

Role of CBNAAT (Cartridge-Based Nucleic Acid Amplification Test) in Detection of Genital Tuberculosis in Endometrial Tissue among Women with Infertility

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Abstract

Background: Female genital tuberculosis is the second most common form of extrapulmonary tuberculosis and is a well-recognized factor associated with infertility among females. It poses a diagnostic dilemma because of its varied presentation and lack of sensitive and specific methods of diagnosis, though CBNAAT gives rapid, accurate, and sensitive results.

Aims and Objectives: To evaluate the role of CBNAAT (cartridge-based nucleic acid amplification test) in the detection of genital tuberculosis in endometrial tissue among women with infertility and to find out the institutional prevalence of genital TB in patients with infertility and to get the diagnostic comparison of endometrial tuberculosis by GeneXpert, HPE, ZN staining and AFB culture.

Material and Methods: 215 patients who presented with infertility were enrolled for the observation study. Premenstrual phase endometrial biopsy was obtained and subjected to histopathological examination, ZN staining, and culture for AFB and CBNAAT.

Results: Out of 215 women 28 were positive for mycobacterium tuberculosis by CBNAAT making the overall institutional prevalence of FG TB in infertile women to an extent of 13%. Endometrial biopsy revealed inflammatory cells in 8.4% and culture was positive in only 2.8% of cases whereas AFB was negative in 100% of cases.

Conclusion: CBNAAT is a specific method for the diagnosis of FG TB. The histopathology examination of all the samples was non-specific. No cases of positive acid fast bacilli on ZN staining were observed. Among the four methods used in the FG TB case, CBNAAT has the highest utility. A comparison of culture and CBNAAT revealed that CBNAAT was more sensitive in detecting positive cases.

Keywords: Genital Tuberculosis, Endometrial Biopsy, Premenstrual Phase, Inflammatory Cells

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Background

Tuberculosis is caused by mycobacterium tuberculosis and may present in two forms: pulmonary tuberculosis and extrapulmonary tuberculosis. Extra pulmonary tuberculosis attribute to 15 to 20% of tuberculosis cases [1,2]. Worldwide genitourinary tuberculosis is attributes to about 27% of cases of extrapulmonary tuberculosis. Due to the asymptomatic nature and underreporting of cases, the exact incidence of female genital tuberculosis remains unknown.

However, ICMR reported an increase in the prevalence of female genital tuberculosis in India from 19% in 2011 to 30% in 2015 [3]. The common genital organ affected in female genital tuberculosis in decreasing order are the fallopian tube (>95%), endometrium (60%), ovaries (20 to 30%), cervix (15%) whereas myometrium (2.5%) and vagina/vulva (1%) are involved rarely [4].

About 40- 80% of cases of FGTB may present with infertility. Multiple modalities are used for diagnosis is often difficult. Serology and microscopy are of less diagnostic yield whereas histopathological examination of specimen is helpful but it is not diagnostic [5]. Culture is time-consuming and the result is often delayed affecting the patient's outcome.

WHO introduced a new technique in 2010 that is CBNAAT. CBNAAT (cartridge-based nucleic acid amplification test) or GeneXpert MTB/RIF which was adopted by RNTCP in 2012. The method is advantageous in the early diagnosis of extrapulmonary tuberculosis with high accuracy. The literature assessing the utility and diagnostic accuracy of CBNAAT for the diagnosis of FGTB is Scarce. CBNAAT is a rapid, simple-to-use nucleic acid amplification test (CBNAAT). The standard assay of GeneXpert utilizes a single-use plastic cartridge with multiple chambers, that are preloaded with liquid buffers and lyophilized reagent beads

necessary for sample processing DNA extraction, and heminested RTPCR.

The Xpert MTB/RIF purifies and concentrates Mycobacterium tuberculosis bacilli from a sample, isolates genomic material from the captured bacteria by sonication, and subsequently amplifies the genomic DNA by PCR. The process identifies most of the clinically relevant rifampicin-resistant mutations in the RNA polymerase beta (*rpoB*) gene in the Mycobacterium tuberculosis genome in a real-time format using fluorescent probes called molecular beacons. Results are obtained from an unprocessed sample in 90 minutes with minimal biohazard and very little technical training required to operate. This test was developed as an on-demand near-patient technology that could be performed even in a doctor's office if necessary.

