

Original Research Article

A study to know the prevalence of genital tuberculosis in female's pulmonary tuberculosis patients and role of cartridge based nucleic acid amplification test in genital tuberculosis from North India

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ABSTRACT

Background: *Mycobacterium tuberculosis*, most commonly, infects the lungs (pulmonary TB). Most cases of female genital TB (FGTB) are found in premenopausal women, theoretically because an atrophic endometrium provides a poor milieu for mycobacterial growth. Female genital TB generally occurs secondary to pulmonary (commonest) and more common in women with reproductive age group. The present study was designed to know the prevalence of genital tuberculosis in female's pulmonary tuberculosis patients and role of cartridge based nucleic acid amplification test (CBNAAT) in genital tuberculosis.

Methods: The patients of female's pulmonary tuberculosis were picked up from out-patient and in patients' section of the hospital at random. After ruling out pregnancy, the endometrial samples were collected from premenstrual endometrium (approximately 2-3 days before menstruation) and were subjected to histopathological examination and CBNAAT. Day 1 or 2 menstrual blood of unmarried females and patients not giving consent for biopsy were taken.

Results: Seventy married and 20 unmarried patients of female pulmonary tuberculosis patients were evaluated for genital tuberculosis. The mean age of married population was 29 ± 7.68 years. Cough with expectoration was most common respiratory symptoms and seen in 94% cases. In present study 89% cases were sputum positive and 11% were sputum negative. The percentage of non MDR and MDR TB cases were respectively 95.7 and 4.2% respectively. Among the gynecological symptom's irregular menstruation, vaginal discharge and pelvic pain were present in 68, 60 and 52% of the study patients respectively. The diagnosis of FGTB by histopathology examination and CBNAAT were 28.6 and 17.1% respectively.

Conclusions: In present study FGTB diagnosed by histopathology examination and CBNAAT were 28.6 and 17.1% respectively and which was statistically significant ($\chi^2=28.25$ and p value=0.00001).

Keywords: Pulmonary tuberculosis, Female genital tuberculosis, CBNAAT

INTRODUCTION

Tuberculosis (TB) is a major public health problem worldwide and is caused by *Mycobacterium TB*. According to WHO estimates, in 2017, an estimated 2.7

million people developed tuberculosis (TB) disease in India and over 400000 people died.¹ Currently, the WHO estimated incidence of rifampicin (R) and MDR-TB in India is around 11 patients per 100 000 population annually as per the global TB report, 2019.²

About 80% cases of tuberculosis are pulmonary and extrapulmonary tuberculosis (EPTB) accounts for rest of all TB cases. In female patients, one of the most common sites of extrapulmonary TB is the reproductive organs, termed genital TB (FGTB) and accounts for about 9% of all EPTB cases.^{3,4} Genital TB in females has been identified to be an important cause for infertility in countries having high prevalence of TB.⁵

The incidence of FGTB varies in different countries from 1% in infertility clinics of USA, 6.1 to 21.1% in South Africa and 1-19% in various parts of India.³⁻⁹ 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% in 2015.¹

Female genital tuberculosis can occur in any age group, but women in the reproductive age group (15-45 year) are the most affected.¹¹

Genital TB is mostly secondary to pulmonary TB or extrapulmonary foci like kidneys, meninges, skeletal system and GIT. TB bacilli infect the genital tract by 4 routes (a) hematogenous route (with lungs as the common primary focus), (b) descending direct spread, (c) lymphatic spread (d) and rarely as primary infection of the genitalia through sexual transmission.¹² Genital organs affected by *Mycobacterium TB* with decreasing frequency are fallopian tubes (95-100%), uterine endometrium (50-60%), ovaries (20-30%), cervix (5-15%), uterine myometrium (2.5%) and vagina/vulva rarely.¹³ Tuberculous infection of the female genital organs can result in infertility, dyspareunia, menstrual irregularities and chronic pelvic inflammatory disease (PID).¹⁴

Diagnosis of genital tuberculosis remains challenging since the number of *Mycobacterium tuberculosis* (MTB) bacilli present in tissues at sites of disease is often low. HPE of the specimens show typical features of TB infection in the form of granulomatous caseous lesions. In 110 FGTB patients Mondal et al reported HPE reports that varied from small-medium epithelioid cells granulomas in different stages of caseation and rare detection of acid-fast bacilli (AFB).¹⁵

Definitive diagnosis of TB requires the isolation of TB bacilli. Convention methods for diagnosis of TB include microscopy and culture (both solid and liquid method). Reliance on culture, the mainstay of diagnosis, often leads to considerable delays, compromising patient care and outcomes.

