



Genital tuberculosis - A diagnostic dilemma

The global prevalence of genital tuberculosis (TB) is estimated to be 8-10 million cases, with a rising incidence in the industrialized and developing countries partly as a result of its association with HIV virus infection. A diagnosis of genital tract tuberculosis has profound implications for the asymptomatic women seeking fertility. The diagnostic dilemma arises because of varied clinical presentations, diverse results on imaging and laparoscopy, and a mixed bag of bacteriological and serological tests. Hence genital tuberculosis is a diagnosis based on the collective evidence from imaging techniques, direct visualization by endoscopy, histopathology of genital tract material, and serology ¹.

Pelvic ultrasound, a useful initial screening test, was able to identify ascites/loculated fluid (100%), adnexal mass (93%), peritoneal thickening (69%), omental thickening (61%), and endometrial involvement (83%) in cases of genital TB ².

Chauhan et al ³ reported genital TB in 7.5% of hysterosalpingo-grammes performed for infertility. In their series, the most common feature was isthmo-ampullary tubal occlusion in 81% cases with terminal hydrosalpinx in 16% cases and the uterus affected by scarring, irregular outline and intravasations in 27% cases ³.

Endoscopy has the dual advantage of pelvic organ visualization and sample collection from inaccessible sites for laboratory diagnosis. The fallopian tubes are almost universally affected, as evidenced by complete tubal block in 80%, adhesions and calcifications in 43%, adherent mass in 35.8%, nodular sclerosis in 11.7%, and miliary tubercles and ascites in 9.4% cases of genital TB ⁴. Diagnostic hysteroscopy allows visualization of tubercles, microcaseation, distorted ostium, synechiae, and out-pouching in the endometrial cavity. Biopsy may also be taken from suspicious sites.

Histopathology

Material for laboratory diagnosis should be collected with two objectives, histology and culture, and the tissue should be divided into two equal parts. One part should be sent as per routine laboratory requirements in a fixative solution (formalin or Bouin's fluid). The other part for culture should be collected in a sterile container. If significant delay is anticipated in transporting the tissue to the laboratory, drying should be avoided by adding saline. Bacterial overgrowth

should be prevented by staining it at a temperature of 4^o to 8^oC. Direct inoculation on the culture medium at the site of collection itself has also been described. A histological diagnosis is made with traditional haematoxylin and eosin (HE) staining as well as with Ziel Nelson staining with a basic fuchsin dye. The classic features are caseous necrosis, giant cells, epithelial cell clusters and lymphocyte infiltration. Lesions are highly indicative of but not exclusive to TB unless tubercle bacilli are seen. A similar picture may also be seen in fungal or sarcoid disease. Fluorescent auramine phenol and rhodamine staining has also been used to identify tubercle bacilli in tissues ⁴.

Culture and PCR

Culture methods are still the gold standard in the detection of genital TB. Culture is traditionally performed on solid egg or agar based media such as Lovenstein Jenson (LJ) or Middlebrook THIO and microinoculated at 37^oC under 5% CO₂. Growth is detected after 4-5 weeks. Colonies are seen if the bacillary count is more than 1000 bacilli. This improves the sensitivity as compared to microscopy alone which requires 10,000 bacilli to be positive. However improvements in media have allowed colonies to grow even when the count is 100 bacilli. This is possible with the use of liquid based media radiometric growth detection such as BACTEC 460 or nonradiometric CO₂ growth detection with BACTEC ALERT 3 D. This also has the added advantage of reduced culture time of only 2 weeks and also provides rapid assessment for drug sensitivity patterns ⁶.

The radiometric culture BACTEC has a sensitivity of 80-90% whereas the LJ medium has a sensitivity of only 30-35%. This high sensitivity is particularly useful in cases of genital TB as traditional methods show poor recovery of acid fast bacilli (AFB). BACTEC uses palmitic acid as the substrate for the growth of AFB. The carbon atoms in this substrate are radio-labeled and thus the CO₂ released is also radio-labeled and this is measured in the form of growth index. A growth index of more than 10 gives a strong suspicion of TB. Then a secondary smear is made from the suspected well to confirm AFB ⁷. The polymerase chain reaction is a technic that shows rapid detection and quantification of few DNA copies with high sensitivity and specificity. Its sensitivity is so high that it requires only < 10 bacteria/mL of specimen to achieve a positive report. It is a

rapid method with results available within a day of the DNA being extracted from the sample. It can also be applied to sterile fluids like peritoneal fluid where the culture is difficult due to a low bacterial load⁸. However the PCR test has its own share of false negative results which are largely due to contamination of the sample with heparin which is a known PCR inhibitor, absence of even a single AFB in the sample collected, and high salt concentration of a specimen which interferes with the PCR results. Also PCR cannot distinguish between live and hiked bacilli and there is a small risk of false positive results⁹.

Multiplex PCR

It means amplification of various sequences simultaneously in one tube. The advantage of this test is that it reveals the false negatives, because each amplification provides an internal control for the other and false positives are very rare due to limitations of pre- and postanalytical variations to a single tube per reaction⁹.

A comparison of the sensitivities of the various bacteria/antigen detection tests

Detection by microscopy needs 10,000 bacilli/mL, by LJ culture 1000 bacilli/mL, by BACTEC 10-100 bacilli/mL, and by PCR < 10 bacilli/mL.

However when the culture and PCR results don't tally, it leads to further confusion.

PCR positive and culture negative

PCR can detect even very few bacilli and even dead bacilli. Hence such a report warrants therapy.

PCR negative and culture positive

This result cannot be dismissed as contamination causing the false negative rate of PCR. Culture remains the gold standard for diagnosis of TB.

Indian scientists have been very active in the development of PCR methods for detection of micobacterium tuberculosis. Different investigators have used separate gene targets like MPB-64, repetitive sequences, GC repeats, DeVR, 38KD, TRC 4, and IS 1081. A patented system has been further modified and a new tested PCR target of this gene has been developed at CDFD, Hyderabad. These assays are promising in confirming the diagnosis of genital TB⁸.

During the last decade, major advances in understanding the genetic structure of mycobacterium have lead to the development of specific gene sequences and several gene probes or gene amplification systems for diagnosis of TB.

DNA probes

These probes have been used in several countries for rapid

confirmation of the identity of mycobacterial isolates. When used with newer methods of growth detection such as BACTEC, Septc-Chek, and MGIT, these are of great help in rapidly confirming the diagnosis within 1 or 2 days.

Ribosomal rRNA based probes

These probes target rRNA, ribosomal DNA, and spacer and flanking sequences which are useful for quick identification of mycobacterial isolates. These probes were earlier radio labeled but have now developed into chemiluminescent technic. rRNA targeting probes are 10-100 fold more sensitive than DNA targeting ones and may be used to confirm the diagnosis directly in the clinical specimens in a good proportion of cases¹⁰.

Isothermal amplification technic

Strand displacement amplification system using isothermal amplification for micobacterium tuberculosis has been very promising with a sensitivity of upto one colony forming unit¹¹.

Conclusion

Tuberculosis, an age old disease, is making a comeback with the HIV pandemic. There is an urgent need for developing definitive diagnostic methods and criteria to be applied to make a conclusive diagnosis of genital TB.

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