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Original Research Article

A study on role of cartridge based nucleic acid amplification test (CBNAAT) in diagnosis of genital tuberculosis among patients of infertility and pelvic inflammatory disease

Versha Keshari[1], Reena Srivastava[1], Pushkar Kesarwani[2,*], Sarita Pandey[3], Devendra Mishra[4]

[1]*Dept. of Obstetrics and Gynaecology, B.R.D. Medical College, Gorakhpur, Uttar Pradesh, India*
[2]*Dept. of Paediatrics, Sir Gangaram Hospital, Delhi, India*
[3]*Dept. of Obstetrics and Gynaecology, Hindu Rao Hospital, Delhi, India*
[4]*Dept. of Paediatrics, Dr. Ram Manohar Lohia Hospital, Delhi, India*

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ABSTRACT

Background: Female genital TB (FGTB)– referring to TB of the uterus, fallopian tubes and/or Ovaries. It poses a diagnostic dilemma because of its varied presentations and lack of sensitive and specific methods of diagnosis, though CBNAAT gives rapid result.
Objective: To study the role of CBNAAT in the Diagnosis of Genital Tuberculosis among infertility and Pelvic Inflammatory Disease (PID) Patients.
Materials and Methods: 102 patient of infertility (52) and chronic PID (50) were enrolled for our cross-sectional study. Mantoux, ESR, Histopathology, CBNAAT was performed in all 102 cases and Hysterosalpingography (HSG), Laparoscopy, Hysteroscopy in selected cases. Patient with clinical features of genital TB, supported with TB suggestive test were diagnosed as high suspicious genital TB (GTB+) and rest Low suspicious GTB (GTB-) cases.
Results: 14/ 52 cases of infertility and 18/ 50 cases of chronic PID were clinically diagnosed as High suspicious genital TB (GTB+). In our study, overall Prevalance of GTB was 31.37%, among infertility patient prevalence was 26.92% and among chronic PID was 36%. 16/32 (50%) mantoux positive, 25/32 (78.13%) had increased ESR. On HSG, 10/52 (19.23%) infertility cases, on laparoscopy 24/32 (75%), on endometrial histopathology only 3/32 (9.37%) cases had finding suggestive of TB. CBNAAT could detect tubercular bacilli only in 25% (8/32) TB cases.
Conclusion: High index of suspicion for FGTB is must for diagnosis. Unlike Pulmonary TB, role of CBNAAT in the diagnosis of female genital TB is limited. PPV of CBNAAT for diagnosis of GTB is almost 100%.

1. Introduction

Tuberculosis is a major public health issue in the world and India, which is a high incidence country. As per the Global TB report 2017, the estimated incidence of TB in India was approximately 28,00,000 accounting for about a quarter of the world's TB cases.[1] Causative organism is Mycobacterium Tuberculosis. The most common form of extrapulmonary TB is genitourinary disease, accounting for 27% (range 14 to 41%) worldwide. Female genital TB (FGTB) affecting the female genital organs in descending order of frequency are fallopian tubes (95-100%), uterine endometrium (50-60%), ovaries (20-30%), cervix (5-15%), uterine myometrium (2.5%) and vagina/vulva (1%).[2]

Tuberculous infection of the female genital organs can result in infertility, dyspareunia, menstrual irregularities and

* *Corresponding author.*
E-mail address: pushkarkesarwani@gmail.com (P. Kesarwani).

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chronic pelvic inflammatory disease (PID). A survey by the Indian Council of Medical Research (ICMR) reported that prevalence of FGTB in India has increased from 19% in 2011 to 30 per cent in 2015.

Unlike pulmonary tuberculosis, the clinical diagnosis of Genital TB is difficult. Because of paucibacillary nature, a very high degree of suspicion is maintained in the majority of the cases and the disease is either asymptomatic or has varied clinical presentation.

