC (Becton Dickinson, Towson, Md.) mycobacteria growth indicator tube 960 culture

The endometrial samples were homogenized using a tissue grinder with a small quantity of sterile saline or water (2–4 mL). The homogenized specimen was decontaminated using the sodium hydroxide-sodium chloride and liquefied completely. The liquefied specimen was then centrifuged at a speed of 3000 rpm or more for 15–20 min and allowed to rest for 5 min. The supernatant was discarded and 1–2 mL of phosphate buffer (pH 6.8) was added to the sediment. 0.5 ml of resuspended pellets was added to the mycobacteria growth indicator tube (MGIT) tubes followed by 0.8 ml of MGIT growth supplement/Polymyxin B, Amphotericin B, Nalidixic acid, and Trimethoprim. The inoculated tubes were then kept in the BACTEC MGIT 960 instrument and incubated at 37°C + 1°C temperature. If the sample had mycobacterium TB, the instrument would signal with a green light indicator showing exact location of the positive tube. The positive tubes were removed and scanned outside the instrument for mycobacterial growth which appeared granular. MGIT tubes were incubated either until the instrument flagged them positive or discarded after a maximum of 6 weeks when the instrument flagged the tubes negative.[14]

Gene Xpert polymerase chain reaction

Using a pipette, 0.7 mL of the homogenized tissue specimen was transferred into to a conical tube and double volume of the Xpert MTB/RIF Sample Reagent (1.4 ml) was added. Then the specimen was incubated for 15 min at room temperature. Between 5 and 10 min of incubation, the specimen was agitated vigorously again 10–20 times. Using a fresh transfer pipette, about 2 mL of the processed specimen was added to the Xpert MTB/RIF cartridge and finally loaded into the Gene Xpert instrument for processing. The results were interpreted by the Gene Xpert DX System from measured fluorescent signals and embedded calculation algorithms were displayed on the window of the Gene Xpert machine.[15]

Mycoreal tuberculosis polymerase chain reaction

A 200 µL of pellet obtained after homogenisation and centrifugation of sample was taken in a 1.5 mL Eppendorf tube for DNA extraction. DNA extraction was done as per the protocol of QIAGEN QI Amp DNA minicolumn extraction kits. After DNA amplification the mix was placed in Roche Cobas Taqman 48 analyser. Positive and negative controls were also put along with the samples. Detection was done by measurement of the SYBR green 1 fluorescence signal and melting curve analysis of the amplified product in utility channel of the Roche real-time PCR. Positive control of M TB H37Rv DNA showed melting peak at 85 degree centigrade. Melting peak, fluorescence level and the crossing point were taken into consideration for final reporting of test samples. Samples negative by real-time PCR were subjected to spiking with known positive DNA to rule out the presence of PCR inhibitor.[16]

Statistical analysis

Statistical analysis were done using R software version 3.6.1.[17]. Diagnostic capability of Gene Xpert PCR and Mycoreal TB PCR was evaluated considering BACTEC MGIT culture as gold standard. Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy of test was calculated. 95% confidence interval (CI) was also calculated.

Results

Women studied belonged to age group of 20 years–45 years with a mean age of 30.8 years. The duration of marriage was between 1 year and 18 years with a mean duration of infertility being 8.1 years. 67.5% of women had primary infertility and 32.5% of women had secondary infertility. This data has been provided in Table 1.

A total of 3229 samples were analysed of which 1754 were evaluated by Mycoreal TB PCR and 1475 were evaluated by Gene Xpert MTB/RIF assay. This has been depicted in Figure 1.

Among the 1754 samples tested 24 samples were positive by Mycoreal PCR. Of the 24 samples tested positive by Mycoreal PCR, 8 were confirmed positive by Bactec culture and rest 16 were negative for culture. Out of 1730 samples that were found negative by Mycoreal PCR, 15 were found to be positive by Bactec culture. This has been specified in Table 2.

The sensitivity of Mycoreal TB PCR technique with Bactec culture as the gold standard was 34.78% (95% CI; 16.38%–57.27%), specificity was 99.08% (95% CI; 98.50%–99.47%), PPV was 33.33% (95% CI; 19.22%–51.23%), NPV was 99.13%

Table 1: Demographic characteristics

Variables	Values
Age (years), mean±SD	30.8±4.69
Primary infertility, *n* (%)	2179 (67.5)
Secondary infertility, *n* (%)	1050 (32.5)
Duration of infertility (years), median (range)	8.1 (1- 18)

