Insight into the diagnosis and management of subclinical genital tuberculosis in women with infertility

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ABSTRACT

Genital tuberculosis (GTB) is an important cause of infertility in India. Lack of an accurate diagnostic test has led to an indiscriminate use of antitubercular treatment in infertile women. Apart from concerns of drug toxicity, this may be a contributing factor in the increasing incidence of multidrug-resistant TB reported in India. We conducted a study to analyze whether a combination of tests could help improve diagnostic accuracy. An algorithm for the management of GTB in infertile women based on the use of multiple tests is presented.

KEY WORDS: Genital tuberculosis, infertility, latent tuberculosis, *Mycobacterium tuberculosis* infection

INTRODUCTION

Tuberculosis (TB) caused by the bacterial pathogen *Mycobacterium tuberculosis* (MTB) is one of the oldest and hardiest diseases known to humans. It finds mention in Ayurveda by Charak in 3000 BC and has also been found in Egyptian mummies. The WHO estimates that one-third of the world's population, approximately 2 billion people, is infected with MTB.[1] Approximately, 10% of those infected develop active TB at a later stage of their life, 5% in the first 2 years after infection, and 0.1%/year thereafter. The risk of progression is highest within the first 2 years of exposure. Impaired immunity such as HIV infection increases the risk to 10%/year and approximately 50%/lifetime. The remaining infected individuals have what is called latent infection: They are asymptomatic and do not spread infection to others.

TB can affect any organ in the body through hematogenous or lymphatic spread from its primary site of infection - the lung. Female genital TB (FGTB) affects the fallopian tubes most commonly (90%), followed by the endometrium (50%–60%) and the ovaries (10%–30%), and cervical involvement is seen in only 5%–15% of the patients.[2]

Patients present with a variety of symptoms, the most common presentation being subfertility or menstrual irregularity. The prevalence of GTB in India is reported to be 18%–19% among infertile women.[3] A recent study suggests that GTB is responsible for 1% of all gynecological admissions in India and 17.4% in infertility clinics.[4]

HOST RESPONSE TO *MYCOBACTERIUMTUBERCULOSIS*

MTB triggers a complex immune response within the body to contain the infection and establish a pool of long-lasting memory T-cells specifically directed against the MTB antigens. Macrophages are the first line of defense against infection because of their ability to ingest and subsequently kill pathogens. Tissue reaction at the site of

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infection leads to the formation of the classic tubercular granuloma that contains macrophages surrounded by T- and B-lymphocytes and fibroblasts. T-lymphocytes kill infected cells and secrete cytokines interferon gamma (IFN-γ) that activates macrophages. MTB bacilli can, however, persist within the macrophages, having developed immune escape mechanisms.[5] The development of immune response takes about 4–6 weeks after the primary infection. Balance between host immunity and bacillary multiplication determines the outcome of infection – primary active infection or development of adequate immunity and latency. Reactivation and development of postprimary TB can occur many years after latency.[6] Tests based on the immune response provoked by MTB have been developed to detect latent disease.

LATENT TUBERCULOSIS INFECTION

The Centers for Disease Control and Prevention[7] states that latent TB designates a condition in which an individual is infected with MTB but does not currently have active disease. Persons with latent TB infection (LTBI) are asymptomatic, have a negative chest radiograph, and are not infectious. Diagnosis is based on a positive tuberculin skin test (TST), a delayed hypersensitivity reaction to the purified protein derivative of MTB or interferon gamma release assay (IGRA), and a T-cell response to MTB-specific antigens. It is believed that MTB remains viable in people with latent infections, not causing disease but maintaining its potential to do so. Altered immune status of the body can lead to reactivation of the bacterium though the mediators of immune control are incompletely understood.

LATENCY AND DORMANCY

The terms latency and dormancy are often used interchangeably; however, dormancy is a stable nonreplicative state of the bacterium where bacteria have reduced metabolic activity, including transcription and translation. Dormancy can occur during antibiotic therapy if the bacterium develops a resistant cell wall preventing penetration of the drug and it is thought to be an evolutionary strategy of the bacterium. Dormant bacteria retain the ability to be resuscitated, i.e., reversal of a stable nonreplicating state into a metabolically active growing population. In mycobacteria, resuscitation can be signaled by exogenous factors as demonstrated in *in vitro* cultures.[8] Toxin-antitoxin systems, or genes with similar functions, may play a role in initiating the cessation of replication in dormant bacteria. Latency, on the other hand, is a clinical term suggesting exposure to infection in the absence of any clinical symptoms. Latency includes a bacterial population that is mostly dormant but contains a subpopulation that is actively replicating.[9] Little is known about the anatomical

location, number, and metabolic state *in vivo* of the infecting tubercle bacilli in LTBI.[10]