Multiple diagnostic modalities can be used for diagnosis of female genital TB. Low sensitivity of smear microscopy, time consuming culture sensitivity limits its role in the diagnosis. To address this issue, there was a need for a simple and rapid diagnostic tool atleast for high-burden countries. A new diagnostic test GeneXpert/ cartridge based nucleic acid amplification test (CBNAAT) that is a rapid molecular beacon was developed. CBNAAT is fully automated and based on polymerase chain reaction (PCR) that detects deoxyribonucleic acid (DNA) directly from the clinical specimens and also detects rifampicin resistance.[3] This diagnostic test is designed to purify, concentrate, amplify and identify targeted rpoB nucleic acid sequences and delivered the results in about 120 minutes. Though in 2012, W.H.O. has recommended the CBNAAT for routine use under programmatic conditionsbut its role in the diagnosis of extrapulmonary TB seems to have limited role.[4]

2. Aim and Objectives

To study the role of CBNAAT in the Diagnosis of Genital

Tuberculosis among infertility and Pelvic inflammatory disease Patients.

3. Materials and Methods

This was a cross- sectional study done over a period of 18 months (from July 1^{st}, 2018 to Dec 31^{st}, 2019). Convenient sample of 102 cases were taken. After obtaining clearance by Ethical and scientific committee of the institution, 102 Women attending department of Obstetrics and Gynaecology OPD and fulfilling inclusion criteria were enrolled for our study as a suspicious FGTB cases.

3.1. Inclusion criteria

1. A woman presenting with infertility (defined as the inability to conceive despite regular unprotected intercourse for 1 year).
2. A woman with provisional diagnosis of Pelvic inflammatory disease (triad of Pelvic pain, cervical motion and adnexal tenderness and presence of fever (>38° c) with or without other features like Pelvic organ tenderness, white discharge, and/ or mucopurulent endocervicitis).

3.2. Exclusion criteria

1. Women who had not given consent.
2. Women with already diagnosed gynaecological problems or chronic discharge due to Fibroid, PCOS, CIN, Dysplasia.
3. Infertility due to male infertility factor.

Clinicosocial history regarding age, Socioeconomic status based on the Modified Kuppuswamy scale, menstrual pattern, type of infertility (primary infertility when a woman has never conceived and secondary infertility is the incapability to conceive in a couple who have had atleast one successful conception in the past), past history of TB in any part of the body or history of TB contact (living in the same household or in frequent contact with a sputum smear positive TB patient in last 2 years). Body Mass Index/ BMI (weight in kilograms divided by the square of height in meters) was calculated.

In PID patients, common causes of PID other than M. Tuberculosis-

Chlamydia, N. Gonorrhea was ruled out by Nucleic acid amplification test. Mantoux test using PPD 23 (5 TU), Erythrocyte sedimentation rate (ESR), Histopathology (HPE) examination of endometrial aspirate(EA)/ endometrial tissue (ET) and CBNAAT of EA/ ET/ pouch of douglas fluid was performed in all 102 cases. According to clinical situation, cases were subjected to one or more of the following examination with aseptic precaution- Endometrial biopsy (EB), Hysterosalpingography (HSG), Hysteroscopy and laparoscopy.

20 EA, 48 EB sample during luteal phase of menstrual cycle and 34 fluid sample from pouch of douglas was taken for CBNAAT. CBNAAT Samples of EB collected in normal saline containing falcon tubes (container) and EAs & fluid from the POD transported in sterile Falcon tubes. For CBNAAT examination the sample reagent were added at a 3:1 ratio to clinical specimens. The closed specimen container was manually agitated twice during a 15-minute period at room temperature, before 2 ml of the inactivated material (equivalent to 0.5 ml of decontaminated pellet) was transferred to the test cartridge.

