

Current concepts of diagnosis for mycobacterial infections in female genital tract

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Abstract

Female genital tuberculosis is a very common cause of infertility not only in India but in other developing countries also. The organ which gets most affected is fallopian tubes (90-100%), followed by endometrium (50-60%), ovaries (20-30%), cervix (5-15%) and vulva vagina (1%). The mode of transmission to the genital tract usually is the haematogenous spread from pulmonary or other sites of tuberculosis. As mycobacterium tuberculosis remains one of the leading cause of female infertility, the mycobacterium species other than tuberculosis (MOTT) are found to be increasingly important pathogens causing genital infections and infertility. The lack of symptoms makes it difficult to diagnose and there are no accepted guidelines for their diagnosis. It shows low sensitivity to bacteriological tests and has poor specificity to most immunological and serological investigations. The samples which are to be taken are menstrual blood, endometrial and ovarian tissues. Diagnosis involves sample collection, processing followed by decontamination and homogenization, staining by stains like ZN, kinyoun and fluorochrome are preferred. Culture techniques involves both liquid and solid medium. For solid culture the media commonly used are L-J egg media, L-J with para-nitobenzoic acid, Middlebrook 7H11 or 7H10, TK medium. Liquid culture is rapid and automated which involves TREK/ESP, MB/BacT system, BACTEC MGIT 960 (Mycobacteria Growth Indicator Tubes), BACTEC 460. Species identification is done by both phenotypic (Biochemicals, Pigmentation, Optimal temperature & time) and genotypic (Micro seq 500 systems, Accuprobe, Inmo-LiPA Mycobacteria assay) techniques. Non culture based methods includes antigen detection methods, phase assay etc. and molecular techniques NAAT (nucleic acid amplification test) along with other newer techniques.

Keywords: Female Genital Tuberculosis, Female Infertility, MTBC, NTM, Deteriorated Fallopian Tubes, Endometrium, NAAT (Nucleic Acid Amplification Test).

Introduction

Female genital tuberculosis (FGTB) is a very common cause of infertility not only in India but in other developing countries also.⁽¹⁻⁵⁾ Tuberculosis (TB) is an important cause of mortality and morbidity all over the world and is particularly relevant in India. According to WHO 9.6 million people fell ill with TB in 2014 and 1.5 million died globally according to the statistics. TB is found to be among top 5 reasons of death of women of child bearing age. Female reproductive system is very vulnerable to this infection. When TB affects genital organs of young females, it produces destructive effects by causing irreversible damage to the fallopian tube resulting in infertility which is difficult to cure both by medical and surgical methods.^(6,7)

The factors apart from tuberculosis which causes infertility in female are abnormal uterus, conditions like fibroid, polyps and adenomyosis which may lead to obstruction of uterus and damage of fallopian tubes. Congenital abnormalities such as septate uterus may lead to recurrent miscarriages or inability to conceive. There may be ovulatory problems, thyroid, epilepsy and other acquired (age, smoking, stress, overweight, underweight, diabetes mellitus, STD, radiation therapy etc.), genetic (chromosomal abnormalities like turner syndrome), and locatory (ovarian factors like PCOS, ovarian cancer, chemotherapy, etc. tubal factors like

endometriosis, pelvic adhesions, pelvic inflammatory diseases etc. uterine factors like uterine malformation, uterine fibroids, ashermans syndrome, implantation failure etc., vaginal factors like vaginal obstruction, vaginismus etc.) factors playing a major role in causing infertility.

Infertility in Indian Scenario

Transition of a woman to a mother is always life changing. Motherhood is a question of pride and honor. A woman without her biological child has to face lot of discrimination and stigma. Such woman is said to be cursed and is socially unaccepted by society. Not only this, the woman with no biological child, is kept away from family rituals as they disappoint the family for the loss of continuity of family generation and is being unable to take their community further. They lead a disrespectful and frustrated life.

