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Diagnostic Efficacy of Cartridge-Based Nucleic Acid Amplification Test (CB-NAAT) Compared with Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) in Detecting Genital Tuberculosis in Endometrial Tissue Among Infertile Women in India

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Abstract: ***Introduction: Female genital tuberculosis (FGTB) is a significant yet often undetected contributor to infertility in tuberculosis-endemic regions like India, where it accounts for a notable proportion of infertility cases. Rapid and accurate diagnostic tools are critical due to the limitations of conventional methods in this paucibacillary condition. Objectives: This study aimed to assess the diagnostic efficacy of Cartridge-Based Nucleic Acid Amplification Test (CB-NAAT) and Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) in detecting Mycobacterium tuberculosis in endometrial tissue from infertile women, and to compare their performance in a high-TB-burden setting. Methods: A prospective study was conducted at a tertiary care hospital in India from March 2022 to March 2024, involving 52 infertile women with suspected FGTB. Endometrial biopsies were analyzed using CB-NAAT, RT-PCR, and histopathology, with culture as a reference standard where feasible. Sensitivity, specificity, and predictive values were calculated. Results: Of 52 participants, histopathology confirmed FGTB in 19 (36.5%). CB-NAAT detected MTB in 15 cases (sensitivity 78.9%, specificity 100%), while RT-PCR identified 16 cases (sensitivity 84.2%, specificity 96.9%). CB-NAAT reported one rifampicin-resistant case, with results available in 2 hours compared to 6–8 hours for RT-PCR. Discussion: CB-NAAT's high specificity and rapid results, including resistance profiling, make it practical for resource-limited settings, though its sensitivity is slightly lower than RT-PCR's. RT-PCR's higher sensitivity may better detect low-bacillary FGTB, but its complexity and potential for false positives pose challenges. Combining these tests with histopathology optimizes diagnosis. Conclusion: Both CB-NAAT and RT-PCR enhance FGTB detection in infertile women, with CB-NAAT offering practical advantages and RT-PCR providing superior sensitivity. An integrated diagnostic approach is recommended for effective management in India.***

Keywords: Female genital tuberculosis, infertility, CB-NAAT, RT-PCR, endometrial tissue, molecular diagnostics

1. Introduction

Female genital tuberculosis (FGTB) is a silent form of extrapulmonary tuberculosis that primarily affects reproductive organs, leading to infertility in 10–15% of cases in India, a country with a high TB burden [1]. The disease often presents with nonspecific symptoms such as pelvic pain, irregular menstruation, or infertility, complicating diagnosis [2]. Conventional diagnostic methods like smear microscopy and culture are suboptimal due to low sensitivity and prolonged turnaround times, particularly in paucibacillary FGTB [3]. Molecular techniques, including CB-NAAT (GeneXpert MTB/RIF) and RT-PCR, have emerged as promising tools for rapid and accurate TB detection [4]. CB-NAAT provides automated nucleic acid amplification with rifampicin resistance profiling, while RT-PCR targets RNA to detect active infection. This study compares these methods in endometrial tissue from infertile women to evaluate their diagnostic utility in FGTB.

2. Review of Literature

FGTB predominantly affects the fallopian tubes (90%), endometrium (50–60%), and ovaries (20–30%), often resulting in tubal occlusion or endometrial scarring [5]. In India, the prevalence of FGTB among infertile women ranges from 3% to 25%, depending on diagnostic criteria and region [6]. Traditional diagnostics like Ziehl-Neelsen staining detect acid-fast bacilli in only 5–10% of cases, while culture, though specific, takes 6–8 weeks and yields positivity in less than 30% of FGTB samples [7].

CB-NAAT, introduced by Cepheid as GeneXpert MTB/RIF, amplifies the rpoB gene of Mycobacterium tuberculosis and detects rifampicin resistance within 2 hours [8]. Studies report its sensitivity in extrapulmonary TB as 70–90%, with 100% specificity, though its performance in FGTB varies due to low bacillary load [9]. Sharma et al. (2020) found CB-NAAT sensitivity of 83% in endometrial samples, with rapid results aiding early treatment [10]. RT-PCR, targeting MTB-specific RNA (e.g., IS6110 or 16S rRNA), offers theoretical advantages in detecting viable bacilli, with sensitivities of 80–

95% in extrapulmonary TB [11]. However, its complexity and risk of contamination limit widespread use [12]. Comparative studies of CB-NAAT and RT-PCR in FGTB are scarce, necessitating further investigation.