Lin and Flynn in 2010[11] described latent infection as a dynamic process of bacterial persistence and immunologic control, and thus LTBI is recognized as a spectrum of responses to MTB infection; in one end of the spectrum, individuals are completely infection free, and at the other end, individuals are incubating actively replicating bacteria in the absence of clinical symptoms [Figure 1]. The dynamic equilibrium between parasite and host (expressed as a long-term asymptomatic infection) and its abrogation (expressed as a reactivation disease) appears to be genetically controlled by both parties.[12] The diverse pathological presentation of mycobacterium tuberculosis infection (MTBI) suggests that the conventional terms of latency and active disease should be changed. It has been suggested that the infection should be viewed as a continuous spectrum extending from sterilizing immunity to subclinical active disease and to fulminant active disease. The conventional designations of latent infection and active disease correspond to partially overlapping regions of biological heterogeneity. The major drawback currently with the changing view of latent TB is that none of the diagnostic assays are sufficiently sensitive or specific to assign/define a particular person to a "place on the spectrum."[6,13]

REACTIVATION OF MILD TRAUMATIC BRAIN INJURY

Immunocompromise is the most important cause of reactivation of dormant bacterium and an increased
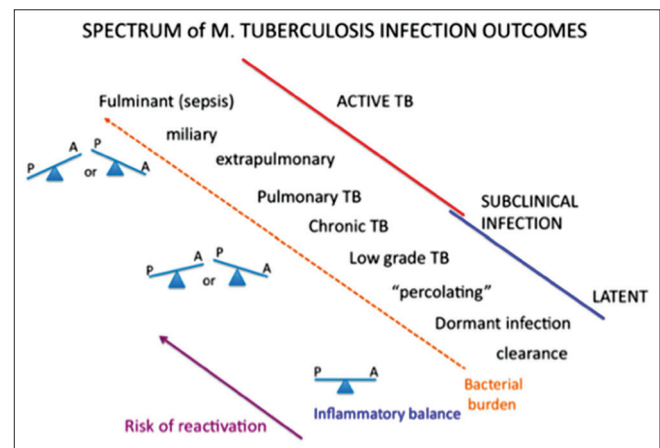

Figure 1: The spectrum of *Mycobacterium tuberculosis* infection outcome. Bacterial burden (dashed orange line) is expected to increase the spectrum of infection. (p) pro- and (a) anti-inflammatory factors in the granuloma. Too much pro-inflammatory or too much anti-inflammatory activity can lead to poor control of bacteria and increased pathology. The purple line reflects the risk of reactivation in the latent spectrum. (Reprinted with permission from Lin PL, Flynn JL. Understanding latent tuberculosis: a moving target. J Immunol 2010;185 (1):15-22.)

susceptibility for fresh infection. Reactivation or susceptibility to infection is seen in immunocompromised HIV individuals and in patients administered with biological agents (tumor necrosis factor alpha antagonists) for the treatment of arthritis. Reactivation can also be initiated during surgical manipulation and has been observed after laparoscopy, hysteroscopy, hysterosalpingography, and pelvic surgery.[14] High-steroid levels and an increased vascularity during ovarian stimulation are thought to be the triggering factors in the infertile population going through *in vitro* fertilization (IVF). Empirical use of steroids and immunotherapies is common in infertile patients with recurrent implantation failure and recurrent pregnancy loss, and these too increase the risk factor for reactivation.

In developed countries where the load of disease is low, there is a drive to eradicate TB by identifying and treating individuals with LTBI. In endemic areas–the developing countries–the load of active disease is very high and the priority is to treat active infection. There is an ongoing dilemma on the treatment of LTBI in these countries as the risk of repeat exposure to infection is very high. Given the adaptive ability of the *Mycobacterium,* an indiscriminate use of antituberculosis treatment (ATT) has an immense potential to promote drug resistance. There are 27 "high-burden drug-resistant TB" countries, and India is among one of them. In India levels of multidrug resistance (MDR) are lower than 2.2% (1.9–2.6) among new cases but as high as 15% among retreatment cases.[15]

DIAGNOSIS OF FEMALE GENITAL TUBERCULOSIS

FGTB is almost always secondary to a tubercular lesion in another part of the body. Diagnosis is difficult and delayed since symptoms appear when significant organ damage has already occurred. The pauci-bacillary status of GTB poses an additional challenge to diagnosis. One of the biggest problems is the diagnosis of latent GTB infection.

TESTS FOR GENITAL TUBERCULOSIS

Both specific and nonspecific tests are available.