Prevalence

FGTB as a cause of infertility is 10–15 times more common in developing countries where incidence of tuberculosis comprises 40 per cent of the total population in India. This is the most common form of extrapulmonary tuberculosis (TB), accounting for about 27% worldwide.⁽⁸⁾ The main burden is in developing countries especially like Asia and Africa with 75% female patients in the most thrifty productive age group

(15–54 years) causing great economic burden on the family and the nation.⁽⁹⁻¹³⁾ The incidence of infertility due to genital TB & other mycobacterial infections worldwide varies from 10-85%.^(8,14-16) According to a study the reported occurrence of the disease varies in different countries from 1% in infertility hospitals in USA, 3.1% in Malaysia, 3.5–23% in Pakistan 0.8%, 2% in tubal factor in Italy, 4.2% in infertility patients in Saudi Arabia, 16.7% in Nigeria and 6.15–21.1% in South Africa, 1–19% in various parts of India.⁽¹⁷⁻³²⁾

TB is the highest health issue that lies around India but what makes it worse is tuberculosis with the issue of drug resistance. TB started off with MDR TB moved on to XDR TB & gradually, the lowest but most dangerous & strongest of them all has evolved in India as TDR TB.

According to a recent study, New Delhi has a prevalence of FGTB in women of infertility was 26% and incidence of infertility in FGTB to be 42.5%, Bihar is the state where prevalence of TB is very high,^(13,17-25) followed by Mumbai, India 39%, Cuttack, Odessa 3%; (41% tubal factor) etc.

Transmission

The organ which gets most affected by the female genital tuberculosis is fallopian tubes (90-100%), followed by endometrium (50-60%), ovaries (20-30%), cervix (5-15%) and vulva vagina (1%).⁽¹⁴⁾

Genital tuberculosis may spread due to sexual contact with an infected person. Transmission typically occurs when infectious people cough, sneeze, talk or spit and thereby propel the tuberculosis bacilli. It is only necessary to breathe in a small number of these mycobacteria to be infected. Infection can spread in females from intra-abdominal sites all the way through fallopian tubes.⁽³³⁾ During primary infection, organisms may spread systemically and may get activated at a genital site at a later stage of life. 90% of transmission to the genital tract is through haematogenous spread from pulmonary or other sites of tuberculosis, descending mode including about 7% which occurs through peritoneum, bowel and lymphatic mesenteric route and lastly through ascending mode where 3% of transmission is via visceral route, coitus with an infected man, sitting on sputum of the infected person, using infected sputum as lubricant before sex.

Initially, the tubes show little change, but as development of the disease occurs, the diameter of the tube increases and becomes larger. Usually, the ampullary region shows the earliest and most extensive changes, the fimbrial processes become greatly inflamed and enlarged, and the ostia remain open or closed. The isthmus and the adjacent interstitial portion of the tube may remain free of TB. As the process continues, the tubes become softer and caseation develops in the inner wall.

After the initial involvement of the tubes, tuberculous infection spreads to the uterus and ovaries

by direct expansion. Expansion to the uterus is all along the endometrium and hardly ever into the myometrium. Direct hematogenous spread to the uterus has infrequently been reported.⁽³⁴⁾

Signs and Symptoms

Female genital tuberculosis is usually a chronic and silent infection as the bacteria may remain latent in body for long as 10 to 20 years which is more often symptomless, non-specific, with mild clinical pictures and low clinical suspicion. But it may show some symptoms which include irregular menstruation, pelvic pain, vaginal discharge, bleeding after intercourse, infertility, pregnancy loss, abdominal pain, abdominal distension, tumour or ovarian abscess, fever, weight loss, bleeding after menopause etc., Sometimes, the lack of symptoms makes it difficult to diagnose genital tuberculosis and there are no accepted guidelines for diagnosing EPTB in view of the low sensitivity of bacteriological tests and the poor specificity of most immunological and serological investigations.^(35,36)

NTM as a cause of infertility

As mycobacterium tuberculosis remains one of the leading causes of female infertility, the mycobacterium species other than tuberculosis (MOTT) or NTM are found to be increasingly important pathogens causing genital infections and infertility. The common non tuberculous mycobacterial genital infections (NTM) among infertile females are *M. kansasii*, *M. fortuitum*, *MAC*, *MAI*, and *M. Scrofulaceum*. The prevalence of NTMB disease is steadily increasing. The diagnosis is difficult or unconvincing Furthermore, given that most NTM strains are resistant to traditional antituberculous agents, it is important to correctly differentiate genitourinary infections caused by NTM from genitourinary TB.

Diagnosis

A positive chest X-ray for healed or active pulmonary tuberculosis, contact history, elevated ESR and positive tuberculin test may indicate the need for further investigations. Moderate rise in the level of CA 125 in genital tuberculosis can be done. Interferon gamma release assays shows poor sensitivity and specificity.