Molecular techniques for the detection of TB are increasingly tested and used currently. Nucleic acid amplification tests for rapid TB diagnosis are increasingly being used. The XpertR MTB/RIF assay (Cepheid Inc., CA, USA) marks an important development in the field of rapid molecular TB diagnostics.¹⁶

CBNAAT is a recently introduced polymerase chain reaction (PCR) based method for detection of TB. It also detects rifampicin resistance as it targets the *rpoB* gene of mycobacteria.¹⁷ CBNAAT is a *Mycobacterium tuberculosis*-specific automated, cartridge based nucleic acid amplification assay, having fully integrated and automated amplification and detection using real-time PCR, providing results within 120 minutes. It is a highly specific test as it uses 3 specific primers and 5 unique molecular probes to target the *rpoB* gene of *M. tuberculosis*, which is the critical gene associated with rifampicin resistance.¹⁸

There is no single diagnostic test available to confirm the diagnosis of FGTB. High degree of clinical suspicion, elaborate history taking, systemic examinations, battery of tests to document *Mycobacterium TB* along with imaging methodologies for characteristic structural changes as needed to diagnose it.¹⁸ So, high indexes of suspicion are required.

Treatment of FGTB is similar to pulmonary TB. Among all new cases or if rifampicin/isoniazid sensitive, whether microbiologically confirmed or clinically diagnosed are given combination of Four drugs isoniazid (H), rifampicin (R), pyrazinamide (Z) and ethambutol (E) is given for two months (HRZE), followed by H, R and E (HRE) daily for four months therapy. For isoniazid-resistant TB patients the uni-phasic regimen (6LfxRZE) is given.¹⁹ For MDR, pre-XDR, XDR TB patients the recommended regimen is bedaquiline, levofloxacin (or moxifloxacin), linezolid, cycloserine, clofazimine.

The present study was done with aimed to know the prevalence of genital tuberculosis in female's pulmonary tuberculosis patients and their clinical presentations and role of using newer diagnostic methods (CBNAAT) in genital tuberculosis.

METHODS

The present study was conducted in the department of tuberculosis and respiratory diseases and department of obstetrics and gynecology, G.S.V.M. medical college, Kanpur, India from December 2015 to November 2017. This prospective study included 70 married and 20 unmarried patients of female pulmonary tuberculosis irrespective of genital symptoms. All participants gave written informed consent to participate in the study and Ethical committee approval was obtained to conduct the study.

Seventy married and 20 unmarried patients of female pulmonary tuberculosis patients

Inclusion criteria

Inclusion criteria included female pulmonary tuberculosis patients.

Exclusion criteria

Exclusion criteria excluded uncooperative patients, patients not giving informed consent, healed pulmonary tuberculosis whether treated or untreated for tuberculosis in past, pregnant women, patients having bleeding disorders and patients having carcinoma cervix or body of uterus.

A detailed clinical history and physical examination including both respiratory and genital examinations was carried out for every subject who entered in the study as per the pre-designed proforma. Patients were examined clinically and radiologically with a view to establish diagnosis. All routine investigation such as hemoglobin, total leucocyte count (TLC), differential leucocyte count (DLC), fasting and post-prandial blood sugar, serum creatinine, SGPT, serum bilirubin, serum protein, serum albumin, sputum for AFB and chest x-ray PA view, were carried out among all the patients.

Urine pregnancy test would be done in patients of childbearing age. An ultrasound examination of abdomen was done and, in some patients, trans-vaginal ultrasound (TVS) examination of the genital system was also done.