The specific tests available for the diagnosis of TB are as follows:
- Acid-fast bacilli (AFB) staining
- Culture methods
- Immunological tests
- Molecular tests – Nucleic acid amplification tests (NAATs)
- Imaging methods
- Endoscopy.

Acid-fast bacilli staining
Ziehl–Neelsen (ZN) staining for AFB requires 10^4–10^6 bacilli/ml of tissue or fluid specimens to give a positive result. The detection rate is generally under 10%.

Tissue culture
Culture remains the gold standard for laboratory confirmation of MTB and it is also required for isolating bacteria for drug susceptibility testing and genotyping. A composition of 10–100 bacilli/ml of sample is required and it takes 2–6 weeks for the growth of *Mycobacterium* in culture. Traditionally, Lowenstein–Jensen culture has been used; in addition, the BACTEC MGIT™ (mycobacteria growth indicator tube) system has shortened the time to diagnosis to 2 weeks though the culture dish is still observed for 6 weeks before a negative result is reported. BACTEC MGIT is a rapid liquid culture method that utilizes fluorescence technology. It senses oxygen reduction in the culture media, which is then centrifuged and stained with ZN stain to identify MTB. Positive cultures are reported usually within 10–12 days.

Immunological tests
There are currently two diagnostic methods that support the diagnosis of LTBI: The TST and IGRAs. Both tests are immunological methods that detect an immune response to antigens and consequently do not allow a direct measure of persistent infection.[7]

Tuberculin skin test
An *in vivo* test is based on the intracutaneous injection of MTB antigens and identification of an immune reaction at the site of injection after 48 h. Limitation of the TST is that the antigens used are not specific to MTB, a local reaction therefore does not differentiate between an existing immune response elicited by previous bacille Calmette–Guérin (BCG) vaccination, exposure to nontubercular mycobacterial (NTM), or MTB infection.[16]

Interferon gamma release assays
IGRAs are *in vitro* assays that detect the presence of cellular immune responses toward MTB-specific antigens. They measure IFN-γ release in response to the region of difference 1-encoded (genomic region of difference) immunodominant antigens such as early secretory antigenic target-6, culture filtrate protein 10, and the TB7.7 antigens. In contrast to the TST, the antigens used in IGRAs are absent in most of the NTM (with the exception of *Mycobacterium flavescens*, *Mycobacterium marinum*, *Mycobacterium Kansasii,* and *Mycobacterium szulgai*), as well as from BCG strains.[17-19]

Although IGRAs cannot distinguish between active TB and LTBI, IGRA results are not confounded by BCG vaccination and are less likely to be confounded by exposure to NTM.[20]

Two commercial IGRAs are available, the QuantiFERON-TB Gold In-Tube assay (Cellestis Ltd., Australia) and the T-SPOT-TB (Oxford Immunotec, UK). Indeterminate results reflect technical factors (e.g., inappropriate storage of blood) or an individual with impaired immune response, and therefore a repeat test is recommended in this situation to differentiate between the two. Unlike TST, IGRAs are ideal for serial testing and can be repeated any number of times without sensitization and boosting.[21] The ECDC guidance document[7] suggests that IGRAs should not replace the standard diagnostic methods for diagnosing active TB. In certain clinical situations (e.g., patients with extrapulmonary TB, patients who test negative for AFB in sputum and/or negative for MTB on culture, TB diagnosis in children, or in the differential diagnosis of infection with NTM), IGRAs could contribute supplementary information as part of the diagnostic workup. A negative IGRA does not rule out active TB, it should be repeated for 4–6 weeks to allow for host immune response to develop.

Sensitivity, specificity, negative and positive predictive values
The IGRAs have an excellent specificity (99.4% confidence interval [CI] 97.9–99.9) that is unaffected by BCG vaccination. TST specificity is high in non-BCG-vaccinated populations but low and variable in BCG-vaccinated populations.[22] Anergy due to advanced disease, malnutrition, and HIV-associated immune suppression may lower the sensitivity of IGRAs.

According to the ECDC guidance document, the positive predictive value (PPV) for the progression of IGRAs may be used as a part of the overall risk assessment to identify individuals for preventive treatment (e.g., immunocompromised persons, children, close contacts, and recently-exposed individuals). Similarly, the high negative predictive value (NPV) for the progression of IGRAs indicates that at the time of testing and in the context of an overall risk assessment, progression to active TB in healthy immunocompetent individuals with negative IGRAs is very unlikely in the next 2 years based on follow-up of patients. Therefore, IGRAs may be used in this context. The individuals were followed up for an average of 2 years to assess whether they remained disease free. The number of studies included in the meta-analysis also included patients at an increased risk of developing TB, such as close contacts of active TB patients. The NPV for progression was 99.8%.

