Tuberculous endometritis: its association with infertility and other gynaecological complaints in Indian women

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Running Title: Detection of M.tb. and M.bo. in endometrial biopsies
Abstract

Endometrial biopsies derived from 393 patients with assorted gynecological complaints were investigated for mycobacterial infection. Employing 4 different techniques mycobacterial pathogens were detected irrespective of the nature / type of clinical complaint. *Mycobacterium tuberculosis* was the predominant pathogen detected among the samples investigated.
Tuberculosis occurs worldwide and causes widespread morbidity and mortality. Pulmonary and extra-pulmonary sites are known to be associated with *Mycobacterium tuberculosis* (*M.tb*.) infection. In fact it is well known that pulmonary tuberculosis patients go on to develop extra-pulmonary tuberculosis. One such manifestation has been the occurrence of female genital tuberculosis (FGTB). The spread of the pathogen to fallopian tubes, endometrium and ovaries, leading to a variety of clinical conditions has been described (1, 8, 15). The present study was undertaken to detect mycobacterial infection in endometrial biopsies (EB) collected from patients registered in the gynaecological OPD.

393 patients attending the Obstetrics and Gynaecology OPD of AIIMS, New Delhi were included in the study. 285 of them were infertility patients, 80 had menstrual dysfunction complaints, 17 had chronic lower abdominal or pelvic pain & the remaining 11 were patients with ovarian cyst, fibroid, prolapse uterus, post recanalization etc. EB were processed as described by Chakravorty and Tyagi (3). Four methods were used to detect mycobacteria in the EB. The processed EB extracts were microscopically examined for AFB, isolation of mycobacteria by inoculation on Lowenstein Jensen media and processed for extraction of target DNA for N-PCR, (12). Culture results at the time of writing were available for 262 samples. 295 EB were processed for histopathological examination by H&E staining. The N-PCR for *hupB* DNA target was carried out as described previously (10). The N-PCR products were electrophoresed on 10% polyacrylamide gel and stained with ethidium bromide. The amplicon size for *M.tb* and *M. bovis (M.bo)* were ~116 bp and 89 bp respectively. Species level identification
of isolates obtained was done by spoligotyping (9) & by standard biochemical tests (16). Randomly selected EB samples showing dual bands (116 and 89 bp) were cloned into the pGEMT vector using TA cloning kit (Promega, USA). The clones were sequenced at the DNA sequencing facility, South Campus Delhi University, New Delhi, India.

The detection and identification of \textit{M.tb} and \textit{M.bo.} in representative EB specimens have been depicted in the Figure. N-PCR amplified products equivalent to 116 bp were obtained in 5 of the 7 samples (Lanes 1-3, 7-8). These samples were considered to be infected with \textit{M.tb}. A representative sample depicting mixed infection with \textit{M.tb.} and \textit{M.bo.} has been shown in lane 14 (Fig.). Samples with dual bands were eluted and sequenced. The sequence of the dual bands corresponding to 116 and 89 bp matched with that of the C-terminal part of the \textit{hupB} gene of \textit{M.tb.} and \textit{M.bo.} respectively as described previously (14).

In EB investigated differences in the detection of AFB / histopathological evidence of tuberculosis infection / isolation by culture and detection of \textit{M.tb.} & / \textit{M.bo.} by N-PCR was observed, (Table). Of the 393 EB collected, AFB was detected in twenty extracts (20 / 393, 5.1%) they included patients with chronic lower abdominal or pelvic pain (2/17, 11.8%) infertility (16 / 285, 5.6%) & menstrual dysfunction complaints (2 / 80, 2.5%). Granulomatous tissue reaction compatible with tuberculosis was observed exclusively in seven EB (7 / 220, 3.2%) derived from infertile patients. Mycobacteria were isolated from eleven samples by culture (11/262, 4.2%). Nine strains were lost on
subculture. Eight of these isolates were derived from patients with complaints of infertility (8/174, 4.6%), two were from patients with complaints of menstrual dysfunction (2/71, 2.8%) & one isolate was obtained from (1/11, 9.0%) a patient with ovarian cyst. Ten isolates have been identified as *M.tb* by spoligotyping and standard biochemical criteria. One isolate was characterized as *M. chelonae* by biochemical criteria. However in comparison to isolation by culture, *M.tb* / *M.bo.* / mixed infection was detected in 123 (123 / 393, 31.3%) samples by N-PCR. Of these 109 were associated with *M.tb* (27.7%) and 31 (7.8%) with *M.bo.* infection. One hundred & eleven of these EB extracts (111 / 285, 39%) were from infertile patients, nine were from (9 / 80, 11.3%) patients with complaints of menstrual dysfunction, one was from a patient with pain in the lower abdomen (1 / 17, 5.9%) the remaining two were from (2 / 11, 18.2%) patients with complaints of post recanalization & fibroid (miscellaneous category, Table). The comparative % of sensitivity of detection of mycobacteria by the 4 methods showed that N-PCR assay has the highest sensitivity of 31.3%. AFB detection by microscopy showed a sensitivity of 5.1% and isolation of these pathogens by the culture technique was found to be 4.2%. However, the least sensitive technique was the histopathological examination for granulomatous tissue reaction compatible with tuberculosis infection (2.4%).
Comparing the various clinical conditions with the techniques used in the study for detection of mycobacteria in the samples, mycobacteria were detected by all the methods used in infertility cases. In cases of patients with menstrual dysfunction mycobacteria could be detected only by 3 methods, namely AFB microscopy, isolation by culture and N-PCR. In these individuals no evidence of granulamitous reaction of an ongoing mycobacterial infection was detected. Taking into consideration that isolation of mycobacteria is the gold standard for diagnosis of tuberculosis, eight isolates were obtained from infertility cases compared to 2 from patients with menstrual disorder and one from other conditions. Similarly comparing the N-PCR results, all categories of patients were positive. The highest percentage of positivity was in infertility cases, (39 %, 111 / 285). These results show that infertility with mycobacterial infection is a significant clinical problem in India. The prevalence of FGTB in infertility clinics has been reported to range from 1-19% (2, 11, 13). Beside *M.tb.*, *M.bo.* infection has been reported in FGTB (4, 7). The failure to isolate *M.bo.* in the present study may be due to use of inappropriate media, (5,6). Non-specific clinical presentation; inefficacy of laboratory diagnostic tests, accessibility of reproductive clinics, has resulted in under reporting of FGTB. Hence patients with complaints of infertility and other gynaecological complaints must necessarily be investigated for tuberculosis of the genital tract. The N-PCR, histopathology and culture results confirm that infertility is a common clinical condition associated with FGTB.
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References:


TABLE: Comparative analysis of smear microscopy for AFB, histopathology, Culture & N-PCR, of 393 patients investigated with various complaints.

<table>
<thead>
<tr>
<th>Clinical category</th>
<th>AFB</th>
<th>Histopathology</th>
<th>Culture</th>
<th>PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infertility</td>
<td>5.6</td>
<td>3.2</td>
<td>4.6</td>
<td>38.9</td>
</tr>
<tr>
<td>Menstrual dysfunction</td>
<td>2.5</td>
<td>0</td>
<td>2.5</td>
<td>11.3</td>
</tr>
<tr>
<td>(80)</td>
<td>(2/80)</td>
<td></td>
<td>(2/71)*</td>
<td>(9/80)</td>
</tr>
<tr>
<td>Chronic lower abdominal or pelvic pain</td>
<td>11.8</td>
<td>0</td>
<td>0</td>
<td>5.9</td>
</tr>
<tr>
<td>(17)</td>
<td>(2/17)</td>
<td></td>
<td></td>
<td>(1/17)</td>
</tr>
<tr>
<td>Miscellaneous</td>
<td>0</td>
<td>0</td>
<td>9.0</td>
<td>18.1</td>
</tr>
<tr>
<td>(11)</td>
<td></td>
<td></td>
<td>(1/11)</td>
<td>(2/11)</td>
</tr>
<tr>
<td>Total</td>
<td>5.1</td>
<td>2.4</td>
<td>4.2</td>
<td>31.3</td>
</tr>
<tr>
<td>(393)</td>
<td>(20/393)</td>
<td>(7/295)*</td>
<td>(11/262)*</td>
<td>(123/393)</td>
</tr>
</tbody>
</table>

- Patients were categorised as complain of infertility, Menstrual dysfunction, Chronic lower abdominal or pelvic pain & miscellaneous etc.
- Detection of AFB by Auramine O staining in biopsy extracts
- H&E stained EB sections examined for tissue reaction compatible with tuberculosis
- Growth on Lowenstein-Jensen with pyruvate on solid media, at 37°C
- 116 bp & 89 bp ampicon generated by N-PCR for *M. tuberculosis* & *M. bovis* respectively.
- EB from patients who have never been able to conceive a pregnancy after a minimum of 1 year of attempting to do so, through unprotected intercourse
- Menstrual dysfunction like Polymenorrhagia, Amenorrhea (Primary Amenorrhea, Secondary Amenorrhea), Oligomenorrhea, Menorrhagia, Irregular, post menstrual, continuous & Dysfunctional uterine Bleeding etc.
- Chronic lower abdominal or pelvic pain
- Miscellaneous complain like ovarian cyst, fibroid, prolapse uterus, endometosis, post Recanalization etc.

* Results available at the time of analysis
Figure Legend:

Fig. Nested PCR for detecting and differentiating *Mtb* and *Mbo* in Endometrial biopsy extracts. The ethidium bromide stained amplification products of *Mtb* and *Mbo* generated by using F and R primers were electrophoresed on non-denaturing 10% polyacrylamide gels. The 116 and 89 bp products obtained in *Mtb* and *Mbo*, respectively are indicated. Sample showing dual infection has been shown (Lane 14). Lane: 1, EB7; 2, EB17; 3, EB26; 4 & 13, *Mtb* positive control; 5 & 12, 100 bp molecular weight marker; 6 & 11, *Mbo* positive control, 7, EB40; 8, EB83; 9, EB55; 10, negative control; 14, EB34.