Imaging methods

- USG
- Computerized axial tomography (CT scan)
- MRI: Useful for tubo-ovarian masses
- PET scan: Tubercular tubo-ovarian masses
- HSG: Endometrial TB can cause synechiae formation, a distorted, obliterated or T shaped cavity and venous and lymphatic intravasation.

Though absolute diagnosis cannot be made from characteristic features in hysterosalpingogram (HSG) or

laparoscopy, laparoscopy is a valuable procedure for obtaining tissue for culture and histopathological examination.⁽³⁷⁻³⁹⁾

Endoscopy:

- Hysteroscopy: The endometrium is pale looking, the cavity is partially or completely obliterated by adhesions of varying grade (grade 1 to grade 4) often involving ostia. There may be a small shrunken cavity.⁽⁴⁰⁻⁴⁸⁾
- Laparoscopy: Most reliable tool to diagnose FGTB especially for tubal, ovarian and peritoneal disease. The test can be combined with hysteroscopy for maximum information. There can be tubercles on peritoneum or tubes, tubo-ovarian masses, caseous nodules, encysted ascites, various grades of pelvic adhesions, hydrosalpinx, pyosalpinx, beaded tubes, tobacco pouch appearance and inability to see tubes due to adhesions.^(47,49)

Sample Collection

As the fallopian tubes cannot be taken out, samples from the ovaries and endometrium is usually taken that too due to the cyclical shedding of the endometrium, granulomas do not have enough time to form, so the granulomatous endometrium may not illustrate evidence of tuberculosis in all the cycles. Pre-menstrual endometrial tissue is suggested for the detection of FGTB.⁽⁵⁰⁾ Use of menstrual blood for bacteriologic or molecular diagnosis has been recommended⁽⁵¹⁾ but was reported to show low sensitivity.⁽⁵²⁾ So the Samples which are to be taken are menstrual blood and other endometrial and ovarian tissues. Menstrual blood is collected within 12 hours of the onset of menses. Tubal biopsy is taken at laprotomy and endometrial tissue is obtained by curettage and is collected in normal saline. Samples will be kept at 4°C before processing in case of any delay. Error during sample collection may give false diagnosis.

Staining

After decontamination and concentration endo-ovarian tissue sediments are spread onto a slide. Two types of acid fast stains are commonly used.

Carbol Fuchsin Stains:

- Ziehl – Neelsen (hot stain)
- Kinyoun (cold stain)

Fluorochrome Stain:

- Auromine O, with or without a second fluorochrome, rhodamine

Culture Techniques

Both liquid and solid medium is used for the cultivation of mycobacteria.

Solid Culture: It is a time consuming procedure of about 8 to 12 weeks. Culture is considered to be the gold standard. The culture media used for identification are as follows -

- L-J egg medium is used for both tuberculous mycobacteria and non-tuberculous mycobacteria. L-J with para-nitobenzoic acid is used for non-tuberculous mycobacterium (NTM) as tuberculosis is inhibited on this medium. To ensure the growth of NTM culture on MacConkey agar is also done. NTM shows both rapid and slow growth.
- Middlebrook 7H11 or 7H10 is another agar based solid media which is commonly used.
- Petragani medium is most inhibitory contaminating bacteria because of high malachite green.
- TK medium is a newly developed media for TB diagnosis. TK Medium uses multi-color dye Indicators that identify M. tuberculosis rapidly. It can also be used for drug-susceptibility testing, and can differentiate a contaminated specimen.

Liquid Culture: It is rapid and automated type of cultivation than solid culture which is more often recommended and detects the bacterial metabolism.⁽⁵³⁾ The maximum time taken by liquid culture is about 1 to 7 weeks. Some of the automated liquid culture techniques are -

- TREK /ESP which detects change in pressure (modification of 7H9 & oleic acid- albumin- dextrose- catalase enrichment in addition to automated hardware.)
- MB /BacT system consist of a bottle with colorimetric sensor. With the increasing amount of carbondioxide the sensor changes color. There is production of carbondioxide by bacteria.
- BACTEC MGIT 960 (Mycobacteria Growth Indicator Tubes) it has modified 7H11 broth, with an indicator at the base of the tube that fluoresces under UV light by which there is consumption of oxygen. Limitation is that blood cannot be inoculated.
- BACTEC 460 (Gold standard) it contains radiolabelled palmitic acid as a carbon source in the medium. This is metabolized by growing organisms, and radiolabelled CO₂ is given off.