Nucleic acid amplification tests
NAATs are used to amplify deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) segments with a high specificity to rapidly identify the microorganisms in a specimen. The turnaround time is 48–72 h. False positives may occur due to contamination of specimens with MTB DNA product from the polymerase chain reaction (PCR) laboratory. One

of the major disadvantages of PCR is the inability to detect a difference between viable and nonviable organisms. Therefore, the test can remain positive for longer periods in patients who are taking anti-TB medications or who have completed TB treatment. It should therefore,not be used for the detection of LGTBI in patients treated for pulmonary or extrapulmonary Koch's previously. False-negative results may occur because of the inefficient extraction of the DNA due to low mycobacterial numbers or the presence of PCR inhibitors. The presence of PCR inhibitors has been reported in sputum, pus samples, and tissue biopsies.[23] Shrivastav *et al.* in 2014[24] found that PCR-negative samples in endometrial tissue were positive by culture methods and concluded that DNA-PCR done alone is not reliable for TB diagnosis, it should be combined with culture. Even for pulmonary TB where bacterial numbers are high and sample collection is easy, it is suggested that NAATs should be interpreted within the context of the patients' signs and symptoms, and they should always be performed in conjunction with AFB smear and culture.

Types of nucleic acid amplification test
Deoxyribonucleic acid-polymerase chain reaction
- Ampiclor: It amplifies a portion of the 16S ribosomal RNA gene that contains a sequence that hybridizes with an oligonucleotide probe specific for MTB complex bacteria
- MTD-2: This test should not be used for patients who have taken TB medications in the last 12 months or who have taken TB medications for more than 7 days
- In-house DNA-PCR tests: For example, MycoReal; many laboratories have developed in-house DNA-PCR tests
- Gene-Xpert: The Xpert MTB/rifampin (RIF) (Xpert) assay (Cepheid Inc., Sunnyvale, CA, USA) is a cartridge-based, semi-automated, rapid molecular assay, which permits rapid TB diagnosis through the detection of the DNA of MTB and simultaneous identification of a majority of the mutations that confer RIF resistance (which is highly predictive of MDR-TB). The entire process is carried out in a closed automated system except for addition of the specimen into the cartridge, thus reducing contamination. However, the limitation of DNA-PCR tests regarding the detection of viable bacteria is not eliminated by the use of this method. Moreover, its use in extrapulmonary TB is still under evaluation.

Single tube-nested reverse transcription polymerase chain reaction
This test detects only the live organisms in the clinical specimen as well as phenotypic drug susceptibility testing. The major drawback is that it needs to be transported in ice to the laboratory within 2 h to prevent the degradation of RNA since the average half-life of bacterial messenger RNA is 3 min.[25]

Background of the study

Given the importance of GTB in the development of infertility, the current status of diagnostic tests, and concern about the rampant misuse of treatment, we decided to undertake a study to evaluate whether IGRA could be used in GTB diagnosis in combination with NAAT and culture to improve diagnostic accuracy and also to develop an algorithm for the treatment of GTB.

Objective of the study

The objectives of this study are as follows:

1. To find the correlation of endometrial biopsy (EB) for DNA-PCR tests and Koch's culture with IGRA in treatment naïve infertile women (Group A)
2. To find the pregnancy outcome in patients who were not given ATT (positive DNA-PCR, negative IGRA, and negative Koch's culture at 6 weeks) compared to those given ATT for positive DNA-PCR alone and a control group who were negative for GTB by all three tests (Group B).

This study was conducted prospectively at a tertiary infertility center.

MATERIALS AND METHODS

Five hundred and fifty patients attending outpatient department for infertility from January 2013 to January 2015 were screened and evaluated for infertility. A detailed history was obtained regarding the duration of infertility, contraception use, menstrual cycle regularity, parity, past medical/surgical intervention, and previous medication. Specific questions were asked about the history of Koch's in the family or staff working in the household or exposure at workplace to determine exposure to MTB. A general and gynecologic examination was performed. Patients who had received ATT for pulmonary or extrapulmonary Koch's were excluded from the study but had an EB for Koch's culture to rule out GTB. Patients who had received ATT for GTB were also excluded from the study. The diagnostic techniques, duration of treatment, and type of drugs used in this GTB-treated group emphasize the need for the development of a standardized approach for the management of GTB in the infertile patients. This group of patients had normal endoscopic findings. Diagnosis/decision to start treatment was based on the following:

- Positive mycobacterial serology IgG and IgM only
- Positive immunocytochemistry for pathogen-associated mycobacterial protein only
- EB and blood DNA-PCR-positive only
- QuantiFERON Gold (Q-Gold)-positive only
- DNA-PCR-positive on the products of conception
- Empirical treatment before IVF and intrauterine insemination in unexplained infertility

- Empirical treatment for recurrent implantation failure
- Empirical treatment after missed abortion
- Beaded look of patent fallopian tubes on laparoscopy
- Presumed "tubercles" on hysteroscopy - (tests negative, good endometrial thickness). Endometrial glands are often mistaken for tubercles
- An inability to gain weight.

