Golestan University of Medical Sciences
Journal of Clinical and Basic Research
Online ISSN: 2538-3736

Comparison of molecular with conventional methods for detection of genital tuberculosis in infertile women: A comparative prospective study

Anupama Hari [1], Anusha Reddy Thota [2], Sravanthi Katamreddy [3]*

1. Department of Obstetrics and Gynecology, Gandhi Medical College and Hospital, Secunderabad, India
2. Department of Obstetrics and Gynecology, Mahatma Gandhi Medical College and Research Institute, Pillayarkuppam, Puducherry, India
3. Department of Obstetrics and Gynecology, Santhiram Medical College and General Hospital, Nandyal, India

* Correspondence: Sravanthi Katamreddy. Department of Obstetrics and Gynecology, Santhiram Medical College and General Hospital, Nandyal, India. Tel: +9985335296; Email: sravs1233@gmail.com

Abstract

Background: Diagnosis of genital tuberculosis (TB) as a risk factor for infertility remains a diagnostic dilemma for clinicians. Employing the best clinical practices for diagnosing and treating genital TB may help optimize fertility rate. The current study aimed to evaluate the molecular and conventional methods to diagnose genital TB in women with infertility.

Methods: A comparative study was conducted on 100 infertile women between 20-35 years of age. Routine laboratory assays and other investigations, such as cervical swab gonorrhea and Chlamydia, Nested TB Polymerase Chain Reaction (TB-PCR), histopathological examination (HPE), culture by Bactec method, and hysterosalpingography (HSG) were performed.

Results: The majority of the cases (52%) were between 21 and 25 years. Moreover, 68% of the participants had patent tubes on HSG, 7% had a right tubal block, 9% had a left tubal block, 14% had a bilateral tubal block, and 2% had a unicornuate uterus. Additionally, 9% of the participants were TB-PCR-positive. Hysterolaparoscopy results suggested a likelihood of TB observed among 8% of the participants. Also, 25% of the patients with TB and 7.9% of the patients without genital TB had positive Mantoux test results. Furthermore, 75% of the patients with genital TB had HSG results suggestive of TB, whereas 23.8% of the patients without genital TB had such results. Laparoscopy (75%) and TB-PCR (75%), as well as HSG (75%), were the most specific tests for ruling out genital TB. The most sensitive tests for diagnosing genital TB were TB-PCR (100%), HPE (100%), and laparoscopy (100%).

Conclusion: Hysterolaparoscopy is useful for early diagnosis of genital TB. PCR is highly helpful in paucibacillary vaginal TB. Positive findings can be obtained with as few as 10 bacteria/ml of material. In addition to clinical and endoscopic examination, the routine use of endometrial bacteriological PCR testing may be significantly promising for enhancing the diagnosis of genital TB. For the best results, early detection and timely treatment are essential.

Article Type: Research Article

Article History

Received: 5 October 2024
Received in revised form: 27 January 2025
Accepted: 4 March 2025
Available online: 11 March 2025
DOI: 10.29252/JCBR.9.1.24

Keywords

Tuberculosis
Endometritis
Polymerase chain reaction
Hysterolaparoscopy
Mantoux test

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Highlights

What is current knowledge?

Genital tuberculosis (TB) is a significant cause of female infertility. The paucibacillary nature of the disease within the female reproductive system frequently complicate its diagnosis. Currently, there is no singular diagnostic test that can reliably identify genital TB.

What is new here?

It is essential that all individuals experiencing infertility undergo regular assessments for genital TB. PCR has emerged as a more promising approach than other methodologies such as Mantoux test, USG, hysterosalpingography, hysteroscopy, laparoscopy, and histopathological examinations. Despite advancements, histopathology remains the most reliable and accessible diagnostic tool. Best clinical practice implementation can improve fertility outcomes in genital TB.

Introduction

Infertility is the inability to conceive following a minimum of one year of unprotected sexual activity. One of the main causes of infectious disease-related morbidity and mortality worldwide is tuberculosis (TB). Genital TB is a major cause of subfertility in South India. Besides causing tubal obstruction and dysfunction, genital TB also impacts implantation due to endometrial involvement and results in ovulatory failure due to ovarian involvement (1). Female genital TB remains one of the main causes of infertility. There are notable differences in the prevalence of female genital TB in infertility clinics across different nations, ranging from 15% to 25%. Female genital TB affects young women aged between 18 and 38 in 80-90% of cases (2).

