JOURNAL OF CLINICAL MICROBIOLOGY, Dec. 2008, p. 4068–4070 Vol. 46, No. 12
0095-1137/08/$08.00+0 doi:10.1128/JCM.01162-08

Association of Tuberculous Endometritis with Infertility and Other Gynecological Complaints of Women in India▽

P. Kumar,[1] N. P. Shah,[1] A. Singhal,[1]† D. S. Chauhan,[4] V. M. Katoch,[4] S. Mittal,[2] S. Kumar,[2] M. K. Singh,[3] S. Datta Gupta,[3] and H. K. Prasad[1]*

Departments of Biotechnology,[1] Obstetrics and Gynaecology,[2] and Pathology,[3] All India Institute of Medical Sciences, New Delhi 110029, India, and National JALMA Institute of Leprosy and Other Mycobacterial Diseases, Tajganj, Agra 282001, India[4]

Received 19 June 2008/Accepted 30 September 2008

Endometrial biopsy samples derived from 393 patients with assorted gynecological complaints were investigated for mycobacterial infection. By employment of four 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 extrapulmonary sites are known to be associated with *Mycobacterium tuberculosis* infection. In fact, it is well known that pulmonary tuberculosis patients go on to develop extrapulmonary tuberculosis. One such manifestation is the occurrence of female genital tuberculosis (FGTB). The spread of the pathogen to fallopian tubes, endometria, and ovaries, leading to a variety of clinical conditions, has been described previously (1, 8, 15). The present study was undertaken to detect mycobacterial infection in endometrial biopsy (EB) samples collected from patients registered in the gynecological outpatient department of the All India Institute of Medical Sciences, New Delhi, India.

Three hundred ninety-three patients attending the obstetrics and gynecology outpatient department of the All India Institute of Medical Sciences were included in the study. Of these, 285 were infertility patients, 80 had menstrual dysfunction complaints, 17 had chronic lower abdominal or pelvic pain, and the remaining 11 were patients with complaints such as ovarian cyst, fibroid, prolapsed uterus, and postrecanalization. The EB samples were processed as described by Chakravorty et al. (3). Four methods were used to detect mycobacteria in the EB samples. The processed EB extracts were microscopically examined for acid-fast bacilli (AFB), processed for isolation of mycobacteria by inoculation on Lowenstein-Jensen medium, and processed for extraction of target DNA by nested PCR (N-PCR) (12). Culture results at the time of this writing were available for 262 samples. Two hundred ninety-five EB samples were processed for histopathological examination by hematoxylin and eosin staining. The N-PCR for the *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 sizes for *M. tuberculosis* and *Mycobacterium bovis* were ~116 bp and 89 bp, respectively. Species-level identification of the isolates obtained was done by spoligotyping (9) and by standard biochemical tests (16). Randomly selected EB samples showing dual bands (116 and 89 bp) were cloned into the pGEMT vector by using a TA cloning kit (Promega). The clones were sequenced at the DNA sequencing facility, South Campus Delhi University, New Delhi, India.

The detection and identification of *M. tuberculosis* and *M. bovis* in representative EB specimens are depicted in Fig. 1. N-PCR-amplified products equivalent to 116 bp were obtained for five of the seven samples (lanes 1 to 3, 7, and 8). These samples were considered to be infected with *M. tuberculosis*. A representative sample depicting mixed infection with *M. tuberculosis* and *M. bovis* is shown in Fig. 1, lane 14. Samples with dual bands were eluted and sequenced. The sequences of the dual bands corresponding to 116 and 89 bp matched those of the C-terminal parts of the *hupB* genes of *M. tuberculosis* and *M. bovis*, respectively, as described previously (14).

Among EB samples, differences between results for detection of AFB, histopathological evidence of tuberculosis infection, isolation by culture, and detection of *M. tuberculosis* and *M. bovis* by N-PCR were observed (Table 1). Of the 393 EB extracts collected, AFB were detected in 20 (20/393; 5.1%), including those from patients with chronic lower abdominal or pelvic pain (2/17; 11.8%), infertility (16/285; 5.6%), and men-

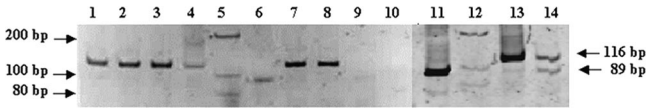

FIG. 1. N-PCR for detecting and differentiating *M. tuberculosis* and *M. bovis* in EB extracts. The ethidium bromide-stained amplification products of *M. tuberculosis* and *M. bovis* generated by using forward and reverse primers were electrophoresed on nondenaturing 10% polyacrylamide gels. The 116- and 89-bp products obtained from *M. tuberculosis* and *M. bovis*, respectively, are indicated. A sample with dual infection is shown (lane 14). Lanes: 1, sample EB7; 2, EB17; 3, EB26; 4 and 13, *M. tuberculosis*-positive controls; 5 and 12, 100-bp molecular weight markers; 6 and 11, *M. bovis*-positive controls, 7, EB40; 8, EB83; 9, EB55; 10, negative control; 14, EB34.

