Recent trends in diagnosis of urogenital tuberculosis

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“the efficient diagnosis of UGTB by reliable tests would certainly reduce the infertility in both men and women and the other sequelae associated with disease.”

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Urogenital tuberculosis (UGTB) is the second most common form of extrapulmonary TB (EPTB) in developing nations with severe epidemic situations. For example, India (comprising 45% of EPTB cases) and the third most common form in locations with a lower incidence of TB [1,2], which include both renal and genital TB. However, concomitant UGTB and pulmonary TB (PTB) are found in 2–10 and 15–20% of patients in developed and developing nations, respectively [2]. In fact, UGTB occurs after a prolonged period of latent infection followed by hematogenous spread to the kidneys, fallopian tubes and epididymis [3]. The clinical manifestations of female UGTB include primary or secondary infertility, menstrual dysfunction and pelvic inflammatory disease [4]. Infertility is the most common complication, which occurs in 5–16% of Indian women caused by irreversible damage to the fallopian tubes. Mostly, fallopian tubes are associated with congestion and miliary tubercles, while endometrium is linked with caseation and ulceration in 50–80% cases, resulting in Asherman's syndrome [5]. Similarly, the most frequent sites for male UGTB are the epididymis and prostate followed by seminal vesicles and testicles originating from renal foci, which also lead to infertility [6]. Owing to asymptomatic nature and varied clinical presentations (ranging from vague urinary symptoms to chronic kidney disease) that mimic several urologic and genital diseases, diagnosis of UGTB is a daunting challenge [3].

Smear/culture, histopathology, interferon- γ release assays (IGRAs), intravenous pyelography, laparoscopy and nucleic acid amplification tests (NAATs) are the main modalities employed in the diagnosis of UGTB [6–8], which have certain limitations. Smear microscopy and culture for *Mycobacterium tuberculosis* identification (on Lowenstein-Jensen medium or BACTEC-460/MGIT-960) are commonly used methods but they lead to poor sensitivities due to paucibacillary nature of specimens [7]. Though urine culture is considered the gold standard, it requires skillful technicians and has a prolonged turnaround time of approximately 6–8 weeks [7] when irreparable damage to the fallopian tubes and epididymis already occurs. Delayed diagnosis also leads to nonfunctioning unilateral kidney, contracted bladder and renal failure [1]. Endoscopic procedures such as laparoscopy/hysteroscopy are often linked with operative risks and mostly cause flaring of infection [5], while histopathological examination can be confirmatory only when it demonstrates acid-fast bacilli in the tissues that are very scarce in endometrial biopsies (EBs) [7]. Intravenous pyelography may be suggestive of UGTB but it is nonspecific and often mimics other pathologic lesions [1].

IGRAs

Immunological procedures such as IGRAs are widely employed for the early detection of UGTB cases. Notably, Kim *et al.* [8] reported a sensitivity of 52.6% by Quantiferon-TB Gold in-Tube assay with the peripheral blood of both male and female UGTB patients using ESAT-6 (Rv3875), CFP-10 (Rv3874) and TB 7.7 as eliciting agents and proposed this test to be a supplementary tool along with urine smear/culture. On the other hand, enzyme-linked immunospot assay for interferon- γ production revealed a higher sensitivity of 86.4% but a lesser

specificity (75.4%) in female UGTB patients [9]. Moreover, T-SPOT.TB test with pelvic effusion exhibited better sensitivity than peripheral blood.

NAATs

Molecular tests such as NAATs, in other words, loop-mediated isothermal amplification (LAMP), PCR, multiplex-PCR (M-PCR), real-time PCR and GeneXpert (MTB/RIF) are quite popular these days for the rapid diagnosis of EPTB cases, including UGTB.

LAMP: Though LAMP test has been well-documented for the diagnosis of PTB, TB meningitis and intraocular TB [7], there is only one report to detect UGTB cases. For example, Sethi *et al.* [7] designed LAMP targeting IS6110 using EBs of female UGTB that exhibited moderate sensitivity and specificity though male UGTB specimens were not exploited. To further enhance the accuracy, we are currently focused to design LAMP test using multiple-gene target approach with 2 or 3 targets from a pool of genes, in other words, IS6110, *mpt64* (Rv1980c), *fbp* (Rv1886c), *esat6* – among others, within urine samples of both men and women. Indeed, LAMP has the potential to revolutionize UGTB diagnosis especially in resource-limited settings as it utilizes a simple dry water-bath for amplification and avoids the use of a thermocycler, which may translate into a simple, reliable and cost-effective point-of-care test.

PCR: PCR tests targeting IS6110, *hsp65* (Rv0440), *mpt64*, *fbp*, *mpt40* (Rv2351c), *TRC4* (conserved repetitive element) – among others, have been devised using endometrial aspirates, EBs, renal biopsies, menstrual blood and urine specimens [2,4,10] but they often lead to false-positive and false-negative results. Detection of male UGTB within semen samples by PCR has also been reported that revealed superiority over MGIT-960 culture [6]. However, Radhika *et al.* [11] improved the sensitivity of monoplex nested-PCR targeting IS6110 by combining with BACTEC-460 culture in diagnosing female UGTB. In a recent meta-analysis of eight studies conducted with the urine samples of UGTB patients from the diverse geographical regions by PCR [2], heterogeneous sensitivities were observed, which could be due to different gene targets employed.