Material and Methods

An observation study: role of CBNAAT (cartridge-based nucleic acid amplification test) in the detection of genital tuberculosis in endometrial tissue among women with infertility presented at the department of obstetrics and gynecology, Gandhi Medical College and associated Sultania Zanana Hospital, Bhopal, during the study period of 12 months. The sample size was 215 cases according to inclusion criteria.

Criteria for Selection of Cases

Inclusion Criteria

- Women with infertility (primary and secondary) visiting OPD in Sultania Zanana Hospital, Bhopal.

Exclusion Criteria

- Known cases of Pulmonary TB or Extra Pulmonary TB.
- Women did not give consent to participate in the study.

After being included in the study, the patients were explained about the procedure and informed consent was obtained. Detailed data on socio-demographic

variables such as age, education, socioeconomic statuses, and the rural or urban background was obtained from all the study participants and entered in Performa. Post that, the patients were inquired about in the detail regarding their clinical histories such as presenting complaints, duration of infertility, number of children, duration of marriage, detailed menstrual history, obstetrics, and gynaecological history, and family history of TB. Detailed sexual history including frequency of intercourse, dyspareunia, impotency, or vaginismus was inquired about. History of tuberculosis, contact with a TB patient, history of STI, and any treatment taken for infertility were obtained and documented.

Observations

Premenstrual phase endometrial biopsy was obtained and subjected to

- Histopathological examination
- Culture for AFB
- CBNAAT

Statistical Analysis

The data was compiled using MS Excel and analysed using IBM SPSS software. The diagnostic accuracy of CBNAAT for the detection of female genital tuberculosis was calculated in the form of sensitivity, specificity, positive predictive value, and negative predictive value and expressed as a percentage.

Table 1: Distribution according to Age

Age (years)	Frequency (n=215)	Percentage
20-24	39	18.1
25-29	111	51.6
30-34	60	27.9
35-39	5	2.3

Table 2: Distribution of patients according to the type of infertility

Type of Infertility	Frequency (n=215)	Percentage
Primary	205	95.3
Secondary	10	4.7

Table 3: Distribution of patients according to socioeconomic status

Socioeconomic status	Frequency (n=215)	Percentage
Upper	11	5.1
Upper Middle	41	19.1
Lower Middle	59	27.4
Upper Lower	49	22.8
Lower	55	25.6

Table 4: Distribution of patients according to CBNAAT

CBNAAT	Frequency (n=215)	Percentage
Negative	187	87.0
Positive	28	13.0

Table 5: Distribution according to findings of endometrial biopsy

Endometrial Biopsy	Frequency (n=215)	Percentage
Normal	193	89.7
Inflammatory Cells	18	8.4
Others	4	1.9

Table 6: Distribution according to smear for AFB

Smear for AFB	Frequency (n=215)	Percentage
Negative	215	100.0
Positive	0	0

Table 7: Distribution according to culture findings

Culture	Frequency (n=215)	Percentage
Negative	209	97.2
Positive	6	2.8

Table 8: Results of various tests on endometrial samples

Test	Number	Positive results (%)
CBNAAT	215	28 (13%)
Smear	215	0 (0)
Culture	215	6 (2.8%)

Results

A total of 215 patients were enrolled in the study. The mean age of women with infertility was 27.6 ± 3.34 years and about 51.6% of women belong to the 25 to 29 years of age group. This was followed by 27.9% of cases belonging to the age range of 30 to 34 years and only 18.1% and 2.3% of patients belonged to 20 to 24 years and 35 to 39 years respectively.

In our study, the majority that is 95.3% women had primary infertility whereas only 4.7% of women had secondary infertility. About 27.5% of women belonged to lower middle socioeconomic status followed by 25.6% and 22.8% cases belonging to lower and upper lower socioeconomic status respectively. Only 19.1% of women belonged to the upper middle and 5.1% of women belonged to the upper socioeconomic class.

CBNAAT was conducted in all the cases of infertility; of the 13% cases were positive in our study. Endometrial biopsy revealed inflammatory cells in 8.4% of cases whereas other findings were revealed in 1.9% of cases however 89.7% of endometrial biopsies revealed normal findings. In our study; none of the patients had a positive smear for AFB i.e., the smear for acid-fast bacilli was negative in 100% of cases. Out of 215 cases of infertility, culture was positive for tubercle bacilli in 2.8% of cases in our study.