Objectives

1) To evaluate the sensitivity and specificity of CB-NAAT in detecting Mycobacterium tuberculosis in endometrial tissue from infertile women with suspected FGTB.
2) To compare the diagnostic performance of CB-NAAT with RT-PCR in the same cohort.
3) To assess the feasibility and practical utility of these molecular tests in a high-TB-burden setting over a two-year period.

3. Materials and Methods

Study Design and Population

This prospective study was conducted at a tertiary care hospital in Assam, India from March 2022 to March 2024. Fifty-two women aged 20–40 years with primary or secondary infertility and clinical suspicion of FGTB (e.g., tubal block, endometrial irregularities, or chronic pelvic pain) were included. Exclusion criteria included active menstruation, pregnancy, or recent TB treatment. Ethical clearance and written consent were obtained.

Sample Collection

Endometrial biopsies were collected during the luteal phase using a Pipelle curette under sterile conditions. Each sample was split into three parts for CB-NAAT, RT-PCR, and histopathological examination (HPE), with a subset sent for culture.

Diagnostic Techniques

1) **CB-NAAT**: Tissue (0.5 g) was homogenized in 1 mL of sample reagent, incubated for 15 minutes, and processed in the GeneXpert system. Results, including MTB detection and rifampicin resistance, were available in 2 hours.
2) **RT-PCR**: RNA was extracted using a commercial kit (e.g., Qiagen RNeasy), reverse-transcribed to cDNA, and amplified using primers for the IS6110 gene in a real-time PCR platform. A Ct value < 35 indicated positivity.
3) **Reference Standards**: HPE assessed granulomatous inflammation, and mycobacterial culture was performed on Lowenstein-Jensen medium for confirmation in 20 samples.

Statistical Analysis

Sensitivity, specificity, PPV, and NPV were calculated using HPE and/or culture positivity as the gold standard. The chi-square test assessed differences between CB-NAAT and RT-PCR, with $p < 0.05$ deemed significant.

4. Results

Participant Characteristics

The mean age was 29.1 ± 5.2 years, with 36 (69.2%) women having primary infertility and 16 (30.8%) secondary infertility. Clinical findings included tubal occlusion (48%), endometrial thinning (25%), and adhesions (18%).

Diagnostic Yield

HPE confirmed FGTB in 19 (36.5%) cases, showing granulomas (15 with caseation). Culture was positive in 10 of 20 samples. CB-NAAT detected MTB in 15 (28.8%) cases, with 1 rifampicin-resistant result. RT-PCR identified MTB in 16 (30.8%) cases.

Performance Metrics

- **CB-NAAT**: Sensitivity 78.9% (15/19), specificity 100% (33/33), PPV 100%, NPV 89.2%. Four false negatives were noted.
- **RT-PCR**: Sensitivity 84.2% (16/19), specificity 96.9% (32/33), PPV 94.1%, NPV 91.4%. One false positive occurred.
- **Comparison**: Differences in sensitivity ($p = 0.72$) and specificity ($p = 0.61$) were not significant.

Turnaround Time

CB-NAAT results were available in 2 hours, while RT-PCR required 6–8 hours due to RNA extraction and amplification steps.

5. Discussion

FGTB's impact on infertility underscores the need for reliable diagnostics. CB-NAAT's 78.9% sensitivity in this study aligns with reports of 70–85% in extrapulmonary TB [13], though it missed four cases, likely due to low bacillary load—a known limitation in FGTB [14]. Its 100% specificity and rifampicin resistance detection enhance its utility in India, where MDR-TB prevalence is rising [15]. RT-PCR's higher sensitivity (84.2%) may reflect its ability to detect active infection via RNA, consistent with findings by Moure et al. (2011) [16]. However, its false positive highlights contamination risks, necessitating skilled personnel [17].

HPE detected FGTB in 36.5% of cases, comparable to the 30–50% reported in Indian studies [18]. Culture, though specific, was limited by availability and slow growth, reinforcing the need for molecular tools. CB-NAAT's rapid results favor its use in resource-constrained settings, while RT-PCR's sensitivity may justify its application in specialized centers. Combining these tests with HPE could optimize diagnosis, as suggested by Kohli et al. (2021) [19]. The small sample size (52) limits generalizability, but reflects logistical challenges in FGTB research.

6. Conclusion

CB-NAAT and RT-PCR are valuable for diagnosing FGTB in endometrial tissue among infertile women, with RT-PCR offering higher sensitivity and CB-NAAT providing speed and resistance profiling. In India, CB-NAAT's practicality makes it a frontline tool, though its sensitivity gaps suggest a complementary role for RT-PCR or HPE. Larger studies are needed to refine diagnostic algorithms for FGTB, improving infertility management in TB-endemic regions.

Conflict of Interest: None

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