SUMMARY
&
CONCLUSIONS
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Female genital tuberculosis, one of the relatively ‘silent’ forms of extra-pulmonary TB has been assuming importance in the recent years and is emerging as a notable cause of infertility in females across the globe. The disease accounts for a large proportion of morbidity and mortality in a developing country like India. It is a frequent cause of chronic pelvic inflammatory disease and infertility. The fallopian tubes are affected in almost 100% cases followed by endometrium in 50%, ovaries in 20%, cervix in 5% and vagina and vulva in <1%. The actual incidence of genital tuberculosis in general population can be accurately measured, as most of the patients are asymptomatic and may remain undiscovered. The diagnosis thus requires high index of suspicion. Early diagnosis of genital tuberculosis and its treatment in young patients is essential and may improve the prospects of care before the tubes are damaged beyond recovery.

Early diagnosis of tuberculosis still relies on acid fast bacilli (AFB) microscopy and culture, despite the fact that both techniques several diagnostic lacunae. Culture, although considered laboratory gold standard, requires a minimum of 10-100 bacilli/ml of clinical sample and a long incubation period. The commonly used ‘laparoscopy’ (the so called clinical gold standard) may be able to detect advanced stages of infection. However, the changes during the early stage of infection may be missed. During the last decade, polymerase chain reaction has emerged as a rapid, sensitive and specific molecular method for detection of mycobacterial DNA. Subclinical or latent infection might give a positive result in PCR, but this is considered insignificant as prompt diagnosis is essential for averting permanent to genital organs and consequent infertility.

Despite significant diagnostic promise of DNA-PCR for confirming clinical diagnosis of genital tuberculosis, this method is not useful for differentiating between active and latent or dormant cases. mRNA-based methods have been shown to act as an important tool for pin-pointing active disease; hence it was worthwhile to evaluate mRNA-based RT-PCR or real-time PCR for confirming active cases of genital tuberculosis; more so because limited studies have been carried-out worldwide, particularly on the innovative diagnostics and drug-resistance components of female genital tuberculosis; Indian data on the subject is almost negligible excepting couple on recent reports on limited
samples. Accordingly, during the present study, we evaluated PCR as a diagnostic test for female genital tuberculosis and also correlated the findings of PCR with laparoscopy and other diagnostic tests. We found that DNA PCR (though may not be fool-proof) can be considered as the method of choice to achieve reliable diagnosis compared to other available methods, particularly due to poor sensitivity of AFB culture; and also has an edge over laparoscopy which is often considered as clinical gold standard.

PCR-based methods that amplify *M. tuberculosis* DNA have proven to be very useful for rapid diagnosis of infection in pulmonary as well as extra pulmonary tuberculosis. However, a DNA-PCR is unable to differentiate between viable and nonviable organisms while bacterial mRNA with a mean half-life of 3–5 min is more prone to destruction than genomic DNA, hence a positive mRNA signal would indicate the presence of viable organisms. On amplification of mRNA-specific for 85B antigen gene, we found that only ~30% of all true positive (laparoscopy and DNA positive) cases of female genital TB are cases of active TB harboring viable tubercle bacilli as evident from the presence of *M. tuberculosis*–specific mRNA. This may help in prioritizing the cases for urgent and intensive therapy. Also, in view of the very low sensitivity of AFB culture, the only reliable method to differentiate between viable and non-viable bacteria in cases of female genital tuberculosis can be messenger RNA detection using RT-PCR. In addition, our study also supports the use of sequence-based confirmation to rule out the possibility of false amplification of mRNA product.

With regard to drug resistance, we subjected all the DNA PCR-positive samples to gene sequencing to amplify the genes responsible for multi drug resistance (MDR); i.e. *rpoB, katG* and *inhA*. On analysis of results, we found that most mutations observed in the *rpoB* and *katG* genes in our study were those already reported earlier. A total of 2 cases of genital MDR TB were noted in our study. It is likely that emergence of drug resistance is relatively less in cases of genital tuberculosis because of the less indiscriminate use of ATT probably due to asymptomatic presentation of the disease in maximum number of cases.
Improved pregnancy rates after anti tubercular treatment have been reported earlier. In our study, ~43% (3/7) conceived spontaneously after ATT indicating that early diagnosis and thus treatment may prevent a woman from undergoing a painful and psychologically daunting infertility treatment.

Conclusions:

- Of an array of currently available diagnostic tools, DNA PCR may be used as the method of choice at present to achieve more reliable diagnosis. AFB culture – the all time “Gold Standard” may not be very helpful for routine diagnosis of genital tuberculosis.
- DNA PCR also has been found to give better results than laparoscopy (the clinical gold standard); hence DNA PCR may be relied upon for diagnosis of Genital tuberculosis.
- ONLY ~12% (51/400) of all cases of female infertility have tubercular etiology.
- mRNA RT-PCR findings of the present study strongly suggest that only ~30% of all cases of female genital tuberculosis are the cases of active tuberculosis harboring viable tubercle bacilli. This may help in prioritizing the cases for urgent and intensive therapy.
- No significant new mutation was observed in the rpoB and katG genes in our study, suggesting that the magnitude of drug-resistance in cases of genital TB may be much less than other forms of TB at present.
- It is likely that emergence of drug resistance is relatively less in case of genital tuberculosis because of the less indiscriminate use of ATT probably due to asymptomatic presentation of the disease in maximum number of cases.
- It may thus be important to give due emphasis to the DNA PCR/ mRNA RT-PCR and gene sequence-based molecular tests for initiating prompt therapy of female genital tuberculosis at an early stage, instead of waiting for laparoscopic changes to manifest for making assessment based on currently practiced clinical diagnosis. The findings of this study may be considered a step forward towards the ultimate object of reducing incidence of female genital tuberculosis in India.