Reactivation of a silent bacillemia, mainly from the lungs, is often the cause of this secondary complication. It usually affects the fallopian tubes (92-100%), ovaries (10-30%), cervix (5%), endometrium (50%), vagina and vulva (<1%); however, it can also occur from the kidney, intestines, etc. (3). The endometrium is the most often implicated location, according to a small number of the study findings. When engaging in sexual activity with a partner who has tuberculous lesions of the genitalia, direct inoculation of tubercle bacilli can also occur over the vulva or vagina. It is quite uncommon for female vaginal organs to become infected (4).

Clinicians have struggled to diagnose genital TB for decades; however, more cases have been identified recently as a result of better diagnostic techniques. In areas without diagnostic facilities, diagnosis is mostly established using a high index of clinical suspicion and the relevant investigations. The majority of cases present without symptoms (4). The aim of this study was to assess female infertility using nested PCR for genital TB. Additionally, we aimed to find any correlation between the results of hysterolaparoscopy and positive cases of genital TB.

[DOI: 10.66224/jcbr.9.1.24]

Methods

Study Design: This was a prospective and comparative study. The source of data included infertile women attending Gynecology OPD at Gandhi Hospital, Secunderabad from December 2013 to August 2015. The number of cases was 100 individuals. Inclusion criteria were infertile women of 20-35 years, primary or secondary infertility for <1 year, and male partners who were willing to provide semen samples. Exclusion criteria were male factor infertility, endometriosis, and polycystic ovarian syndrome. Bimanual examinations were performed after obtaining written informed consent, a comprehensive history, a general examination, an abdominal examination, and a full gynecological examination with a speculum. Chest X-ray, ultrasound, erythrocyte sedimentation rate (ESR), CBP, and Mantoux tests. Serum prolactin and thyroid profile were also assessed. The husbands' semen was collected to rule out male factor infertility.

Investigations were conducted on cervical swabs for detection of gonorrhea and Chlamydia. Endometrial aspiration sample was taken for nested TB-PCR for amplification of IS6110 locus, histopathology, sample features suggestive of TB were collected, the +ve tubercle bacilli, caseous necrosis, giant cells, epithelial cell clusters and lymphocytic infiltration; and culture by Bactec method. HSG was performed during the pre-ovulatory period, which lasts from the sixth to the eleventh day, on patients who have conventional testing and negative TB-PCR results. In TB-PCR-positive cases, a thorough assessment of the hysterolaparoscopic results was conducted to look for any signs of genital TB, including caseosalpinx, nodular salpingitis, and miliary tubercles on the uterus and tubes. Peritubal, periovarian, omental, and bowel adhesions, hydrosalpinx, and free fluid in the Douglas pouch.

In order to find characteristics suggestive of TB, such as the presence of tubercles, microcaseation, deformed ostia, calcifications, and synechie (Grades 1 to 3), a hysteroscopy was performed using normal saline as the distention medium. Grade 1: involvement of <1/4 of the uterine cavity. Grade 2: ostia and fundus partially involved; 1/4th to 3/4th of uterine cavity involved. Grade 3: The ostia and upper cavity are obstructed, with more than three-quarters of the uterine cavity is affected.

Results

Fifty-two percent of the participants were between 21 and 25 years; 40% were between 26 and 30 years; and the remaining 8% were between the ages of 31 and 35 years. Of the study participants, 47% had been married for less than 5 years, followed by 6-10 years (40%) and 11-15 years (13%). Ten cases (83.3%) out of the 12 positive cases of infertility in genital TB cases had primary infertility, whereas two cases (16.6%) had secondary infertility. Ten percent had positive results from the Mantoux test. Moreover, 68% of the participants had patent tubes on HSG, 7% of the cases had a right tubal block, 9% had a left tubal block, 14% had a bilateral tubal block, and 2% of the cases had a unicornuate uterus (Table 1).

Four percent of the participants had puffy ends on hysteroscopy, three percent had uterine adhesions, and one percent had a right ostia block. According to histopathological analysis, 51% of the participants were in the proliferative phase, 46% were in the secretary phase, and three percent had TB endometritis. Nine percent of the participants had a positive TB-PCR (Table 2).