Patient with clinical features of genital TB, supported with TB suggestive test like mantoux/ ESR / Hysterosalpingography/ laparoscopy/ hysteroscopy were diagnosed as high suspicious genital TB (GTB+) and remaining were diagnosed as Low suspicious GTB (GTB-) cases as depicted in flow Chart 1. Sputum for AFB examination and Chest X- ray was done in all GTB+ cases to rule out pulmonary Koch.

Microsoft word and Excel have been used to generate graphs and tables. Sensitivity and specificity was calculated.

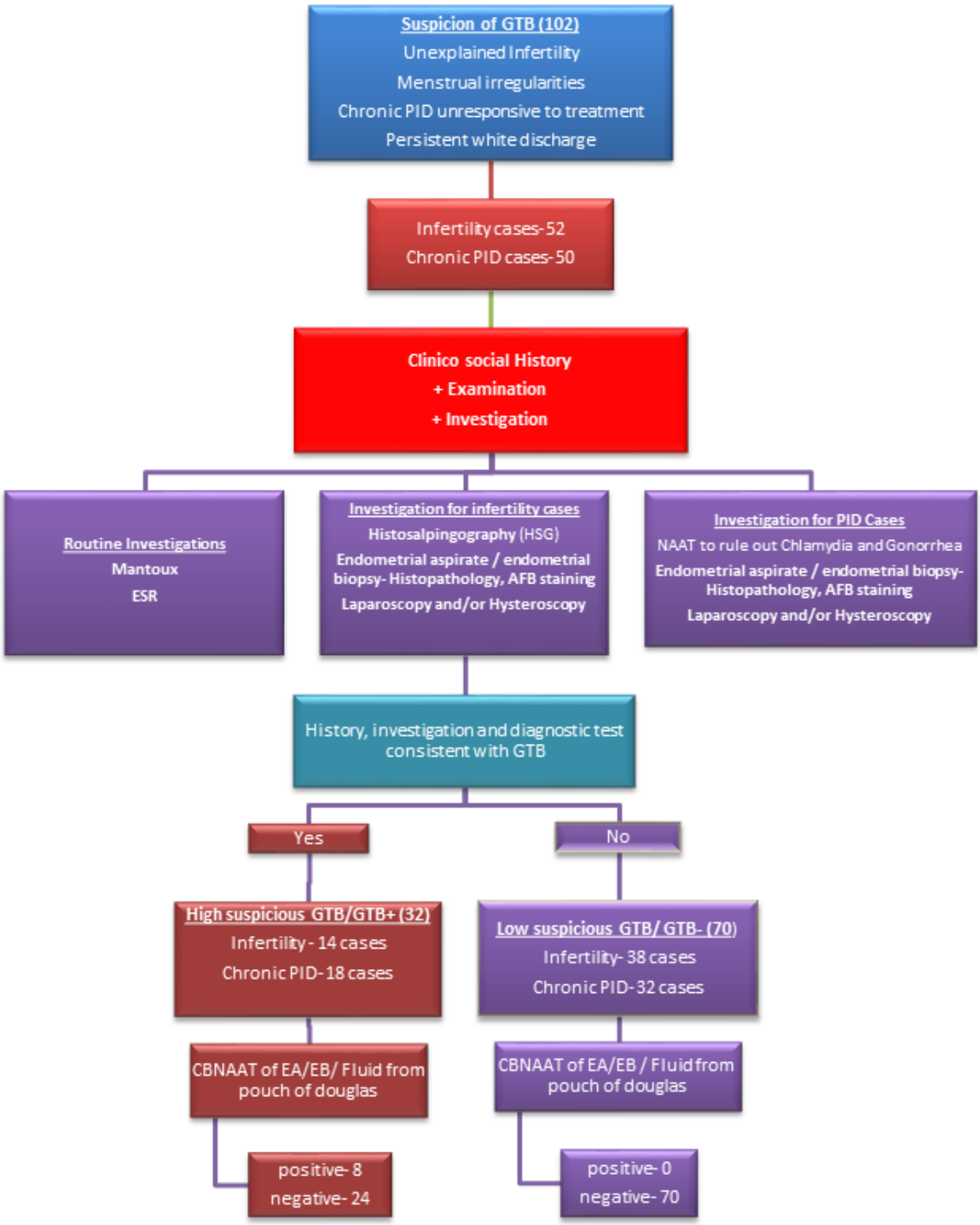

Chart 1: Flow diagram showing method to diagnose high suspicious genital TB (GTB+).