* Corresponding author. Mailing address: Department of Biotechnology, All India Institute of Medical Sciences, Ansari Nagar, New Delhi 110029, India. Phone: 91 11 26594994. Fax: 91 11 26588663. E-mail: hk_prasad@hotmail.com.

† Present address: Laboratory of Molecular Pathology of Tuberculosis, Pasteur Institute, 642 rue Engeland, Brussels B-1180, Belgium.

▽ Published ahead of print on 8 October 2008.

TABLE 1. Comparative analysis of smear microscopy results for AFB detection, histopathological examination, culture, and N-PCR for 393 patients investigated with various complaints

Clinical category (no. of patients)	No. of positive samples/no. tested (%)			
	AFB detection[a]	Histopathology[b]	Culture[c]	PCR[d]
Infertility[e] (285)	16/285 (5.6)	7/220 (3.2)[i]	8/174 (4.6)[i]	111/285 (38.9)
Menstrual dysfunction[f] (80)	2/80 (2.5)	0	2/71 (2.5)[i]	9/80 (11.3)
Chronic lower abdominal or pelvic pain[g] (17)	2/17 (11.8)	0	0	1/17 (5.9)
Miscellaneous[h] (11)	0	0	1/11 (9.0)	2/11 (18.1)
Total (393)	20/393 (5.1)	7/295 (2.4)[j]	11/262 (4.2)[j]	123/393 (31.3)

[a] Detection of AFB was done by auramine O staining of biopsy extracts.
[b] Hematoxylin-and-eosin-stained EB sections were examined for tissue reactions compatible with tuberculosis.
[c] Growth on Lowenstein-Jensen with pyruvate on solid medium at 37°C.
[d] Amplicons of 116 and 89 bp were generated by N-PCR for *M. tuberculosis* and *M. bovis*, respectively.
[e] EB samples were taken from patients unable to become pregnant after a minimum of 1 year of attempting through unprotected intercourse.
[f] Menstrual dysfunctions such as polymenorrhagia; amenorrhea (primary or secondary); oligomenorrhea; menorrhagia; and irregular, postmenstrual, continuous, or dysfunctional uterine bleeding.
[g] Chronic lower abdominal or pelvic pain.
[h] Miscellaneous complaints, such as ovarian cyst, uterine fibroid, prolapsed uterus, endometosis, and postrecanalization.
[i] Results available at the time of analysis.

strual dysfunction complaints (2/80; 2.5%). Granulomatous tissue reactions compatible with tuberculosis were observed exclusively in seven EB samples derived from infertile patients (7/220; 3.2%). Mycobacteria were isolated from 11 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%), and one was obtained from a patient with an ovarian cyst (1/11; 9.0%). Ten isolates were identified as *M. tuberculosis* by spoligotyping and standard biochemical criteria. One isolate was characterized as *Mycobacterium chelonae* by biochemical criteria. However, in comparison to what was found with isolation by culture, *M. tuberculosis*/*M. bovis* mixed infection was detected in 123 samples by N-PCR (123/393; 31.3%). Of these, 109 (27.7%) were associated with *M. tuberculosis* and 31 (7.8%) with *M. bovis* infection. One hundred eleven of these EB extracts were from infertile patients (111/285; 39%), nine were from patients with complaints of menstrual dysfunction (9/80; 11.3%), one was from a patient with pain in the lower abdomen (1/17; 5.9%), and the remaining two were from patients with complaints of postrecanalization and fibroid (2/11; 18.2%) (Table 1, miscellaneous category). Comparison of the percentages of sensitivity for detection of mycobacteria by the four methods showed that the N-PCR assay has the highest sensitivity (31.3%). AFB detection by microscopy showed a sensitivity of 5.1%, and that for detection by isolation of the pathogens by the culture technique was found to be 4.2%. The least sensitive technique was histopathological examination for granulomatous tissue reactions compatible with tuberculosis infection (2.4%).

Results for the various clinical conditions and techniques used in the study for detection of mycobacteria in the samples revealed that mycobacteria were detected by all the methods used in infertility cases. In cases of patients with menstrual dysfunction, mycobacteria were detected by only three methods, namely, AFB microscopy, isolation by culture, and N-PCR. In these individuals, no evidence of granulomatous reactions compatible with ongoing mycobacterial infection was detected. With acknowledgment that isolation of mycobacteria is the gold standard for diagnosis of tuberculosis, eight isolates were obtained from infertility cases, two from patients with menstrual disorders, and one from a patient with an ovarian cyst. Similarly, N-PCR results for patients in all categories were positive. The highest percentage of positivity was for infertility cases (111/285; 39%). 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 to 19% (2, 11, 13). In addition to *M. tuberculosis* infection, *M. bovis* infection has been reported to occur in FGTB (4, 7).The failure to isolate *M. bovis* in the present study may be due to use of inappropriate media (5, 6). Nonspecific clinical presentation, inefficacy of laboratory diagnostic tests, and inaccessibility of reproductive clinics have resulted in underreporting of FGTB. Hence, patients with complaints of infertility and other gynecological 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.