Duration, Number and Type of ATT used
- Duration of ATT given: Less than 3 months to 18 months/course. In some patients, it was given for 15 days after embryo transfer (ET) to improve implantation
- Number of courses of ATT: 1–4 courses
- Type of drug: Standard HZRE- Rifampicin, INH, Ethambutol, Pyrazinamide. Addition of Streptomycin (SM), third and fourth generation drugs was also seen.

Study patients

One hundred and eighty patients who had not been evaluated or empirically treated for GTB were included in the study (Group A). The tests done to diagnose GTB were QuantiFERON Gold TB, EB for DNA-PCR, and Koch's culture. For Group B (to evaluate pregnancy outcome), 136 patients were included. This group was further divided into three groups: Group B1 - 34 patients (from Group A) who had a positive DNA-PCR not given ATT, Group B2 - 30 patients previously treated with ATT, DNA-PCR positive on EB (taken from the screened group of 550 patients), and a control Group B3 of 72 patients from Group A who were tested negative for all GTB tests [Figure 2].

Ethical clearance was obtained and consent was taken from the patients to participate in the study. Patients were given
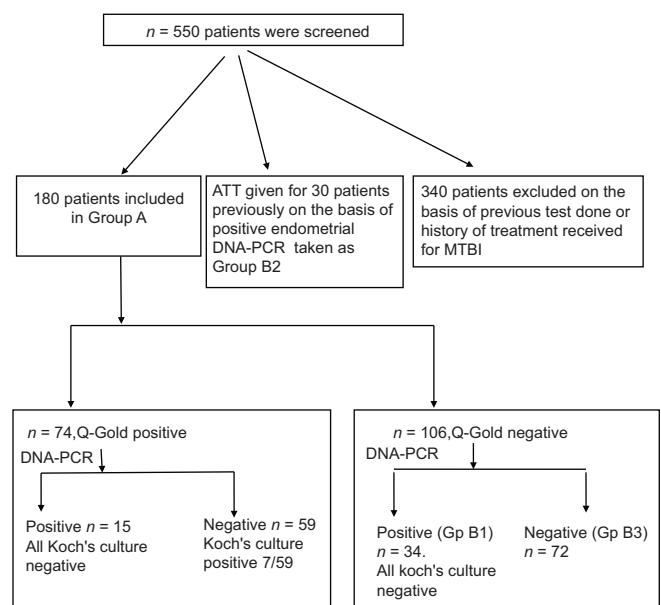

Figure 2: Flow chart of study group with results of TB tests

complete background information on GTB and were given the option to take ATT based on positive DNA-PCR if they desired (in which case they would be excluded from the study).

Tests done

- QuantiFERON Gold (Q-Gold) - Blood sample
- EB for AFB culture (BACTEC method) and DNA PCR sent to Oncquest Laboratory. Endometrial tissue was collected either premenstrual if there was no history of sexual contact or on D1/D2 of menstrual cycle using a Pipelle (Gynetics). At the time of endometrial sampling, precautions were taken to avoid contamination. Cervix was cleaned with saline twice before aspiration. The tissue obtained was rinsed in saline to remove blood and then put in a prelabeled container.

RESULTS OF GROUP A

Incidence of latent tuberculosis infection

a. By IGRA: Positive on IGRA/total patients evaluated: 74/180 (41%): The number of cases exposed to primary MTBI.

Incidence of subclinical/active genital tuberculosis infection

a. By EB for DNA-PCR: Positive DNA-PCR/total patients: 49/180 (27%): The number of cases having subclinical GTB (SGTB).
b. By EB for Koch's culture: Positive culture/total patients: 7/180 (3.8%): The number of proven GTB cases.

Of the seven patients who had positive cultures, none had DNA-PCR positive, and of the 49 patients showing DNA-PCR positivity, only 15 patients had a corresponding Q-Gold positive.

Correlation of tests

If Koch's culture is considered the gold standard for diagnosing GTB, the sensitivity and specificity of DNA-PCR are 0% and 71.6%, respectively, the PPV is 0% and NPV is 94.6%, the agreement between tests is 68% (Group A) [Tables 1a and b]. The sensitivity of Q-Gold is 100% and specificity is 61.2%, PPV is 9.46%, NPV is 100%, and agreement is 62.7% (Group A) [Tables 2a and b].