SD=Standard deviation

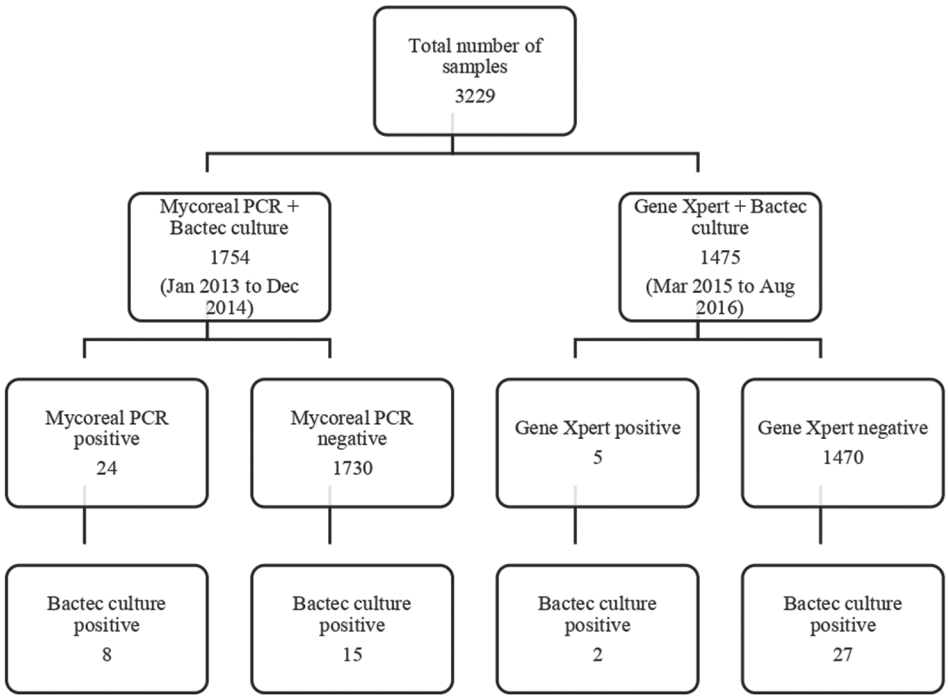

Figure 1: STARD (Standards for reporting of diagnostic accuracy studies) flow diagram showing the number of positive based on mycoreal polymerase chain reaction and GeneXpert considering Bactec culture as gold standard

Table 2: Comparison of mycoreal polymerase chain reaction and culture

	Culture positive	Culture negative
Mycoreal PCR positive	8	16
Mycoreal PCR negative	15	1715

PCR=Polymerase chain reaction

Table 2a: Diagnostic Parameters of Mycoreal PCR

	Value (%)	95% CI
Sensitivity	34.78	16.38- 57.27
Specificity	99.08	98.50- 99.47
PPV	33.33	19.22- 51.23
NPV	99.13	98.83- 99.36
Accuracy	98.23	97.50- 98.80

PPV=Positive predictive value, NPV=Negative predictive value, CI=Confidence interval

(95% CI; 98.83%–99.36%) and accuracy was 98.23% (CI 95%; 97.50%–98.80%). This has been provided in Table 2a.

Among the 1475 samples evaluated with Gene Xpert (MTB/RIF) technique only 5 samples were PCR positive, of which only 2 were confirmed positive by culture and the other 3 were culture negative. Out of 1470 samples that were found negative by Gene Xpert (MTB/RIF), 27 were found to be positive by Bactec culture. This has been illustrated in Table 3.

The sensitivity of Gene Xpert (MTB/RIF) TB PCR technique with Bactec culture as the gold standard was 6.90% (95% CI; 0.85%–22.77%), specificity was 99.79% (95% CI; 99.39%–99.96%), PPV was 40.00% (95% CI; 10.37%–79.34%), NPV was 98.16% (95% CI; 97.98%–98.33%) and accuracy was 97.97% (CI 95%; 97.11%–98.62%). This has been illustrated in Table 3a.

Discussion

Genital TB is the second most common site of TB after pulmonary TB. FGTB alone accounts for 9% of EPTB. FGTB often goes undiagnosed as patients are usually asymptomatic. It is very difficult to diagnose FGTB because it has been quoted to be a paucibacillary disease.[18]

This study evaluated two different PCR techniques namely Mycoreal and Gene Xpert (MTB/RIF) and it was observed that Mycoreal TB PCR technique was more sensitive than Gene Xpert in detection FGTB. The use of Mycoreal PCR technique seemed to detect true positives better than Gene Xpert. However both the PCR techniques had similar specificity indicating that both the tests could rule out true negatives equally well. The sensitivity of Mycoreal TB PCR technique with culture as gold standard was 34.78%, specificity was 99.08%. The sensitivity of Gene Xpert (MTB/RIF) TB PCR technique with culture as gold standard was 6.90%, specificity was 99.79%.