Positive results of various tests on endometrial samples in cases of infertility are as follows: overall CBNAAT was positive in 13% of cases; whereas HPE and culture were positive in only 10.3% and 2.8% of cases. Smear was negative in 100% of cases.

Discussion

Genital tuberculosis is one of the most common forms of extrapulmonary tuberculosis and its prevalence is increasing rapidly worldwide [5-10]. As this form of tuberculosis is mainly asymptomatic, the exact incidence remains unknown [11,12]. Though any genital organ can be affected in female genital tuberculosis, the fallopian tube (>95%), endometrium 60% and ovaries (20-30%) are the most common organs affected while endometrium and vagina/vulva are rarely affected [13].

Infertility is the most common presentation and may be observed in as high as 80% of the cases with FGTB. Apart from this, menstrual irregularities and recurrent abortions may also be a presenting feature [14]. Various pathophysiological mechanisms have been identified as contributory factors leading to infertility in cases with FGTB. These include Tubal factors: tubal blockage, loss of tubal motility due to ciliary damage, adhesions, tubal-ovarian mass formation,

hydrosalpinx, and peri-salpingitis. Uterine factors: decrease endometrial receptivity, failed implantation, endometrial atrophy, synechiae, vascularization of the endometrium. Ovarian dysfunction: chronic anovulation, luteal phase defects, the antigonadotrophic effects of tuberculosis, implantation failure, and intrinsic oocyte factor defect. The present study was conducted to evaluate the role of CBNAAT in the detection of genital tuberculosis in endometrial tissue among women with infertility. A total of 215 women with primary or secondary

infertility were enrolled in infertility clinics during the study period. Literature suggests that the mean age of developing the tuberculosis of female genital tract in developing countries like India is 20-30 years i.e., a decade earlier than compared developed countries (40 years). The early age at presentation in India could be attributed to early marriage and early childbearing. In our study, the mean age of women with infertility was 27.6 ± 3.34 years. However, the majority of females belong to 25-29 years of age (51.6%).

Table D.1: Comparison of age at presentation of females with other studies

Studies	Mean age (years)	Most common age group
Jahromi BN <i>et al</i> (2001)[15]	30.4 years	-
Mondal SK <i>et al</i> (2008)[16]	25.6 years	-
Agarwal S <i>et al</i> (2016)[17]	26.33 years	20-30 years (86.2%)
Kumar A <i>et al</i> (2021)[18]	29 ± 7.68 years	-
Keshari V <i>et al</i> (2021)[19]	30.81 ± 6.46	25 -34 years (64.7%)
Present study (2021)	27.6 ± 3.34 years	25 to 29 years (51.6%)

Socio-economic status (SES) has been observed as an important determinant of the reproductive health behaviour in females [20]. Low socioeconomic status and poor genital hygiene have been long linked with genital tuberculosis [21]. In the present study maximum females belonged to lower middle socioeconomic status (27.5%) followed by 25.6% cases belonging to lower socioeconomic economic status. Our study was conducted at the tertiary care centre, which caters to the population from all socio-economics strata but the population of lower socioeconomic status contributes to a major proportion of patients seeking care. However, in a study by Keshari V *et al* (2021) [19], about 50% of the patients belonged lower middle class followed by 28.12% belonging to a lower class. The findings of our study were supported by the findings of Surekha T. *et al* (2013) [20].

CBNAAT is a real-time polymerase reaction (RT-PCR) method that is used for the early identification of pulmonary and extrapulmonary cases with high accuracy.

CBNAAT is helpful in the detection of rifampicin resistance as it targets the *rpoB* gene [22]. Previous studies by Alwani H *et al* (2019) reported the 71% sensitivity of CBNAAT for EPTB with 98 to 100% specificity [23]. Our study documented CBNAAT to be positive in 13% of cases of infertility irrespective of the type of infertility. Our study findings were supported by the findings of Kumar *et al* 2021 in which CBNAAT was positive in 17.1% of genital tuberculosis [18]. Kousar S *et al* (2021) reported a CBNAAT positive in 1 out of 200 endometrial samples and its utility in the diagnosis of genital TB was questioned [24]. Keshari V *et al* (2021) reported a CBNAAT positive rate in 25% of the cases of genital tuberculosis.