M-PCR/real-time PCR: To improve the sensitivity of PCR, Bhanothu *et al.* [10] designed M-PCR using *mpt64*+*LpqH* (Rv3763)+*TRC4*+*mpt59-α* (*fbp*) within EBs, ovarian biopsies and pelvic aspirates of suspected female UGTB cases. Compared with smear/culture and histopathology, M-PCR showed an improvement for the detection of UGTB cases. Since the collection of menstrual blood is a noninvasive procedure, M-PCR targeting *mpt64*+IS6110 has been devised within menstrual blood specimens and compared results with EBs [12], where menstrual blood revealed better sensitivity than EBs. Similarly, a comparison was made between the menstrual blood and EB specimens by M-PCR using IS6110+*dnaJ* (Rv0352)+*hsp65* targets [13], which showed lesser sensitivity but higher specificity with menstrual blood, while the reverse was true with EBs. The utility of M-PCR targeting *hsp65*+*esat6*+*its* (16S–23S internal transcribed spacer sequence) for differential detection of *M. tuberculosis* and *M. avium* complex from the suspected female UGTB cases has been documented, which exhibited better sensitivity than laparoscopy/hysteroscopy [4]. Interestingly, diagnosis of renal TB from renal biopsy specimens by real-time PCR targeting IS6110 was demonstrated using 35- and 40-cycle threshold (CT) cut-off values, which revealed higher sensitivity but lesser specificity with CT-40, while the reverse was noted with CT-35 [14]. Taken together, positive M-PCR results along with clinical findings is a good choice to start antitubercular therapy in suspected UGTB cases, when the other conventional tests almost fail.

GeneXpert: GeneXpert assay, a semi-nested real-time PCR using *rpoB* (Rv0664) has been a major breakthrough during the last decade for the identification of both *M. tuberculosis* and rifampin resistance [15,16] though limited information is available to detect UGTB specimens by this assay. While analyzing premenstrual EBs for diagnosing 240 infertile women, Xpert revealed a low sensitivity (46.6%) but a high specificity of 100% [16]. In another study [15], a moderate sensitivity of 63% but a high specificity (98.8%) was attained by Xpert within urine samples of male and female UGTB patients. These findings indicate that Xpert can be used as an excellent rule-in test but not as rule-out test. Moreover, wide utilization of this test in developing countries like India is restricted primarily due to high cost.

Immuno-PCR

Notably, NAATs are restricted to detect nucleic acid molecules only; however, there are abundant protein/lipid molecules in circulation during UGTB infection, which remain unexplored. Although ELISA can detect non-nucleic acid molecules, it fails when the target antigen concentration is low. Contrarily, Immuno-PCR (I-PCR) assay can detect ultralow amount of target antigens [17]. In fact, I-PCR comprises the versatility of ELISA with

the enormous amplification capacity of PCR, thereby exhibiting many-fold higher sensitivity than ELISA [17]. Moreover, urine is quite easy to obtain that has a lesser risk of infection to the laboratory workers but it carries low concentration of *M. tuberculosis* biomarkers [18]. Detection of mycobacterial lipoarabinomannan (LAM) within urine samples is considered as an attractive biomarker, which has been demonstrated in HIV-coinfected and HIV-uninfected TB individuals by lateral flow immunochromatography and ELISA methods though poor sensitivities were observed in HIV-uninfected TB individuals [17,19]. However, urinary extracellular vesicles (EVs) isolated from TB patients can furnish intact pathogen-derived biomarkers and also concentrate those biomarkers [19]. We recently detected CFP-10 and LAM from the urinary EVs of PTB and EPTB patients by I-PCR, which revealed significantly higher sensitivities than ELISA [19]. Furthermore, sensitivities attained with LAM detection by I-PCR were better than CFP-10 detection, but urinary EVs of UGTB patients were not examined. To further improve the diagnosis of UGTB, we recommend detection of a cocktail of *M. tuberculosis* biomarkers, for example, LAM+CFP-10/MPT64 within urinary EVs of UGTB patients by I-PCR/nanoparticle-based I-PCR assay, which may match the WHO criteria of high-priority target product profile [19].

Circulating cell-free DNA

Identification of circulating cell-free DNA by PCR/real-time PCR in bodily fluids, for example, plasma, urine and pleural fluids of TB patients has recently been documented [20]. The prospective of circulating cell-free DNA as a noninvasive diagnostic method seems to be encouraging for UGTB as it may allow the diagnosis of disease from easily available blood/urine samples and that can develop into a point-of-care test.

In brief, the efficient diagnosis of UGTB by reliable tests would certainly reduce the infertility in both men and women and the other sequelae associated with disease.

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