In a prospective study comparing AFB smear microscopy, culture and PCR, in diagnosis of FGTB,

Table 3: Comparison of Gene Xpert (*mycobacterium tuberculosis*/rifampicin) tuberculosis polymerase chain reaction and culture

	Culture positive	Culture negative
GeneXpert PCR positive	2	3
GeneXpert PCR negative	27	1443

PCR=Polymerase chain reaction

Table 3a: Diagnostic Parameters of Gene Xpert MTB/RIF

	Value (%)	95% CI
Sensitivity	6.90	0.85- 22.77
Specificity	99.79	99.39- 99.96
PPV	40.00	10.37- 79.34
NPV	98.16	97.98- 98.33
Accuracy	97.97	97.11- 98.62

PPV=Positive predictive value, NPV=Negative predictive value, CI=Confidence interval

227 endometrial samples were studied. They concluded that PCR was more sensitive compared to the conventional methods of diagnosis like microscopy and culture, however they also said that DNA PCR is not reliable for diagnosis of genital TB due its limitations of false negativity.[19] There was another study showing similar results which evaluated the sensitivity of PCR. In this study they compared PCR culture and histopathological examination (HPE) in diagnosis of FGTB in 72 patients. The sensitivity of PCR, HPE and culture were 57.1%, 10.7% and 7.14% respectively. The study concluded that PCR was useful in diagnosing the disease early but had an important limitation of false negative results.[20]

In our study PCR also missed nearly 50% of cases that eventually came positive on culture keeping in line with the previously quoted studies showing a high false negative result as a limitation. This could be because of inefficient extraction of the DNA due to low mycobacterial numbers or the presence of PCR inhibitors, which would falsely give a negative result. As a result early detection of the disease by PCR would not be possible.

Several studies and meta analysis have proven that Gene Xpert has increased sensitivity for diagnosing pulmonary and EPTB compared to other techniques. Most of the studies on role of Gene Xpert in EPTB have not included FGTB.[11] In a recent study evaluating the role of Gene Xpert in diagnosis of FGTB including 240 women, the positivity rate of Gene Xpert was 2.9% versus 21.6% with other PCR technique. The study concluded that Gene Xpert has a high specificity but a low sensitivity (46.6%).[9] In a recent study comparing AFB smear versus culture (both Lowenstein-Jensen [LJ] and BACTEC (Becton Dickinson, Towson, Md.) culture) versus PCR concluded that PCR (53.3%) was the most sensitive method followed by BACTEC and LJ culture (33.3%) in diagnosis of genital TB.[14]

In India, endometrial TB PCR is used as one of the screening methods for diagnosis of genital TB but it demands more evaluation as it is debatable whether PCR positivity indicates infection or disease of the genital tract as FGTB is a paucibacillary disease. A number of reports have described the importance of endometrial TB PCR for early diagnosis of genital TB and other EPTB. PCR has high false positivity and alone is not recommended for making diagnosis of genital TB or to start the treatment and it's not recommended by WHO and NTEP (National TB Elimination Program of India).[21] However, in 2015, the WHO recommended the use of GeneXpert MTB for the detection of pulmonary and EPTB due to its high sensitivity and specificity.[4,10] Although there are studies evaluating the usefulness of GeneXpert (MTB/RIF) assay in diagnosis of EPTB, these studies have not included genital TB. Hence, we wanted to know the sensitivity and specificity of GeneXpert as a tool for the diagnosis of genital TB. The present study has evaluated the diagnostic capability of PCR technique in FGTB in a large population of 3229 infertile females.

One of the notable differences with other studies is that endoscopic procedures were not performed to look for evidence of TB. There were a few limitations noted in the study. Firstly, the study was a retrospective study. Secondly the two different PCR tests were not performed in the same endometrial sample from the patient. Hence, the data were not directly comparable. Ideally both the tests should have been done on all cases for better comparison of sensitivity and specificity. Thirdly, use of culture as a gold standard might not have been suitable reference standard since culture has a low sensitivity of 40% and FGTB is a paucibacillary disease.

Conclusions

GeneXpert does not appear to have similar diagnostic accuracy in EPTB as in pulmonary TB. It is probably impossible to have a single diagnostic test for such a paucibacillary disease where sampling cannot be done from the fallopian tube which is mostly affected. The way forward is a combination of clinical/lab/radiological testing.

Mycoreal TB PCR technique is more sensitive than Gene Xpert (MTB/RIF) TB PCR technique in the diagnosis of genital TB. However, both techniques have equal specificity and accuracy in the detection of genital TB. Further studies evaluating the above said techniques on the same sample are recommended in multicentric settings to draw definitive conclusions regarding the use

of best PCR technique to effectively diagnose genital TB.

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Conflicts of interest

There are no conflicts of interest.

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