In females of childbearing age group along with amenorrhea after ruling out pregnancy using urine pregnancy test an endometrial biopsy was taken. Day 1 or day 2 menstrual blood of unmarried females and patients not given consent for endometrial biopsy was taken.

The endometrial samples for the study were collected from premenstrual endometrium (approximately 2-3 days before menstruation). All samples were subjected to histopathological examination and CBNAAT.

For histopathological studies, a portion of the endometrial tissue from the lesion was fixed in 10 per cent formalin; routine processing was done and stained with hematoxylin and eosin. Presence of caseating granulomas surrounded by epithelioid cells, lymphocytes, plasma cells and giant cells were diagnostic of tuberculosis. For microscopic examination of acid-fast bacilli (AFB), biopsy material was ground well using homogenizer and the concentrated mix was taken for smear and was stained with Ziehl-Neelsen stain. For CBNAAT examination, the material from the remaining aspirate was added with buffer in 1:2 ratio, collected in to pre-sterilized falcon tubes and incubated at room temperature for 25 to 30 min. Two ml of the reagent sample mix was then transferred to an Xpert cartridge using a Pasteur pipette and the cartridge was loaded onto Xpert (Cepheid, Dx system version 4.0c) machine. Results were reported as positive or negative for *M. tuberculosis* as CBNAAT gives semiquantitative estimate of the concentration of bacilli as defined by the Ct (cycle threshold) range (high, <16; medium, 16-22; low, 22-28; very low, >28).

Statistical analysis

The data were entered into Microsoft excel and statistical analysis was performed using the statistical package for social sciences (SPSS version 20). Data were expressed as mean \pm standard deviation or number with percentage. Difference between variables in the groups was compared using Pearson's chi-square and Fisher Z test.

RESULTS

Seventy married and 20 unmarried patients of female pulmonary tuberculosis patients were evaluated for genital tuberculosis. The mean ages of married population were 29 ± 7.68 years. Most of study patients had almost equal distribution of rural (51%) and urban (49%). Cough with expectoration was the most common respiratory symptom (94%) followed by fever in 81%, weight loss in 56% and hemoptysis in 19% cases. Diabetes mellitus was found in 5 (7%) cases.

In present study 62 (89%) were sputum positive and 8 (11%) were sputum negative. Of the total cases, the percentage of non MDR tuberculosis and MDR tuberculosis cases were 95.7 and 4.28% respectively. The percentage of patients in 20-30 years (age group 1), 31-40 years (age group 2) and age >41 years (age group 3) were 59, 33 and 8.6% respectively. The sputum positive patients were high in all groups and equally distributed among all age groups. This difference in positivity in different age groups was compared between age group 1 and age group 2, age group 1 and age group 3, age group 2 and age group 3, by applying Fisher Z test, none of the difference was statistically significant (p and Z value=0.73 and 0.34, 0.64 and 0.47, 0.82 and 0.23 respectively).

Among the gynecological symptom's irregular menstruation, vaginal discharge and pelvic pain were present in 68, 60 and 52% of the study patients respectively. Normal menstruation was reported in 31% patients. Out of 68% patients with irregular menstruation, amenorrhea in 26% and menorrhagia in 24% were more common symptoms. In present study infertility were observed in 64.3 % cases.

In present study pelvic USG findings reveals irregular fallopian tube, thickened endometrium, tubo-ovarian mass, encysted fluid/fluid in pouch of Douglas, bulky uterus, in 90, 83.5, 80, 80 and 50% cases respectively and normal study in 5 (10.6%) cases of genital tuberculosis.

Irregular fallopian tube, thickened endometrium, tubo-ovarian mass, encysted fluid/fluid in pouch of Douglas, normal study on ultrasonography was found to have statistically significant association ($\chi^2=23.9, 10.9, 7.9, 7.9, 14.3$ and p value=<0.001, <0.001, 0.0048, 0.0048, <0.001 respectively) with genital tuberculosis by applying chi-squared test. While bulky uterus on ultrasonography was statistically non-significantly

($\chi^2=0.66$, p value=0.42) associated with genital tuberculosis.