When Q-Gold and DNA-PCR were correlated, it was seen that out of 49 DNA-PCR-positive patients, only 15 (30%) were Q-Gold positive. The sensitivity of DNA-PCR is 20.27% and specificity is 67.9% if Q-Gold is taken as the standard. The PPV is 30.6% and NPV is 54.9% and agreement between tests is 48.3% (Group A) [Tables 3a and b].

Table 1a: Correlation between deoxyribonucleic acid-polymerase chain reaction and Koch's culture taking Koch's culture as gold standard (Group A)

DNA-PCR	Koch's culture positive (%)	Koch's culture negative (%)
Positive (*n*=49)	0	49 (100)
Negative (*n*=131)	7 (5.3)	124 (94.6)
Total (*n*=180)	7	173

DNA-PCR=Deoxyribonucleic acid-polymerase chain reaction

Table 1b: Sensitivity, specificity, negative predictive value, positive predictive value, and agreement between deoxyribonucleic acid-polymerase chain reaction and Koch's culture (Group A)

DNA-PCR	Sensitivity	Specificity	PPV	NPV	Agreement
DNA-PCR	0%*	71.6%*	0%*	94%*	68%

*95% CI, taking Koch's culture as gold standard. PPV=Positive predictive value, NPV=Negative predictive value, CI=Confidence interval, DNA-PCR=Deoxyribonucleic acid-polymerase chain reaction

Table 2a: Correlation between Q-Gold and Koch's culture taking Koch's culture as gold standard (Group A)

Koch's culture	Q-Gold positive (%)	Q-Gold negative (%)
Positive (*n*=7)	7 (100)	0
Negative (*n*=173)	67 (38.7)	106 (61.3)
Total (*n*=180)	74	106

Table 2b: Sensitivity, specificity, negative predictive value, positive predictive value, and agreement between Q-Gold and Koch's culture (Group A)

Q-Gold	Sensitivity	Specificity	PPV	NPV	Agreement
Q-Gold	100%*	61%*	9.4%*	100%*	62.7%

*95% CI, taking Koch's culture as gold standard. PPV=Positive predictive value, NPV=Negative predictive value, CI=Confidence interval

Table 3a: Correlation between deoxyribonucleic acid acid-polymerase chain reaction and Q-Gold taking Q-Gold as standard (Group A)

DNA-PCR	Q-Gold positive (%)	Q-Gold negative (%)
Positive (*n*=49)	15 (30)	34 (69)
Negative (*n*=131)	59 (45)	72 (55)
Total (*n*=180)	74	106

DNA-PCR=Deoxyribonucleic acid-polymerase chain reaction

Table 3b: Sensitivity, specificity, negative predictive value, positive predictive value, and agreement between deoxyribonucleic acid-polymerase chain reaction and Q-Gold (Group A)

DNA-PCR	Sensitivity	Specificity	PPV	NPV	Agreement
DNA-PCR	20.2%*	67.9%*	30.6%*	54.9%*	48.3%

*95% CI, taking Q-Gold as standard. CI=Confidence interval, PPV=Positive predictive value, NPV=Negative predictive value, DNA-PCR=Deoxyribonucleic acid-polymerase chain reaction

Primary exposure of MTBI induces an immune response in the body that is measured as a T-cell response by IGRAs. The IGRAs especially have an excellent specificity (99.4% CI: 97.9–99.9) unaffected by BCG vaccination and remain positive after completing TB treatment. Extrapolating from

this, these 34 patients could be having a false-positive DNA-PCR or a low IFN-γ value that occurs when there is clearance or resolution of infection.[26] A review of IGRA results shows that although the overall mean production of IFN-γ production is greater in active disease than among latent patients, there is a tremendous degree of variability within the latent as well as active groups.[27] In our study, seven patients were culture positive; their DNA-PCR was negative, but all had a positive Q-Gold confirming host response in active disease. These results emphasize the importance of sending endometrial tissue for culture, as patients with active GTB would be completely missed if only DNA-PCR is done. Doing a combination of tests would alert the physician to look more assiduously for the evidence of disease or provide assurance regarding absence or clearance and effective containment of disease.

RESULTS OF GROUP B

Clinical outcome was evaluated in 136 patients in Group B. Patients were divided into three groups – Group B1-34 patients of DNA-PCR positive not treated, Group B2-30 patients of DNA-PCR positive, received ATT, and a control group of 72 patients negative for GTB by all the three tests. Patient demographics between the three groups were similar. The

demographics of the three groups and pregnancy outcome are shown in Tables 4 and 5, respectively. Seven patients in Group B1 and 5 patients in Group B3 with ≤2 years of infertility were advised expectant management. There was one spontaneous pregnancy in both groups. In Group B2, because of high patient anxiety, only two patients agreed to go with expectant management for 6 months, there was no spontaneous pregnancy. Rest of the patients went through IVF-ET. Statistical evaluation using Chi-square test was done to calculate the *P* value of pregnancy outcome between Group B1 and Group B2 and between Group B1 and Group B3. There was no statistically significant difference in outcome between Group B1 and Group B2 ($P = 0.61$) and between Group B1 and Group B3 ($P = 0.48$). Thus, positive DNA-PCR may be false positive or may suggest clearance or containment of infection not impacting pregnancy.