4. Results

Total 102 women (52 infertility and 50 chronic PID) were enrolled for the study. 32 cases with clinical features of genital TB, supported with TB suggestive test like ESR / Hysterosalpingography/ laparoscopy/ hysteroscopy were diagnosed as high suspicious genital TB/ GTB+ and remaining 70 cases of low suspicious TB/ GTB.

66 (64.7%) of total 102 cases were of 25 -34 years of age (Mean +SD) 30.81+6.46 (Figure 1). According to modified kuppuswamy scale, 16 (50%) of GTB+ belonged to upper lower class, 9 (28.12%) lower class, 5 (15.62%) lower middle and 2 (6.25%) to upper middle class. Among 32 GTB+ cases, 15 (46.87%) were undernourished (BMI <18.5 kg/m^2), 14 (43.75%) had normal nutrition (BMI= 18.5-23 kg/m^2) and 3 (9.37%) had over nutrition (BMI >23 kg/m^2).

18/32 [56.25%] GTB+ cases had regular cycle and 14/32 [43.75%] had irregular cycle.

12 (85.7%) out of 14 high suspicious GTB cases with infertility had primary and only 2 (14.3%) had secondary infertility.

History of TB contact was present in 12 (37.5%) GTB+ cases. 7 (21.88%) GTB+ cases had previous history of pulmonary kochs, 1 (3.12%) had Abdominal TB and bone TB each.

Among GTB+ cases in infertility and PID group, 16 (50%) were mantoux positive (induration ≥10mm after 72 hour), 13 (40.63%) were mantoux negative (induration <5 mm) and 3 (9.38%) were indeterminate (induration 5-9 mm). Among low suspicious GTB cases in infertility and PID group, 23 (32.85%) were mantoux positive, 45 (64.29%) mantoux negative and 2 (2.86%) were indeterminate. Out of total 102 infertility and PID cases, 39 (38.24%) were mantoux positive, 58 (56.86%) were mantoux negative and 5 (4.9%) were Indeterminate. Sensivity and specificity of mantoux was 50% and 58.9% respectively for genital TB. Positive Mantoux alone was not taken as TB suggestive test.

Among 32 High suspicious GTB cases, 25 (78.13%) had increased ESR (>20 mm in 1 hour) while among 70 low suspicious GTB cases, 16 (22.85%) had increased ESR. Sensitivity and specificity was 78.1% and 77.14%. Out of total 102 infertility and PID cases, 41 (40.19%) had increased ESR (>20 mm in 1 hour).

All 52 infertility cases underwent hysterosalpingography (HSG). 10 (19.23%) had finding suggestive of GTB and belonged to high suspicious GTB cases. Fallopian tube block in 7 (50%), Uterine cavity adhesion was present in 1 (7.14%) and beaded appearance was present in 2 (14.29%).

On hysteroscopy, 17 (53.13%) GTB+ cases had finding suggestive of TB. Synechiae, poor endometrium was present in 3 each, tubercle, distorted ostia in 4 each, cervical stenosis in 2 and fibrosis was seen in 1 case.

On laparoscopy, 24 (75%) GTB+ cases had finding suggestive of GTB. 24 (29.26%) out of 82 cases had finding suggestive of GTB and these 24 belonged to high suspicious GTB group. Tubal block was seen in 7(21.88%), Sacculations/ beaded tubes, Tubercle + Peritubal adhesions, absence of dye spillage, TO mass was seen in 4 (12.5%) cases each, Hydrosalpinx in 3 (9.38%) and pelvic adhesion was seen in 2 (6.25%) cases. 4 cases of TO mass also had tubal block in same patient.

