Molecular Biology in Intestinal Tuberculosis — Chopping Old Blocks

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Tuberculosis (TB) is endemic in India. The overall incidence of TB is likely to rise with increasing incidence of HIV in our country. Intestine is a common site of extra-pulmonary TB. Though TB may affect any part of the intestine, ileocecal (IC) junction is the most commonly affected area.

The diagnosis of IC-TB is based on clinical presentation (chronic disease, abdominal pain, fever, diarrhoea, anaemia of chronic infection, elevated ESR); barium studies (ulcers, strictures, deformed IC junction); CT scan (wall thickening, dilated loops); colonoscopy (ulcer, nodule, stricture); tissue biopsy (caseating granuloma); microbiology (AFB staining, growth on culture); polymerase chain reaction (PCR) and a trial of anti-TB drugs. Till date the gold standard for the diagnosis includes: detection of AFB, positive culture and growth on culture); polymerase chain reaction (PCR) and a trial of anti-TB drugs. Tunnel the date gold standard for the diagnosis includes: detection of AFB, positive culture and caseating granuloma. Most clinicians in India use some of the above available investigations in a complementary manner to diagnose Mycobacterium tuberculosis (MTB).

In recent years the incidence of Crohn’s disease (CD) is rising in urban population. There is considerable overlap in the clinical presentation, radiological, colonoscopic and histological features of CD and TB of intestine. As the treatment of both diseases is poles apart it is mandatory to establish the diagnosis as accurately as possible.

In this issue Amarapurkar et al have selected 60 patients of intestinal TB and 20 patients of CD based on the standard clinical criteria. The clinical evaluation included extra-intestinal manifestations in both groups. About 40% of TB patients had pulmonary involvement. The study was performed in joint collaboration of two teaching institutes from Mumbai.

PCR was performed from old formalin-fixed, paraffin-embedded tissue blocks for MTB. The test was positive in 13 of 60 (21.6%) patients with intestinal TB and in one of 20 (5%) patients with CD. The authors suggest PCR for MTB is a useful method to differentiate intestinal TB from CD.

MTB has been diagnosed in the past by performing PCR on body fluids (sputum, pleural fluid, ascitic fluid, CSF and blood), tissues (pleural, peritoneal, intestinal, joint, skin, bone marrow, lymph node and pancreas) and cells (peripheral blood mononuclear, endothelial, fibroblast). Currently available PCR use the amplification of segment IS 6110 in MTB chromosome. The sensitivity of PCR to detect MTB from intestinal tissues in the current study was 20% which is much lower than ~ 60% reported in other studies. The possible reasons for low sensitivity may be tissue or PCR based.

The tissue variables include 1) sample size: PCR test is more often positive when complete tissue is used for the assay; 2) type of fixation: unbuffered or neutral buffered formalin inhibits the efficacy of PCR; 3) type of section: frozen sections are superior to paraffin-embedded tissue; 4) sampling error: tissues obtained using well biopsy technique, jumbo forceps and after balloon dilation give submucosal samples required for the evaluation of MTB; 5) age of tissue: PCR sensitivity may decrease if tissue storage time is more than 6 years; 6) intralesional heterogeneity: granulomatous lesion give higher PCR positivity; 7) bacterial load: PCR sensitivity for detection of MTB bacilli may vary from nine organisms to 10^9 colony forming units in a 5-um tissue. One of the drawbacks of the retrospective analysis will be a smaller sample size available for re-evaluation. Most of the tissue blocks in the current study were only 3 years old and hence unlikely to be the cause of lower sensitivity. Amarapurkar et al found no correlation between the presence or absence of granuloma and PCR positivity. Interestingly PCR was positive in a few patients who had no histological features of TB. Similar observations were made in another study.

The sensitivity of PCR may be improved by: high speed centrifugation of aspirate (increases bacterial concentration), tissue wash with guanidium isothiocyanate (removes DNA inhibitors), tissue incubation in Lowenstein-Jensen substrate for 2-3 days (increases bacterial load) and DNA release by heating in the presence of detergents and chelex-100 resin.

PCR for MTB is more sensitive than detection of AFB and culture. PCR test on aspirates (pus) give higher positivity than tissue as most tissues have some inhibitory substances. Amarapurkar’s study has shown AFB in eight of 60 (10.3%) tissues and all were PCR positive. PCR in comparison to culture is quick (3 days Vs 2-6 weeks), can be performed on paraffin-embedded tissue and identify gene resistance to rifampicin. AFB culture is more reliable for differentiating active from past infection and post-chemotherapy evaluation.

The current applications of PCR for MTB include: to identify if MTB plays any role in other granulomatous diseases like sarcoidosis and CD, to detect gene resistance to rifampicin, to detect MTB early in immunocompromised patients and military TB to establish nosocomial source of infection, to identify source/ site of latent infection and to identify MTB from bone marrow aspirate in patients with PUO.

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Though PCR techniques are not available at all centers, current data suggest that tissue blocks and sputum on a filter paper can be transported without any loss of PCR sensitivity. Thus the test could be carried out in a major city from a tissue obtained in a village few days back. If this can be documented in a prospective way, it will surely help improve health care services even in remote parts of our country.

A recent study on PCR has used magnetic beads for extraction of MTB DNA from tissue samples over the conventional reagents like phenol/chloroform as the later is time consuming and carries the risk of occupational hazard. Multiple PCR using three primers for detection of MTB, *Mycobacterium avium* and slow-growing *Mycobacterium* would be extremely useful in patients with immunocompromised status and identification of drug resistance.

Despite all advances, the clinicians, treating MTB must use an integrated approach using every available facility to identify disease early. We should not shut eyes on molecular biology techniques but use them judiciously to improve healthcare. Amarapurkar has chopped the tissue blocks and opened the doors for prospective studies to help differentiate two granulomatous diseases (MTB and CD) with many similarities in presentation but distinctive differences in treatment.

**REFERENCES**

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