Infertility may be primary or secondary and genital tuberculosis is associated with both primary and secondary infertility. It has been observed that even after the management of genital tuberculosis, the conception rate and pregnancy success rate remain low [25,26]. In our study, about 95.3% of women had primary infertility

whereas only 4.7% of women had secondary infertility. Out of 28 cases with positive CBNAAT cases, the majority of cases were of primary infertility. Genital tuberculosis is associated mainly with primary infertility and this finding was supported by previous studies. Also, the majority of patients irrespective of genital

tuberculosis who seek care at our institute have primary infertility. This could be attributed to the want of a child and the stigma related to females with no child as compared to females with at least one child. However, we observed no significant association of CBNAAT with the type of infertility.

Table D.2: Comparison of type of infertility in genital TB with other studies

Studies	Primary infertility	Secondary infertility
Khanna A <i>et al</i> (2011)[27]	58%	42%
Agarwal S <i>et al</i> (2016)[17]	81.5%	18.5%
Zahoor D <i>et al</i> (2019)[28]	84.6%	15.4%
Keshari V <i>et al</i> (2021)[19]	85.7%	14.3%
Present study (2021)	92.9%	7.1%

Since we aimed to assess genital tuberculosis in a patient with infertility, and no gold standard method is available for identification of EPTB, we obtained the biopsy sample from endometrium on which AFB smear and culture were done apart from CBNAAT. Endometrial biopsy revealed culture was positive in 2.8% of cases whereas smear was negative in all cases.

Our study findings were concordant with the findings of Farhana A *et al* (2019) in which none of the patients were positive with Ziehl Nelson staining whereas culture was positive in 4.6% of the cases [28]. Zahoor D *et al* (2019) also observed smear negativity in 100% of cases with genital tuberculosis with non-specific findings on histopathological examination.

Amongst culture and CBNAAT was observed to be more sensitive in detecting genital TB-positive cases as compared to culture in this study [29]. Our study findings were also supported by the findings of Tiwari K *et al* (2020), in which 18 cases out of 176 infertility cases were positive for tuberculosis. Of them, culture was positive in 3 out of 18, histopathological in 2 out of 18 and CBNAAT was positive in 2 out of 18 cases [30].

Conclusion

Infertility is a common complaint for which females seek care in Gynaecology OPD. Genital tuberculosis is the second most common cause of infertility among females. Mycobacterium tuberculosis may cause irreversible damage to the endometrium and other female genital organs. Therefore, early diagnosis and treatment of tuberculosis are crucial as they may improve fertility outcomes. Thus, screening for female genital tuberculosis should be done routinely for evaluating infertility.

Molecular diagnosis of M. tuberculosis by CBNAAT has a great potential to improve the ability to diagnose female genital tuberculosis. CBNAAT is a rapid, sensitive, and specific test that can be used for early diagnosis of FGTB and to start treatment with the added advantage of the detection of drug-resistant Mycobacterium tuberculosis. CBNAAT has a higher detection rate as compared to other conventional methods. This study shows that these conventional methods like microscopy and culture were less sensitive when compared to CBNAAT.

None of the endometrial biopsy specimens had histopathological findings specific to tuberculosis. As well as none of the samples submitted to the lab were positive on AFB microscopy whereas culture for M. tuberculosis was positive in 2.8% of cases.

The reason could be the paucibacillary nature of the Mycobacterium in an endometrial sample. It makes detection of AFB difficult in microscopy and culture. However, among the various detection methods of FGTB, culture, and CBNAAT were more reliable.

The major drawback of culture is the long duration in obtaining results which is an asset for CBNAAT. Thus, among the four methods used in FGTB cases, CBNAAT has the highest utility. A high degree of suspicion and a combination of all the methods such as CBNAAT, culture, histopathological examination, and the microbiological test is important for the detection of genital tuberculosis. Thus, through this study, we found that the use of all four methods especially CBNAAT and culture to detect AFB in female genital tuberculosis is necessary. When all the methods specially CBNAAT and culture are applied together, diagnostic yield may be improved, and early treatment can be started.

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