Only 3 out of 102 high suspicious GTB cases had their endometrial histopathology report positive for evidence of tuberculosis and belonged to high suspicious GTB. Only 4 (12.5%) out of 32 clinically suspicious sputum sample sent were AFB positive. Though there was evidence of FGTB clinically and on investigations in 32 but CBNAAT was positive only in 8 (25%) cases and all 8 CBNAAT positive cases were rifampicin sensitive.

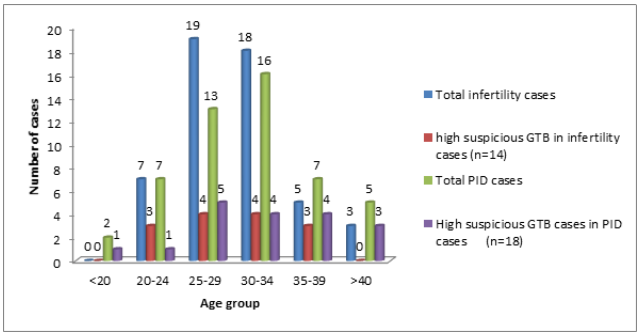

Fig. 1: Study group distribution on the basis of age

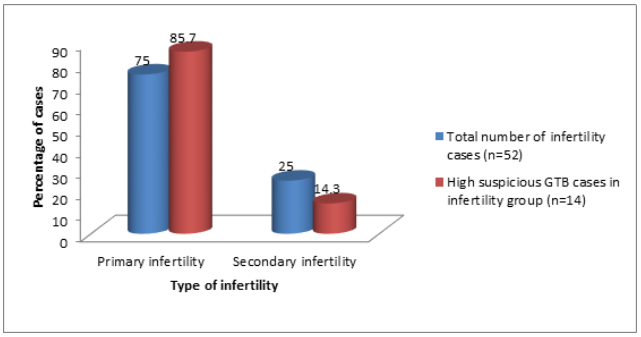

Fig. 2: Distribution of infertility group on the basis of nature of Infertility

5. Discussion

In our study, CBNAAT could detect tubercular bacilli only in 25% (8/32) high suspicious genital TB cases that was diagnosed clinically.

Mean age observed in our study was (Mean ± SD): 30.81± 6.46 which is comparable to the study of Arpitha

Table 1: Result of different diagnostic test

Test		High suspicious GTB/ GTB+ (n=32)		Low suspicious cases/ GTB- (n=70)		Among Total cases (n=102)	
		Cases	Positive/ abnormal result	Cases	Positive/ abnormal result	Cases	Positive/ abnormal result
Mantoux		32	16 (50%)	70	23 (32.86%)	102	39 (38.23%)
Increased ESR		32	25 (78.13%)	70	16 (22.85%)	102	41 (40.19%)
Sputum AFB		32	4 (12.5%)	0	0 (0%)	32	4 (12.5%)
HSG		14	10 (71.43%)	38	0(0%)	52	14 (26.92%)
Laparoscopy		32	24 (75%)	50	0 (0%)	82	24 (23.53%)
Hysteroscopy		32	17 (53.13%)	50	0 (0%)	82	17 (20.73%)
HPE		32	3 (9.38%)	70	0 (0%)	102	3 (2.94%)
	Total	32	8(25%)	70	0(0%)	102	8 (7.84%)
CBNAAT	Infertility	14	4 (28.57%)	38	0 (0%)	52	4 (7.69%)
	PID	18	4 (22.22%)	32	0 (0%)	50	4 (8%)

VJ et al.,[5] (Mean ± SD): 29.89±4.23 years. Our results defer from results of Sileshi Abdissha et al.[6] in which most of the study participants were in the age group of 34–40 years (mean age being 38.37±10 years). Most of the women (41.17%) belonged to lower class according to modified kuppuswamy scale. 53 (51.96%) had normal nutrition, 40 (39.21%) had under nutrition and 9 (8.82%) had over nutrition while among 32 GTB+ cases, 15 (46.87%) were undernourished and 14 (43.75%) had normal nutrition.