Ten percent of the cases had positive Mantoux test results. Moreover, 30% of the cases had positive HSG test results. Hysteroscopy was positive in 8% of the cases. The laparoscopy results were favorable in 48% of the cases. Three percent of the participants had positive HPE test results and 9% of the cases had positive PCR results. Hysteroscopy results, showing a likelihood of TB, were observed in 8% of the cases (Table 3) (Figure 1). Laparoscopy findings and their distribution, indicating the probability of TB are illustrated in Figure 2.

The relationship between hysteroscopy findings and genital TB is summarized in Table 4. The highly specific tests used in this investigation to rule out genital TB were TB-PCR (75%), laparoscopy (75%), and HSG (75%). According to Table 5, HPE (100%), laparoscopy (100%), and TB-PCR (100%) were the most sensitive tests for identifying genital TB. The comparison of all methods for detecting genital TB and their specificities is summarized in Table 5.

Table 1. Distribution based on findings of laparoscopy

Laparoscopy	Frequency	Percentage
Uterus		
Normal	98	98%
Unicornute	2	2 %
Tubes		
Normal	85	85 %
Right beaded	3	3 %
Left beaded	1	1 %
Bilateral beaded	4	4 %
Bilateral hydrosalpinx	2	2 %
Left adhesions	1	1 %
Right clumping	2	2 %
Ovaries		
Normal	57	57 %
Polycystic ovary	39	39 %
Left adhesions	2	2 %
Bilateral adhesions	1	1 %
Pouch of Douglas		
Normal	89	89 %
Adhesions	10	10 %
Free fluid	1	1 %
Chromo perturbation test		
Patent	77	77 %
Right tubal block	9	9 %
Left tubal block	9	9 %
Bilateral block	5	5 %
Others		
Normal	92	92 %
Adhesions	8	8 %

Table 2. Distribution of findings of hysteroscopy, histopathological examination and TB-PCR

Variables	Frequency	Percentage
Hysteroscopy		
Normal	90	90 %
Fluffy end	4	4 %
Uterus-adhesions	3	3 %
Right unicornuate uterus	2	2 %
Right ostia block	1	1 %
TB-PCR		
Positive	9	9 %
Negative	91	91 %
Histopathology		
Proliferative phase	51	51 %
Secretory phase	46	46 %
TB endometritis	3	3%

[DOI: 10.66224/jcbr.9.1.24]

Table 3. Distribution of cases based on high index of suspicion and definitive for genital TB

High index of suspicion and definitive diagnosis	Frequency N=70	%
High index of suspicion suggestive		
Mantoux test	10	10 %
Probable		
HSG	30	30 %
Hysteroscopy	8	8 %
Laparoscopy	48	48%
Definitive tests		
HPE	3	3 %
TB-PCR	9	9 %
BACTEC	00	00

Abbreviations: HPE: Histopathological Examination; HSG: Hysterosalpingography; TB-PCR: Tuberculosis-Polymerase Chain Reaction

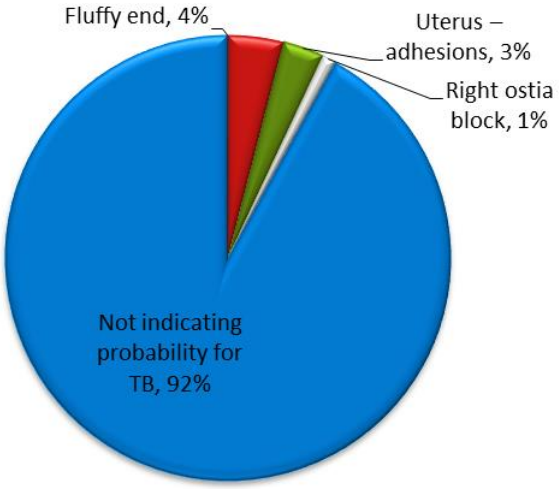

Figure 1. Distribution of the cases according to hysteroscopy findings, indicating the probability of TB

