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Commentary

Female genital tract tuberculosis: How long will it elude diagnosis?

India is a country with one of the highest burden of tuberculosis (TB) accounting for one fifth of the global incidence annually. Although pulmonary TB is the primary and the most common presentation of tuberculosis in India, there is a significant number of cases of extra-pulmonary TB reported annually. Among these, tubercular meningitis is the gravest manifestation while female genital tract TB poses a diagnostic challenge.

Genital tract TB is a chronic disease that often presents with low grade symptomatology and very few specific complaints. Presenting symptoms are generally varied; infertility being the most frequent clinical presentation (43-74%). Other clinical presentations include oligomenorrhoea (54%), amenorrhoea (14%), menorrhagia (19%), abdominal pain (42.5%), dyspareunia (5-12%) and dysmenorrhoea (12-30%)[1]. The tubercle bacilli reach the genital tract mainly by haematogenous spread from foci outside the genitalia. Haematogenous spread of TB bacilli to the fallopian tubes results in involvement of the submucosa (endosalpingitis) at the outer ends with gradual spread medially to the endometrium. Direct spread of infection to the fallopian tubes results in exopsalpingitis with tubercle on the surface. The fallopian tubes are involved in most cases of genital TB and together with endometrial involvement cause infertility in patients. The global prevalence of genital TB is estimated to be 8-10 million cases, with rising incidence in industralized and developing countries partly as a result of its association with HIV infection[2]. Reportedly about 9 per cent of all extra-pulmonary tuberculosis cases are genital tract TB[3]. The diagnostic dilemma arises because of the varied clinical presentation of the disease confounded by diverse results on imaging, laparoscopy, histopathology and a mixed bag of bacteriological and serological tests, each of which has its limitation in diagnostic sensitivity and specificity.

Histology demonstrates the typical caseous granulomatous lesions with giant epitheloid cells. Such lesions are highly suggestive of TB but are not diagnostic, as these also appear in fungal infections and sarcoidosis. Microscopy for alcohol and acid fast bacilli (AFB) can provide a quick diagnosis of poor sensitivity and fluorescent auramine O staining marginally improves the same. Conventional bacteriology for isolation and identification of Mycobacteria has its specific advantages of being a conclusive diagnostic test. Nevertheless, even the liquid culture methods such as BACTEC system or more recent introduction of BacTAlert 3D neither reduce the turn around time below 12 days nor improve the rate of positivity beyond a limit.

In that context the molecular diagnostic methods hold the key to the future of better and efficient diagnosis of genital and other forms of extra-pulmonary TB. Fundamentally all the available molecular tests are based on the principle of polymerase chain reaction (PCR). PCR is a rapid, sensitive and specific molecular biological method applied in the laboratories to diagnose multitudes of diseases. PCR based diagnosis of TB has been evaluated to be useful and important in the detection of pulmonary as well as extra-pulmonary TB. PCR assays targeting various gene segments such as 64kDa protein encoding gene[4], the IS6110 element[5] and *mpt64*[6] have considerably reduced the delay in laboratory diagnosis for definitive mycobacteriological detection. Several authors have explored the application of PCR based diagnosis of female genital tract TB infections to aid in rapid and improved diagnosis. Using *mpt64* based PCR Bhanu *et al*[7] reported that 56 per cent of patients suffering from infertility were PCR positive for TB while AFB (one smear) positivity was only 1.6 per cent and culture positives were only

3.2 per cent. Among the samples tested, endometrial biopsy yielded 53.3 per cent positivity, endometrial aspirate yielded 47 per cent positivity and POD fluid showed 16 per cent positivity[7].

In another study, Kumar *et al*[8] processed endometrial biopsy specimen from 393 patients with assorted gynaecological complaints. Using nested PCR (N-PCR) for *hupB* DNA target they detected both *Mycobacterium tuberculosis* and *M. bovis.* In their series, 38.9 per cent of infertility cases were N-PCR positive(+) for TB whereas only 5.6 per cent were smear +, 4.6 per cent culture + and only 3.2 per cent were histopathology +. Among the menstrual dysfunction cases 11.3 per cent were N-PCR + as against 2.5 per cent smear +, 2.5 per cent culture + and 0 per cent histopathlogy +. Further, among the cases presenting with chronic lower abdominal /pelvic pain, 5.9 per cent were N-PCR + as against 11.8 per cent smear +, 0 per cent culture + and 0 per cent histopathology +. Of note in the study was the detection of *M. bovis* infection in 7.8 per cent of the cases[8].

These and other studies surely underline the importance of PCR diagnostics to improve detection of the cases of female genital tract tuberculosis. However, false negative results and low positivity need attention if PCR diagnosis has to take its due place as a robust diagnostic modality. In that context, the article by Thangappah *et al*[9] in this issue[9] has shown a direction that should be taken to improve the positive detection rate of a PCR based method. The authors have used two sets of primers, IS6110 and TRC_4 to apply PCR detection method on the clinical specimens obtained from female genital tract. Among all the samples tested, the overall PCR positivity has been reported as 36.7 per cent using both the primer sets whereas the smear examination was 8.3 per cent positive, culture 5.6 per cent + and histopathology 6.9 per cent +. What was interesting was when the cases were segregated into clinically suspected TB and clinically not suspected TB, the rate of PCR positivity rose to 57 per cent per cent in the clinically suspected group and remained at 9.5 per cent in the clinically not suspected group. Although this result is only a marginal improvement on the other studies, it certainly shows a possibility for development of multiplex PCR for more sensitive detection of genital TB without compromising on the specificity of the test. This also brings out a very significant observation that clinical suspicion can facilitate improved diagnosis from the laboratory.

Surely the future of better and reliable early diagnosis of genital TB lies in development of multiplex PCR. A combination of suitable primers targeting repetitive sequences and species specific gene sequences would be ideal to detect more number of cases. In addition, instead of keeping the focus only on *M. tuberculosis*, it would be desirable to include primers/probes to detect other mycobacteria such as *M. bovis* as has been shown by Kumar *et al*[8] that a good number of cases of genital TB could be due to *M. bovis* infection. *M. bovis* being pyrazinamide refractory correct detection of the causative organism will facilitate initiation of appropriate treatment and improve cure rate.

To conclude, good history taking, along with correct sampling using various imaging modalities and use of multiplex PCR will certainly turn around the diagnostic difficulty of genital TB.

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