In present study genital tuberculosis diagnosed by histopathology examination and CBNAAT were 28.6 and 17.1% respectively, which was statistically significant association between CBNAAT and HPE by applying chi-square test ($\chi^2=28.25$ and p value=0.00001). While patients suspected of genital tuberculosis by ultrasonography were 31.4%, which was statistically non-significant ($Z=1.77$, p=0.076).

In present study, menstrual blood for AFB culture (BACTEC method) was sent in 20 unmarried cases of pulmonary tuberculosis. BACTEC culture for *M. Tuberculosis* was positive for single case only. (details of other's parameter are given in Table 1).

Table 1: Details of demographic and diagnostic parameter among study populations (n=90).

Parameters	%		
Mean age (year)	29.4		
Residence	Rural	51.4	
	Urban	48.5	
Clinical features (FGT)	Infertility	Primary	21.4
		Secondary	38.5
	Menstruation		
	Normal	31.4	
	Abnormal	Hypomenorrhea	18.5
		Amenorrhea	25.7
Menorrhagia		24.2	
Pelvic pain	52.8		
Abnormal vaginal discharge	60		
USG findings (FGT)	Normal study	62.8	
	Encysted fluid pockets/fluid in pouch of Douglas	4.2	
	Thickened endometrium	5.7	
	Bulky uterus	5.7	
	Irregular fallopian tube	14.2	
	Tub ovarian mass	7.1	
Sputum for AFB	Positive	88.5	
	Negative	11.4	
Drug sensitivity (DST) for MTB	Non MDR	95.7	
	MDR	4.2	
Diagnosis female genital tract TB	HPE	28.5	
	CBNAAT	17.1	

DISCUSSION

Female genital tuberculosis (GTB) is a common health problem in developing countries and most often silent and sometime causes chronic pelvic inflammatory disease, menstrual disturbances and infertility. Diagnosis by culture of the tubercle bacillus from tissue sampled of

the genital tract is the yardstick for diagnosis and remains the gold standard.

In present study, we included both married and unmarried female patients. Further we have investigated the married female patients with active pulmonary tuberculosis for the presence of genital tuberculosis using newer diagnostic methods namely CBNAAT (GenXpert).

In present study, mean age of the patients was 29.4±8 years (range 19-52). While in other studies age of female populations, varied from 18-60 years.^{4,10}

In present study, irregular menstruation, vaginal discharge and pelvic pain were found in 68, 60 and 52% of the study patients respectively.

Carter et al had done a study among female genital tuberculosis and they reported infertility, pelvic pain, vaginal bleeding, amenorrhea, vaginal discharge and post-menopausal bleeding in 44, 25, 18 and 5% respectively.²⁰

A study from India was, reported menorrhagia, hypomenorrhea, oligomenorrhoea, primary amenorrhea and secondary amenorrhea seen in 22.5, 20, 17.5, 5 and 5% respectively, in 40 infertile women with genital tuberculosis.⁵

Subsequent study from India, reported that secondary amenorrhoeic, oligomenorrhoea and menorrhagia were seen in 25% of cases and oligomenorrhoea was the commonest menstrual abnormality noted in genital tuberculosis patients.²¹

A study had done in Pakistan to know the prevalence of female genital tract tuberculosis (FGT) among 150 infertile patients. She further reported that infertility was main complaint, followed by lower abdominal pain (n=3, 10%), menstrual disorder (n=5, 16.7%), vaginal discharge (n=3, 10%), pelvic mass (n=4, 13.3%) and weight disturbances (n=1, 3.3%)²². According to Prasad et al menstrual dysfunction in the form of amenorrhea, oligomenorrhea, menorrhagia and dysmenorrhea was seen in 8%, 13.33, 7.3 and 0.67% of women, respectively among genital tuberculosis patients.²³ Another study had done from India in patients with genital tuberculosis and they observed oligomenorrhoea, hypomenorrhea and menorrhagia in 82.9, 14.6 and 2.4% cases respectively.²⁴

In present study genital tuberculosis was diagnosed by histopathology examination (HPE) and CBNAAT in 28.6 and 17.1% cases respectively. The prevalence of genital tuberculosis in present study is 24.7%.