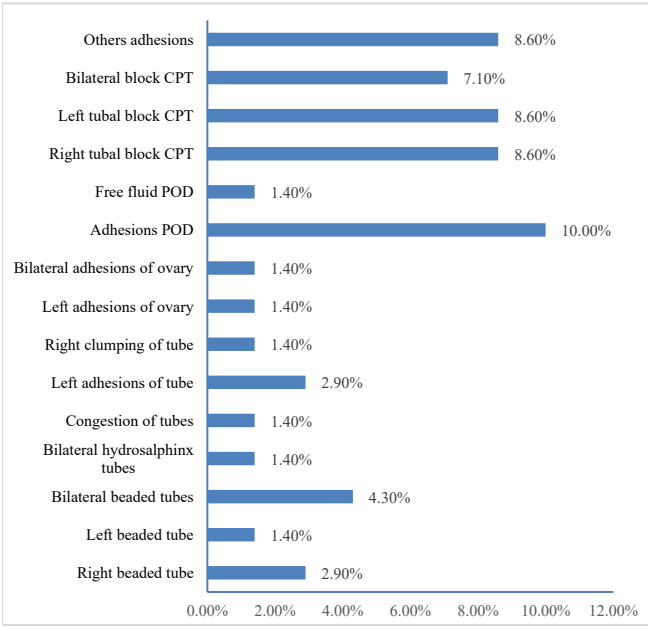

Figure 2. Distribution of the cases according to laparoscopy findings, indicating the probability of TB

Table 4. The relationship between hysteroscopy findings and genital TB

Variables	Genital TB		P-value
	Yes	No	
Age group			
21 -25 years	8 (66.6%)	44 (50.0%)	0.40
26 - 30 years	4 (33.4%)	36 (40.9%)	
31 - 35 years	0	8 (9.1%)	
Married life			
≤ 5 years	6 (50.0%)	41 (46.6%)	0.34
6 -10 years	6 (50.0%)	34 (38.6%)	
11 - 15 years	0	13 (14.8%)	
Mantoux test			
Positive	3 (25.0%)	7 (7.9%)	0.06
Negative	9 (75.0%)	81 (92.1%)	
Hysteroscopy			
Normal	12 (100%)	78 (88.6%)	0.82
Fluffy end	0	4 (4.5%)	
Ostia block	0	1 (1.1%)	
Unicornuate uterus	0	2 (2.3%)	
Uterine adhesions	0	3 (3.4%)	
HSG			
Patent	3 (25.0%)	65 (73.8%)	0.001
Right tubal block	3 (25.0%)	4 (4.5%)	
Left tubal block	0	9 (10.2%)	
Bilateral block	6 (50.0%)	8 (9.1%)	
Unicornuate uterus	0	2 (2.3%)	

Table 5. Comparison of various tests used for detecting genital TB

Tests	Genital TB		Specificity	Sensitivity	P-value
	Yes (n-12)	No (n=88)			
Mantoux Test	3 (25.0%)	80 (91.9%)	25 %	90 %	0.001
USG	0	82 (93.5%)	0 %	93 %	0.001
HSG	9 (75.0%)	67 (75.8%)	75 %	76 %	0.93
Hysteroscopy	0	78 (88.7%)	0 %	88 %	0.01
Laparoscopy	9 (75.0%)	88 (100%)	75 %	100 %	0.00001
HPE	3 (25.0%)	88 (100%)	25 %	100 %	0.01
TB-PCR	9 (75.0%)	88 (100%)	75 %	100 %	0.00001

Discussion

As a chronic illness, genital TB frequently manifests as low-grade symptoms with few specific complaints. Infertility is the most common clinical manifestation, accounting for 43-74% of all presenting symptoms (5). An estimated 5-13% of Indian women visiting infertility clinics are thought to have genital TB (6). Infertility was the most frequent early indicator of female genital TB (7). In regions where TB is endemic, more cases of genital TB would be observed if this condition was taken into account when evaluating each infertile patient (7). Since 80%-90% of the cases with female genital TB are identified in individuals aged 20 to 40 years, frequently during workup for infertility, the disease is generally considered to affect young women.