In our study, out of total 102 cases, 62 (60.78%) had regular menstrual cycle while among 32 GTB+ cases, 18 (56.25%) had regular cycle and 14 (43.75%) had irregular cycle. Hypomenorrhoea was the most common menstrual abnormality, present in 5 (15.62%) patients followed by oligomenorrhoea in 4 (12.5%) of patients, menorrhagia in 2 (6.25%) patients, amenorrhea in 3(9.37%). These results are comparable with the study of Arpitha VJ et al[5] in which 53(76.8%) had regular menstrual cycle and rest had menstrual abnormality. Hypomenorrhoea was the most common menstrual abnormality present in 6 (8.7%) followed by oligomenorrhoea in 4(5.8%), menorrhagia in 4(5.8%), amenorrhea in 2(2.9%) patients. Our results defer from results of Thangappah et al[7] in which most patient had oligomenorrhea and Sileshi Abdissha et al.,[6] in which 112(73.7%) of the study participants had menstrual disturbance.

In our study, out of 52 infertility group, 39 (75%) had primary infertility and 13 (25%) had secondary infertility as shown in Figure 2. 12 (85.7%) primary infertility and only 2 (14.3%) secondary infertility were highly suspicious for GTB. Similar results were reported by Gupta N et al.[8] (primary infertility 30(75%), secondary infertility 10(25%), Arpitha VJ et al[5] primary infertility 45 (65.2%), secondary infertility 24 (34.8%) but Bharati malhotra et al[9] reported primary infertility 450 (92.21%) slightly higher than our result.

Among high suspicious 32 GTB cases, history of TB contact was present in 12 (37.5%). Thangappah et al[7] found history of close contact with tuberculosis only in 4.16% cases.

In our study, out of total 102 infertility and PID cases, 9 (8.82%) had past history of pulmonary TB and among 32 High suspicious GTB cases, 7 (21.88%) had pulmonary kochs, 1 (3.12%) had Abdominal TB and bone TB in each in the past (1 to 15 years back). Similar results were obtained by Arpitha VJ et al.[5] In 69 patients in their study, previous history of pulmonary TB was seen in 4(5.9%), abdominal TB in 2(2.8%) and 1(1.4%) patient had history of TB of bones. Similarly, Thangappah et al[7] also found only 4(5.5%) infertility cases with past history of tuberculosis such as axillary adenitis, abdominal TB and pulmonary tuberculosis

In our study, sensivity and specificity of mantoux was 50% and 58.9% respectively for genital TB. These results are comparable to the study of Raut et al[10] which had a sensitivity and specificity of 55% and 80% in women with laparoscopically diagnosed tuberculosis. Khanna A et al.[11] reported Mantoux test sensitivity 61.50% and specificity 66.21%.

In our study, Sensitivity and specificity of the ESR test was 78.1% and 77.14%. Khanna A et al[11] reported sensitivity of raised ESR 57.69% and specificity 63.51%. Our results are similar to the study of Thangappah et al.[7] ESR was elevated in 11 cases (15%) and there was corroborative evidence of tuberculosis by other clinical parameters in 10 (90.9%) cases.