After the confirmation of positive culture, it is subcultured on blood agar and Gram and AFB staining is done.

Species Identification

MTB complex does not require Species identification. But for NTM,

Phenotypic Techniques:

- **Biochemical Tests** like Niacin, Catalase, Urease, Nitrate, Arylsulphatase, Tween 80 Hydrolysis, Iron Uptake, Pyrazinamidase are done.

- **Pigmentation** growth in the presence and absence of light.
- **Optimal temperature & time** of growth helps in further species identification of NTMs.
- HPLC, MALDI-TOFMS, Lateral Flow Immunochromatography.

Genotypic Techniques:

- **Micro seq 500 system** is based on amplification of a portion of the 16S r DNA gene. Certain NTM's are difficult to identify and similarly cannot differentiate between different members of the MTB complex.
- **Accuprobe** targets ribosomal RNA, each species requires separate probe. It is a rapid and reliable technique.
- **Inno-LiPA Mycobacteria assay** involves hybridization of PCR products of a portion of 16-23S r DNA spacer region to oligonucleotide probes on a membrane strip which identifies MTB and common NTM.

Non culture based Methods

Antigen Detection Methods: LAM (lipoarabinomannan) mycobacterial cell wall component is excreted in urine. Recently a kit is available to detect urinary LAM in by ELISA.

Phase Assay: Use of mycobacteriophages for TB diagnosis is recent development.

Molecular Techniques

PCR (Polymerase Chain Reaction): Rapid (1-2 days), sensitive and specific method for detecting mycobacterial DNA (mpt 64 gene) with high pickup rate but can be false negative due to contamination or false positive as it can pick up even single, mycobacterium tuberculosis and may not be able to differentiate between infection and disease.^(46,47) Hence ATT should not be started just on the basis of positive PCR unless there is some other evidence of FGTB on clinical examination or on investigations like presence of tubercles or other stigmata of TB on laparoscopy.

Rapid Tests

There is an urgent requirement of the rapid diagnostic tests, allowing for advance and appropriate treatment of the disease by inhibiting the transmission of infection and slash the expenditure. This is particularly for the developing countries where tuberculosis and HIV is prevalent. Newer tests for TB assure greatly improved diagnostic accuracy.^(54,55) The new generation tests for the diagnosis of TB are:

Adenosine Deaminase (ADA) levels: ADA levels have shown immense change in the diagnosis of extrapulmonary tuberculosis particularly pleural

effusion and pericardial effusion. Its value for diagnosing tuberculous meningitis is restricted.⁽⁵⁴⁾

NAAT (Nucleic Acid Amplification Test): It is a newly and widely used technique. It directly detects and distinguishes MTBC organisms in specimens. Identify specific NTM and rapidly detect drug resistance. Results are available within hours to a few days. It amplifies nucleic acid sequences using a nucleic acid probe. Such assays complement the conventional laboratory approach to the diagnosis of active disease. AFB smears are speedy, but are deficient in - sensitivity and specificity. Culture is both sensitive and specific, but results are obtained after 2-8 weeks. NAA assays, however, gives rapid, sensitive and specific diagnosis of *M. tuberculosis*. The sensitivity of NAA assays is at least 80% in presently used tests and the specificity is 98-99%. The sensitivity of these assays in AFB smear negative samples is lower than for smear-positive samples.⁽⁵⁶⁾

The most common targets are IS6110 for MTB and a16S r DNA.

The Amplified Mycobacterium Tuberculosis Direct (MTD) Test and its reformulated form – AMTDII or E-MTD for enhanced MTD.

1. **AMPLICOR *M. tuberculosis* Test:** uses DNA polymerase chain reaction (PCR) to amplify nucleic acid targets.
2. The MTD assay is an isothermal approach for recognition and detection of *M. tuberculosis* r RNA.