According to Bicha et al.'s study, the age of the patients with genital TB was 35 years (8). Fifty to sixty percent of patients with genital TB involve the endometrium due to direct tube-borne transmission. Because frequent menstruation minimizes endometrial

[DOI: 10.66224/jcbr.9.1.24]

involvement, the initial phases are unremarkable. Advanced stages of caeseation and ulceration result in endometrial destruction, amenorrhea, and the production of synechiae (Asherman's syndrome). There are no distinct indications or symptoms of genital TB. The correct diagnosis of TB depends on effective screening practices, radiographic studies, histological, and bacteriological examinations. A strong index of suspicion and, if feasible, definitive laboratory testing are essential for the diagnosis of genital TB. Every diagnostic technique complements the others.

The diagnostic criteria for genital TB in this study are granulomatous lesions on histology A and culture, as well as TB-PCR. In this study, we compared TB-PCR with HPE and culture. Different combined diagnostic tests, such as hysteroscopy and laparoscopy, were utilized in a number of additional investigations; however, the results may lack specificity (9). The incidence of genital TB was 12% in this study. According to several other sources, the incidence was between 15 and 25% (6). TB-PCR was used to diagnose 75% of cases with genital TB in our study, whereas other authors have reported varying TB-PCR positivity rates (10,11). Our study's high genital TB prevalence rate may have resulted from the use of nested TB-PCR, which is known to boost test sensitivity. However, neither the PCR type nor the gene region under investigation has been reported by the authors of the aforementioned work.

The highest detection rate among suspected patients was 43.1% with TB-PCR, compared to 11% with histopathology, 7.8% with culture, and 5% with AFB staining, according to a study comparing AFB smear, culture, histopathology, and TB-PCR. In contrast to other authors' findings of 11.5%, our study's histopathological identification of genital TB was 25% (12). The Mantoux test is not very useful for detecting active genital TB in women of childbearing age. However, early laparoscopic examination is necessary for infertile women who have a positive Mantoux test. The Mantoux test's specificity was 80%, while its sensitivity was just 55% (13).

A positive test result only indicates a history of infection. Following BCG vaccination and in cases of non-tubercular mycobacterial infection, positive outcomes are observed. In India, almost 60% of the population has tuberculin. Immunosuppression and severe sickness can also produce false-negative results. An induration of 5 mm is measured in individuals who are HIV-positive and tuberculin-positive. The Mantoux test had a sensitivity of 90% and a specificity of 25% in the current investigation. The Mantoux test had a specificity of 66.21% and a sensitivity of 61.5% in diagnosing TB-PCR-positive subjects (11). Of note, 25% of the cases in our research not only were TB-PCR-positive but also were positive based on histological analysis.

Since HSG might cause post-procedure sepsis and a flare-up of the disease, it should never be performed in suspected cases of genital koch's (6). In the current study, 75% of the cases with genital TB exhibited aberrant tubal block HSG findings, indicating that 12.5% of the cases had unilateral block and 62.5% had bilateral block. An ultrasonography cannot definitively diagnose pelvic TB because of its usual appearance (14). A very helpful method for diagnosing genital TB, particularly for tubal, ovarian, and peritoneal illnesses, is laparoscopy and chrome perturbation which can be used in conjunction with hysteroscopy. Nowadays, laparoscopy is a widely accepted method for diagnosing TB in infertile women with various findings. In the sub-acute stage, the pelvic organs may have adhesions, edema, and congestion with numerous fluid-filled pockets. Laparoscopic findings were observed in 62.5% of the patients in the current study who were positive for TB. In addition, adhesions were observed in 62.5%, hydrosalpinx in 12.5%, pelvic organ congestion in 12.5%, tubal beading in 25%, and tubal clumping in 12.5% of the patients. The results of the remaining 37.5% of patients were normal. According to a previous research, majority of patients showed no endoscopic abnormalities, while 48 patients exhibited abnormalities that suggested TB (15).