Out of total 102 cases, hysteroscopy was performed in 82 patients and 17 (20.73%) had abnormal hysteroscopic finding**.** Among 32 GTB+ cases, 17 (53.13%) had abnormal finding. Synechiae, poor endometrium was present in 3 (9.34%) each, tubercle, distorted ostia was seen in 4 (12.50%) each, cervical stenosis was seen in 2(6.25%) and fibrosis was seen in 1 (3.13%). Our results defer from the study of Gupta N et al[8] who found adhesions in 7 (17.5%) cases of infertility on hysteroscopy while study conducted by Arpitha VJ et al.[5] hysteroscopy revealed normal findings in 51 of patients (73.9%), 2 patients (2.9%)

each had distorted ostia and fibrosis, poorly vascularised endometrium was seen in 8 patients (11.6%), bald areas was seen in 4 patients (5.8%), synechiae was seen in 1 patient (1.4%), cervical stenosis in 1 patient (1.4%).

In our study, all 52 infertility cases underwent hysterosalpingography. 10 (19.23%) cases had finding suggestive of GTB and were kept in GTB+ group. Uterine cavity adhesion was present in 1 (7.14%), Fallopian tube block in 7 (50%) and distorted ostia was present in 2 (14.29%) cases. Our results defer from results of Thangappah et al[7] who on Hysterosalpingogram found characteristic features suggesting genital tuberculosis such as distorted endometrial cavity, beaded appearance of the tubes, retort shaped hydrosalpinx, calcified areas and cornual blocks in 35 out of 59 cases (54.2%). In our study, 82 out of total 102 infertility and PID cases underwent laparoscopy. Out of 82 cases, 24 (29.27%) had finding suggestive of TB and kept in high suspicious GTB group (75%). Similar results were seen in the study of Thangappah et al.[7] and Arpitha VJ et al[6] in which 43(59.7%) and 41(60%) of patient on laparoscopy had abnormal finding suggestive of GTB respectively.

In our study, histopathology sample (HPE) was sent in all 102 cases. Only 3 (2.94%) cases had evidence of tuberculosis. Our results are similar to the study results of Thangappah et al.,[7] HPE positive in 6.9% cases and Geetika et al[12] HPE positive in 2.38% cases. In our study, 32 sputum sample from GTB+ cases was sent for AFB detection. Only 4 (12.5%) sputum sample were AFB positive.

Only 8 (7.84%) out of 102 CBNAAT sample were reported to be positive for mycobacteria tuberculosis and these cases were from High suspicious GTB group (21.9%). Sensitivity of CBNAAT for FGTB was 25%. PPV was 100% and NPV was 74.4%. Very few study has directly tested the sensitivity and specificity of CBNAAT in the diagnosis of genital TB while role of PCR in the GTB diagnosis was done in many studies. Zahoor et al[13] did Gene Xpert in 69 High suspicious GTB patients. Of these, 5 (7.25%) came positive. Our results are similar to review article published by Jai Bhagwan Sharma et al[14] stating that CBNAAT has 35% sensitivity and 100% specificity in the detection of FGTB.

In our study, prevalance of GTB was 31.37% and prevalanc of GTB among infertility patient was 26.92% while study of other researchers reported prevalence between 3-39% in infertility group. Thangappah et al[7] reported incidence of 38.89% among infertility patients. Prevalance of GTB among PID patient was 36% in our study. Sileshi Abdissha S et al. reported prevalence of endometrial TB 4.6% by PCR in patient who underwent endometrial biopsy because of various gynaecological causes.

While GTB was diagnosed in 32 infertility and chronic PID cases clinically but CBNAAT was positive in only 25% cases.

6. Conclusion

1. High index of suspicion for FGTB is must for diagnosis.
2. Unlike Pulmonary TB, role of CBNAAT in the diagnosis of female genital TB is limited.
3. Clinical diagnosis is still the most important way of diagnosing FGTB.
4. Positive predictive value (PPV) of CBNAAT for FGTB is 100% while negative predictive value (NPV) is 74.4%.

7. Source of Funding

None.

8. Conflict of Interest

The authors declare that there is no conflict of interest.

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Author biography

Versha Keshari, Senior Resident

Reena Srivastava, Professor

Pushkar Kesarwani, Senior Resident

Sarita Pandey, Senior Resident

Devendra Mishra, Senior Resident

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