Mani et al reported tubercular endometritis from the samples obtained from 110 infertile women in 3.6% cases on histopathology.²⁵ Kumar et al. studied on association of tubercular endometritis with infertility and other gynecological complaints of women in India and revealed

endometrial tuberculosis in 3.2% (n=220).²⁶ A study by Thangappah et al on 72 infertile women with tubal factor infertility, adnexal mass on ultrasound, recurrent refractory pelvic inflammatory disease and those with unexplained infertility, where they found 6.9% of endometrial samples positive for tuberculosis.²¹

A study of 100 infertile women by Kohli et al showed 4% positive results on HPE.²⁷ Goel et al done a retrospective study and they compare histopathological examination (HPE), acid-fast bacilli (AFB) smears, Lowenstein-Jensen (LJ) culture, BACTEC culture and polymerase chain reaction deoxyribonucleic acid (PCR-DNA) for diagnosing endometrial tuberculosis in 546 infertile women. They highlighted that Bactec, HPE, LJ, PCR and AFB smear were positive in 3.7, 2.4, 1.8, 1.5 and 0.2% cases respectively.²⁸ Shende et al had done a prospective study to know the prevalence of genital tuberculosis in 120 women presenting with infertility. Endometrial curettage was done and sample was sent for HPE and for TB-PCR. They further concluded that, GTB was diagnosed by hystero-laparoscopy, TB PCR and HPE in 94, 54 and 10% cases respectively.²⁹

Rozati et al had done a study to evaluate the 65 women clinically suspected cases of genital tuberculosis with infertility and investigated endometrial aspirate for AFB smear, HPE, culture, and PCR for the *Mycobacterium tuberculosis*. She further highlighted that of the TB-PCR, HPE, culture for MTB and AFB smear were positive in 43, 26, 18 and 12% cases respectively.³⁰ Aka et al have done a study to investigate the prevalence of genital tuberculosis in 57 female patients with active pulmonary tuberculosis using menstrual blood culture, HPE and hysterosalpingography. They further concluded that hysterosalpingography, HPE and menstrual blood culture were positive in 8.7, 8.7 and 1.8% cases respectively.³¹ Eryani et al conducted a prospective observational study to identify the prevalence of genital tuberculosis among 61 women with infertility. Of these, 55.7% (34/61) were positive by histopathology, 57.6% (19/33) were positive by laparoscopy and 44.1% (15/34) were positive by polymerase chain reaction.³² Another study from Chennai India reported that female genital tract tuberculosis came to be positive by laparoscopy, PCR and HPE in 59.7, 36.7, 6.9 cases respectively.³³ Shahzad had done a study to know the prevalence of female genital tract tuberculosis (FGT) among 150 infertile patients. They highlighted that, tuberculous endometritis was found in 20 out of a total of 25 (80%) cases, while tuberculous salpingitis and tuberculous oophoritis were found both in 2 (8%) of the cases, respectively.²² However, the results of our study were greater than what is reported above.

In present study, menstrual blood for *M. tuberculosis* (BACTEC method) was positive for single case only, among unmarried females.

In the study by Patil et al where they studied 123 infertile women suspected to have genital tuberculosis and

subjected them by testing menstrual blood samples as well as endometrial aspirates with gen-probe amplified *Mycobacterium tuberculosis* direct (MTD) test which is a nucleic acid amplification test (NAAT) and Versa TREK culture. They found *M. tuberculosis* in only 0.8% and mycobacterium other than tuberculosis (MOTT) in 2.43% samples and was identified to be *Mycobacterium intermedium*.²⁴ Sindhoora et al, where they had 2% positivity by TB-PCR on menstrual fluid obtained from 50 patients for the evaluation of infertility.³⁴

CONCLUSION

A definitive bacteriological diagnosis of FG TB is challenging and early detection and treatment of genital tuberculosis considerably improves the chances of restoring fertility. As genital tuberculosis, is almost always secondary to pulmonary tuberculosis, so all female pulmonary tuberculosis patients should be screened for evidence of genital tract involvement.

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