DISCUSSION

FGTB can lead to serious damage to the reproductive organs leading to infertility and subsequent sterility. Diagnosis is difficult and delayed given the low bacillary load and inaccessibility of the fallopian tube, the first structure to be affected, for tissue diagnosis. The term SGTB should be used instead of LGTB in the context of female GTB since

Table 4: Patient demographics (Group B)

	DNA-PCR positive, untreated (Group B1), *n*=34 (%)	DNA-PCR positive, treated (Group B2), *n*=30 (%)	GTB negative (Group B3), *n*=72 (%)
Mean age±SD	32.0±4.4	33.66±3.5	32.9±5.08
Cause of infertility			
Male factor	8 (23)	7 (23.3)	20 (27.7)
PCOS	3 (8.8)	3 (10)	5 (6.9)
POR	4 (11.7)	4 (13)	11 (15.2)
Unexplained infertility	16 (47)	13 (43.3)	30 (41.6)
Endometriosis	3 (8.8)	3 (10)	6 (8.3)
Total	34	30	72

SD=Standard deviation, DNA-PCR=Deoxyribonucleic acid-polymerase chain reaction, GTB=Genital tuberculosis, POR=Poor ovarian reserve, PCOS=Polycystic ovarian syndrome

Table 5: Pregnancy outcome (Group B)

	DNA-PCR positive, untreated (Group B1), *n*=34 (%)	ATT received on the basis of DNA-PCR (Group B2), *n*=28 (%)	GTB negative by all means (Group B3), *n*=72 (%)	*P* Group B1 versus Group B2	*P* Group B1 versus Group B3	*P* Group B2 versus Group B3
		Two patients lost to follow-up				
Clinical pregnancy (gestational sac seen)	16/34 (47.0)	15/28 (53.5)	38/70 (54)	0.610	0.489	0.949
Missed abortion	1 (2.9)	2 (7.4)	4 (5.7)	0.443	0.553	0.764
Delivered	15 (44.1)	13 (46)	34 (48)	0.856	0.765	0.943
term + preterm	12+3 (34 weeks PIH - 2 and PPROM)	12+1 (IUD abnormal karyotype)	32+2 (1 cervical incompetence and PPROM)			
Negative	17	10	32	0.259	0.529	0.427
Ectopic	0	0	0			
Biochem	1 (2.9)	2 (7.4)	0	0.443	0.144	0.022*

PPROM=Preterm premature rupture of membranes, ATT=Antituberculosis treatment, GTB=Genital tuberculosis, DNA-PCR=Deoxyribonucleic acid-polymerase chain reaction, IUD=Intra-uterine death, PIH=Periventricular-intraventricular hemorrhage

we label infertility as a symptom. LTBI diagnosis is based solely on a positive IGRA or TST without symptoms and signs (X-ray) of disease.[7]

Our results indicate that a combination of tests must be carried out to diagnose GTB to avoid unnecessary treatment or miss out diagnosis. Drugs used for ATT have serious side effects, especially drug-induced hepato- and neuro-toxicity, hence adequate confirmatory steps should be taken before prescribing them. Indiscriminate use of ATT can also lead to drug resistance with serious consequences. Counseling and a full discussion with patients before giving ATT are of paramount importance, given the increasingly litigative nature of society. DNA-PCR in isolation is the most frequently carried out test for the diagnosis of GTB. Our study showed a very poor correlation between EB DNA-PCR and Koch's culture which is the gold standard for GTB diagnosis. Other authors[24,28,29] have also reported this and reiterated the need to perform concomitant culture.

There was also a low correlation between IGRA and DNA-PCR in our study. Since immune response in the host occurs with primary infection, IGRA should be positive in all cases of positive DNA-PCR. However, variability has been demonstrated, active TB cases have been associated with greater production of IFN-γ compared with latently infected patients.[30,31] Low IFN-γ value occurs when there is clearance or resolution of infection,[26] this too can account for IGRA reversal. Using multiple tests rather than a single diagnostic test may be useful for accurate diagnosis and has been suggested by many authors.