In terms of *Mycobacterium* detection, PCR was shown to be the fastest and most sensitive approach (94.28%), three times more sensitive than culture and nine times more sensitive than smear analysis. Since genital TB is a paucibacillary, PCR is the next line of research. Results can be obtained within a day or two. In our investigation, the PCR's sensitivity and specificity, using real-time PCR, were 100% and 75%, respectively. Thus, our study has a high detection rate. Positive TB-PCR results strongly correlated with the presence of ostial fibrosis, corneal block, tubal beading, tubercles, intrauterine adhesions, and periovarian adhesions. The endoscopic evaluation's overall predictive value for genital TB diagnosis was 42.52%. In 71% of the cases, there was agreement between the two primers' results. Comparing TRC4 probe to clinical criteria, its sensitivity was 46.4%, while IS6110's was 25%. Because the so-called South Indian strain of *M. tuberculosis* only possesses one copy of IS6110, it may be the cause of this low positive by IS6110 in comparison to the TRC4 probe. In our investigation, the prevalence of genital TB is 12% because 12 out of 100 cases were found to have the disease. Although we could distinguish between MTB and MOTT using the culture method, PCR results might be negative since it could not identify the *Mycobacterium* species. For the early diagnosis of genital TB, PCR is a quick, sensitive, and precise diagnostic method. PCR is used to detect infections when clinical suspicion is strong, smear results are negative, and *Mycobacterium* signs and symptoms are visible because other methods are time-consuming. The prevalence of genital TB is 12% since in our investigation, 12 out of 100 cases were found to have the disease.

A thorough history, a detailed systemic and gynecological examination, and prudent application of diagnostic techniques such as endometrial biopsy for HPE, culture, and PCR, in addition to imaging techniques and endoscopic visualization, are used for final diagnosis. While we could distinguish between MTB and MOTT using the culture method, PCR results might be negative since it could not identify the *Mycobacterium* species. For the early diagnosis of genital TB, PCR is a quick, sensitive, and precise diagnostic. PCR is used to detect infections when clinical suspicion is strong, smear results are negative, and *Mycobacterium* signs and symptoms are visible because other methods are time-consuming.

Strength of the study:

1) The ongoing investigation has uncovered a greater number of cases with TB using nested PCR techniques.

2) Although the TB-PCR test is quite expensive, participants in this study are not financially impacted, as the research is conducted free of charge.

3) The sensitivity of the TB-PCR test is exceptionally high.

4) As the procedure is performed on an outpatient basis, patients experience minimal disruption.

5) Compared to endometrial biopsy, endometrial aspiration is more accurate, less invasive, and more comfortable. We used endometrial aspiration to collect samples for our study. Given that other diagnostic methods can be lengthy, PCR is employed to identify the infection when there is a strong clinical suspicion, negative smear results, and clear signs and symptoms of *Mycobacterium*.

Conclusion

Hysterolaparoscopy plays a vital role in the early detection of conditions, evaluation of disease severity and assessment of reproductive outcomes. This procedure includes visual diagnosis, sample collection for histological analysis and confirmation, adhesiolysis, and any other necessary interventions, thereby underscoring its diverse applications in gynecological practice. Molecular diagnostic methods, especially PCR, are crucial for diagnosing paucibacillary genital TB, as they can detect positive results from samples with as few as ten bacteria per milliliter. In contrast to traditional culture techniques, which take two to four weeks for results, PCR can deliver outcomes in just 1 to 2 days. With its remarkable sensitivity and specificity, PCR is a promising method for diagnosing genital TB. The routine use of endometrial bacteriological PCR assays, in conjunction with clinical evaluations and endoscopic assessments, has significant potential to improve the accuracy of genital TB diagnoses.

Acknowledgement

None

Funding sources

The authors did not receive any support from any organization for the submitted work.

[DOI: 10.66224/jcbr.9.1.24]

Ethical statement

Study was conducted after approval of institutional ethical clearance. Study was in accordance with the Declaration of Helsinki. The subjects were provided written informed consent from the patient/spouse/attendee. The current study protocol was approved by the Institutional Ethics Committee.

Conflicts of interest

The authors declared no conflict of interest.

Author contributions

AK and AH collected data, designed the experimental framework, and conceptualized the study. AT and AH revised and analyzed the data. AK, AT, and AH drafted the manuscript. All authors read and approved the final version of the manuscript.

Data availability statement

The data supporting the findings of this study can be obtained from the corresponding author upon request.

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How to Cite:

Hari A, Thota AR, Katamreddy S. Comparison of molecular with conventional methods for detection of genital tuberculosis in infertile women: A comparative prospective study. *JCBR.* 2025;9(1):24-8. http://dx.doi.org/10.29252/JCBR.9.1.24

[DOI: 10.66224/jcbr.9.1.24]