Senior Research Fellowships to P.K. and A.S. from the Indian Council of Medical Research and the University Grants Commission and the technical assistance of Shailendra Kumar are acknowledged. The financial support of DBT, Government of India, is acknowledged.

Mycobacterium tuberculosis H37Rv DNA was obtained from the Tuberculosis Vaccine Testing and Research Materials program (NIAD/NIH grant AI-75320).

REFERENCES

1. **Abebe, A., M. Lakew, D. Kidane, Z. Lakew, K. Kiros, and M. Harboe.** 2004. Female genital tuberculosis in Ethiopia. Int. J. Gynecol. Obstet. **84:**241–246.
2. **Bhanu, N. V., U. B. Singh, M. Charaborty, N. Suresh, J. Arora, T. Ranu, D. Takkar, and P. Seth.** 2005. Improved diagnostic value of PCR in the diagnosis of female genital tuberculosis leading to infertility. J. Med. Microbiol. **54:**927–931.
3. **Chakravorty, S., M. Dudeja, M. Hanif, and J. S. Tyagi.** 2005. Utility of universal sample processing methodology, combining smear microscopy, culture, and PCR, for diagnosis of pulmonary tuberculosis. J. Clin. Microbiol. **43:**2703–2708.
4. **Chowdhury, N. N. R.** 1996. Overview of tuberculosis of the female genital tract. J. Indian Med. Assoc. **94:**345–347.
5. **Corner, L. A., and C. Nicolacopoulos.** 1988. Comparison of media used for

the primary isolation of Mycobacterium bovis by veterinary and medical diagnostic laboratories. Aust. Vet. J. **65:**202–205.
6. **Cousins, D. V., B. R. Francis, and B. L. Gow.** 1989. Advantages of a new agar medium in the primary isolation of Mycobacterium bovis. Vet. Microbiol. **20:**89–95.
7. **Gatongi, D. K., G. Gitau, V. Kay, S. Ngwenya, C. Lafong, and A. Hasan.** 2005. Female genital tuberculosis. Obstet. Gynaecol. (London) **7:**75–79.
8. **Gupta, N., J. B. Sharma, S. Mittal, N. Singh, R. Mishra, and M. Kukreja.** 2007. Genital tuberculosis in Indian infertility patients. Int. J. Gynaecol. Obstet. **97:**135–138.
9. **Kamerbeek, J., L. Schouls, A. Kolk, M. van Agterveld, D. van Soolingen, S. Kuijper, A. Bunschoten, H. Molhuizen, R. Shaw, M. Goyal, and J. van Embden.** 1997. Simultaneous detection and strain differentiation of *Mycobacterium tuberculosis* for diagnosis and epidemiology. J. Clin. Microbiol. **35:**907–914.
10. **Mishra, A., A. Singhal, D. S. Chauhan, V. M. Katoch, K. Srivasatava, S. S. Thakral, S. S. Bharadwaj, V. Sreenivas, and H. K. Prasad.** 2005. Direct detection and identification of *Mycobacterium tuberculosis* and *Mycobacterium bovis* in bovine samples by a novel nested PCR assay: correlation with conventional techniques. J. Clin. Microbiol. **43:**5670–5678.
11. **Parikh, F. R., N. Naik, S. G. Nadkarni, S. B Soonawala, S. A Kamat, and R. M. Parikh.** 1997. Genital tuberculosis—a major pelvic factor causing infertility in Indian woman. Fertil. Steril. **67:**497–500.
12. **Prabhakar, S., A. Mishra, A. Singhal, V. M. Katoch, S. S. Thakral, J. S. Tyagi, and H. K. Prasad.** 2004. Use of the *hupB* gene encoding a histone-like protein of *Mycobacterium tuberculosis* as a target for detection and differentiation of *M. tuberculosis* and *M. bovis*. J. Clin. Microbiol. **42:**2724–2732.
13. **Roy, A., S. Mukherjee, S. Bhattacharya, S. Adhya, and P. Chakraborty.** 1993. Tuberculous endometritis in hills of Darjeeling: a clinicopathological and bacteriological study. Indian J. Pathol. Microbiol. **36:**361–369.
14. **Shah, N. P., A. Singhal, A. Jain, P. Kumar, S. S. Uppal, M. V. P. Srivatsava, and H. K. Prasad.** 2006. Occurrence of overlooked zoonotic tuberculosis: detection of *Mycobacterium bovis* in human cerebrospinal fluid. J. Clin. Microbiol. **44:**1352–1358.
15. **Verma, T. R.** 1991. Genital tuberculosis and subsequent fertility. Int. J. Gynecol. Obstet. **35:**1–11.
16. **Vestal, A. L.** 1977. Procedures of isolation and identification of mycobacteria, p. 65–98. U.S. Department of Health, Education, and Welfare publication no. (CDC) 77-8230. Centers for Disease Control, Atlanta, GA.