We looked at the pregnancy outcome in patients who were DNA-PCR positive and not given ATT (Group B1) and compared them with patients treated on the basis of DNA-PCR (Group B2) and patients negative for GTB (Group B3) and found that the clinical pregnancy rate, live birth rate, and miscarriage rates were similar. However, larger studies need to be done to confirm our findings.

Management of genital tuberculosis in infertile women

Suggested course of management adding results of IGRA to tissue testing is presented. In endemic areas, in the context of genital Koch's, all cases should be treated with a full course of ATT and not with 3–4 months of isoniazid (INH) or RIF, and INH as the risk of developing drug-resistant TB is very high.

Active genital tuberculosis: Histopathological (HP) diagnosis and Mycobacterium tuberculosis growth on culture

Management providing a full course of ATT for 6 months. Wherever possible, drug sensitivity should be carried out. Culture must be repeated after completing 3 months of treatment (2 months of active phase and 1 month of maintenance). In case associated pelvic damage (assessed on endoscopy) is present, treatment may be extended to 8 months.

Sub-clinical GTB

Patients with Unexplained Infertility.

a) Endoscopy normal, IGRA positive. EB NAAT positive, Koch's culture negative. Treatment should be offered to the patient since this could be an early pauci-bacilliary stage of the infection or even a dormant phase that could get re-activated during infertility treatment.
   Management: A full course of treatment for 6months is recommended in endemic countries to prevent MDR TB.

b) Normal Endoscopy, Negative IGRA, EB Koch's culture negative, EB NAAT positive
   This could be a false positive. Treatment not advised in the absence of any other signs or symptoms. Repeat IGRA after 3 months to check for conversion. If index of suspicion is high a repeat EB for DNA PCR using 'Gene Xpert Test' can be carried out.

c) Only IGRA is positive:
   This indicates exposure to MTBI it does not indicate GTB in the absence of tissue diagnosis. In endemic countries latent TB is not treated because of a high possibility of repeated exposures. In case the physician elects to treat on the basis of IGRA, then a full course of ATT is advisable.

Patients with tubal and/or uterine factor infertility

Healed/contained/dormant genital tuberculosis: Pelvic adhesions on laparoscopy and/or intrauterine adhesions with negative tissue culture, NAAT, and AFB stain can be classified as healed/contained TBI. The healing process involves fibrosis, the *Mycobacterium* being sealed within.

Management: Management decision in these cases is difficult as these adhesions can be a result of bacterial infection or a previous surgical trauma. However, in India, since the possibility of Koch's is high and isolation of MTB is fraught with difficulty, a course of ATT for 6–8 months should be considered, especially if there is no previous history of pelvic infection, vaginitis, or surgical interference. The bacterium may still be viable within the fibrotic lesion and may get activated at a later time when patient immunity is low. IGRA may be of help in confirming exposure to infection.

CONCLUSION AND KEY POINTS

1. The term SGTB should replace LGTB
2. SGTB is an important cause of infertility in India
3. MTB attacks the fallopian tubes in 90% cases followed by the endometrium leading to gross damage in the

absence of specific symptoms

4. Diagnosis is difficult because of the pauci-bacillary status and lack of specific symptoms

5. Diagnosis should be based on a combination of IGRA, EB for DNA-PCR, and Koch's culture to avoid unnecessary administration of ATT. A decision on management based on a combination of tests is more prudent

6. For active TB, i.e., culture positive, Histopathology diagnosis, or AFB stain positive, ATT should be advised for 6 months. Culture should be repeated after 3 months of treatment. Drug sensitivity initially (if possible) or in the event of a repeat culture positive should be carried out

7. In patients of unexplained infertility having an EB positive for DNA-PCR, negative Koch's culture, and IGRA, treatment should be withheld. A repeat IGRA may be done after 3 months to look for conversion. A repeat EB for DNA-PCR using gene Xpert can also be performed

8. If the patient becomes pregnant, IGRA may be repeated at 8–12 weeks of gestation as the activation of MTB may occur during pregnancy when immunity is altered

9. In patients of unexplained Infertility, if both IGRA and EB for NAAT are positive, a full course of ATT should be offered. Neither of the tests should be repeated, as they remain positive even after treatment. Repeat treatment on the basis of a positive EB for NAAT is not advised

10. In patients with TF, Asherman's syndrome, or frozen pelvis, ATT should be offered even when tissue diagnosis is negative. IGRA may be helpful in proving exposure as previous surgical intervention or non-MTB infection may also lead to adhesions. Decision on ATT in such a situation rests with the treating physician

11. ATT can lead to hepato-neurotoxicity. Monitoring is important during drug administration.

There is an urgent need for accurate tests to diagnose SGTB and treat persons with a high risk of reactivation and avoid unnecessary treatment of those who do not carry such a risk.

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Conflicts of interest
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