The E-MTD has an improved sensitivity. Processing time is 3 hours approximately. It is labor intensive for microbiologist. It is often not available in hospital labs. E-MTD is particularly helpful for confirming disease in intermediate- and high-risk people and not including the cases of low-risk patients.⁽⁵⁶⁾

Limitations of NAAT: They are not perfect as there may be inhibitors in the clinical specimens which are directly used, which may prevent amplification. Expert MTB/RIF includes a sample processing control to check for the presence of inhibitors likewise other NAAT's have manual with instructions on how to test inhibitors. Another limitation is that none of these tests will specify the organism dead or alive, so they should not be used to follow patient on treatment.

Xpert MTB/RIF (Gene-Expert): FDA approved in 2013. Using culture as gold standard. It has a Specificity of 99%. Also test rifampin resistance. Automated cartridge based test. The PCR is used during this technique. No contamination occurs and the results are in almost 2 hours.

T Spot-TB Test: This is a latest and absolutely new test that diagnoses both type of active and latent

tuberculosis. It uses a new method called ELISPOT. It is an ex vivo T-cell-based assay for the detection of cell-mediated immunity, which was used in detecting *M. tuberculosis*. The technique detects and takes amount of peripheral blood gamma-interferon secreting T cells in response to TB antigens. These t cells are counted as spots on a test plate.⁽⁵⁴⁾

QuantIFERON-TB Gold (QFN Gold): This test is a more definitive form of QFT. are usually PPD, the test uses antigens that are highly precise to MTB. These antigens consist of culture filtrate protein (CFP-10) and the early secretory antigenic target 6 (ESAT-6). These low molecular weight proteins, encoded within the region of difference 1 (RD1) of the *M.tuberculosis* genome, are more particular to *M. tuberculosis* than PPD, and they are not common with the BCG sub strains and most NTM species (with the exception of *M.kansasii*, *M. marinum* and *M. szulgai*). Tests based on these TB specific antigens are called RD1 based IFN- assays.^(55,56)

QuantIFERON-TB Test: This test measures a component of cell-mediated immune reactivity to *M.tuberculosis*. The basis of this test is the detection in serum of the release of IFN-gamma on stimulation of sensitized T cells by antigens of *M. tuberculosis*.⁽⁵⁷⁾ Whole blood is incubated overnight with purified protein derivative (PPD) from *M. tuberculosis* and control antigens. The IFN- released from sensitized lymphocytes is afterwards quantified by ELISA. The antigens included in the test kits are PPD from *M.tuberculosis* (tuberculin) and PPD from *Mycobacterium avium* (avian sensitin). The kits also include phytohemagglutinin (a mitogen used as a positive assay control), and saline (negative control or nil).⁽⁵⁷⁾

Advantages:

- Results within 24 hours.
- Does not boost responses measured by subsequent tests, which can happen with tuberculin skin tests (TST).
- Is not exaggerated by previous BCG (Bacille Calmette-Guérin) vaccination.

Disadvantages and Limitations:

- Blood samples must be processed within 12 hours after collection while white blood cells are still viable.
- Errors may come at any stage of processing.

Loop-mediated isothermal amplification (TB-LAMP): Apart from PCR, A viable molecular assay Loop amp MTBC Detection Kit based on loop-mediated isothermal amplification was developed by Eiken Chemical Company Ltd (Tokyo, Japan) for the diagnosis of *Mycobacterium tuberculosis* complex (TB-

LAMP). Loop-mediated isothermal amplification (LAMP) is a fresh nucleic acid amplification method in which reagents react under isothermal state of affairs with high specificity, efficiency, and rapidity.⁽⁵⁸⁾

TB-LAMP is a labor-intensive assay that require not more than one hour to perform and can be read with the naked eye under ultra violet light. Following review of the up-to-the-minute confirmation, WHO recommends that TB-LAMP can be used as a substitute for microscopy for the diagnosis of TB with special reference to pulmonary one. It can also be measured as a follow-on test to microscopy in adults with signs and symptoms of pulmonary TB, especially when supplementary testing of sputum smear-negative specimens is required.⁽⁵⁸⁾

Abbreviations

- MTB: Mycobacterium Tuberculosis
- FGTB: Female Genital Tuberculosis
- ESR: Erythrocyte sedimentation rate
- CA: Cancer antigen
- USG: Ultrasonography
- CT: Computed tomography
- MRI: Magnetic resonance imaging
- PET: Positron emission
- NAAT: Nucleic Acid Amplification Test
- RIF: Rifampicin
- NTM: Non-tuberculous Mycobacterium
- HSG: Hysterosalpingogram
- WHO: World Health organization

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