

IS TESTING OF MENSTRUAL BLOOD JUSTIFIABLE FOR DIAGNOSIS OF ENDOMETRIAL TUBERCULOSIS AMONG INFERTILE WOMEN?

Anushree. D. Patil^{1*}, Sushil K Shinde², Geetanjali M. Sachdeva³, Achhelal R. Pasi⁴, Shanta M. Chitlange⁵,
Nomita Chandhiok⁶, Rahul K. Gajbhiye⁷, Sanjay L. Chauhan⁸

¹Scientist C, ²Project Research Officer, ³Head of Department of Primate Biology,

⁴Dept. of Clinical Research, ⁵Former Head of Department of Clinical Research,

⁶Deputy Director, ⁷Scientist B, ⁸Head of Department of Clinical Research,

National Institute for Research in Reproductive Health (ICMR), Mumbai

***Corresponding Author:**

E-mail: rajshrinm@yahoo.co.in

ABSTRACT

Introduction: *Diagnosis of endometrial tuberculosis is often carried out by using commercially available molecular diagnostic tests on endometrial biopsy samples and menstrual blood samples of infertile women in many infertility clinics.*

Aim: *To determine whether menstrual blood is a good alternative sample for diagnosis of endometrial tuberculosis.*

Methods: *Women (n=123) presenting with infertility were investigated for endometrial tuberculosis by testing menstrual blood samples as well as endometrial aspirates with GEN-PROBE AMPLIFIED Mycobacterium Tuberculosis Direct (MTD) test which is a Nucleic Acid Amplification Test (NAAT) and VersaTREK culture. Results of the two tests for detection of M. Tuberculosis were compared in menstrual blood and endometrial aspirate samples.*

Results: *Out of 123 menstrual blood samples tested M. tuberculosis was detected by culture in only 0.8 per cent of women. Mycobacterium other than Tuberculosis (MOTT) was detected in 2.43 per cent samples of menstrual blood on culture reports and was identified to be Mycobacterium inter medium.*

Conclusion: *Menstrual blood was not found as a good alternative sample for diagnosis of endometrial tuberculosis. Results of MTD tests on menstrual blood sample for diagnosis and management of endometrial tuberculosis (as is widely used in infertility clinics) should be interpreted with caution. Pathological significance of Mycobacterium inter medium in female infertility, though a possible contaminant, needs further research.*

Keywords: *Endometrial tuberculosis, Infertility, Menstrual blood samples.*

INTRODUCTION

Endometrial tuberculosis is an important cause of female infertility. Various Indian studies have shown that tuberculosis endometritis and salpingitis account for 4-9 per cent of all infertility cases¹⁻⁴. Endometrial tuberculosis is almost invariably secondary to a primary lesion elsewhere in the body. In the majority, the infection reaches the endometrium by the haematogenous route where it either persists in the basal layer, which is not shed during menstruation, or it gets reinfected from the tubes following menstruation. Most women with endometrial tuberculosis are asymptomatic and present with infertility.

Diagnosis of endometrial tuberculosis remains a difficult task. The direct demonstration of *Mycobacterium tuberculosis* in endometrial samples has very low sensitivity because most lesions are paucibacillary⁵⁻⁷. Though endometrial sample obtained by curettage is considered the ideal sample for testing for endometrial tuberculosis, interventional procedures like curettage can further lead to flaring of the existing pathology hence testing of menstrual blood was proposed as a potential less invasive sample which could be easily obtained in asymptomatic cases presenting with infertility⁸. Any method that is used to diagnose endometrial

tuberculosis should be highly sensitive to diagnose the disease reliably in its early stage, so that treatment may improve the prospects of cure before the tubes are damaged beyond recovery⁹.

There are only few published studies where the menstrual blood has been evaluated for diagnosis of endometrial tuberculosis¹⁰. However expensive molecular diagnostic tests are widely done on menstrual blood samples of infertile women in many fertility clinics to diagnose endometrial tuberculosis and women are managed depending on results obtained. Whether menstrual blood sample is a good alternative for detection of *M. Tuberculosis* needs to be researched.

The present study assessed the clinic based prevalence of endometrial tuberculosis among women attending infertility clinic at National Institute for Research in Reproductive Health (NIRRH), Mumbai. We also explored the utility of these expensive tests on less invasive sample like menstrual blood for diagnosis of endometrial tuberculosis.

MATERIALS AND METHODS

This cross sectional study included 123 infertile women who were enrolled over a period of 13 months (May 2012 to June 2013). Sample size of

200 was calculated based on the reported prevalence of endometrial tuberculosis (0.3)¹¹, power of 80%, confidence interval of 95% and annual clinic attendance of 300. However due to logistic difficulties and because menstrual blood sample results were not encouraging, enrollment was restricted to 123 women.

Institution Ethics Committee approval was obtained to conduct the study. Women presenting with infertility and who were willing to participate in the study were recruited after written informed consent. Women with primary or secondary amenorrhea (more than six months) where no menstrual blood sample could be obtained were excluded from the study.

After a detailed history and clinical examination, all patients were investigated as per the infertility work up with a pelvic sonogram and hysteron-salpingography and laparoscopy where indicated and this was used as a data set for clinical correlation. Sampling was done by convenience sampling technique. A total of 123 menstrual blood samples from infertile women were tested and in 42 out of them, endometrial aspirate was also tested. The participants were divided in two groups:

Group 1: 81 participants in whom only menstrual blood at one visit was collected and tested with GEN-PROBE AMPLIFIED Mycobacterium Tuberculosis Direct (MTD) test for tuberculosis (TMA) & VersaTREK culture for detection of *M. Tuberculosis*. Menstrual blood sample was collected from the cervical so by a syringe in sterile containers. The sample was collected during the menstrual phase of the cycle on the day of maximum menstrual flow.

Group 2: In 42 out of 123 non-randomly selected participants, menstrual blood was tested as in group 1. In addition, these participants were called for a second visit in the next menstrual cycle. Menstrual aspirate was collected from the uterine cavity with a probe on day one of menses and tested with MTD and culture for detection of *M. Tuberculosis*. This was to compare the results of the menstrual blood with that of the accepted standard which was endometrial aspirate.

The tests were carried out at Metropolis healthcare laboratory which is a standard, laboratory accessible to the clinic. The results of the two tests and also of the samples obtained by the two methods were compared.

GEN-PROBE AMPLIFIED Mycobacterium Tuberculosis Direct (MTD) Test:

MTD test is a FDA approved target-amplified nucleic acid probe test for the *in vitro* diagnostic detection of *Mycobacterium tuberculosis* complex rRNA in acid-fast bacilli (AFB). This test has also been validated at Metropolis for extra pulmonary samples which include body fluids such

as pleural fluids, ascitic fluids, pericardial fluid, endometrial biopsy etc. MTD test utilizes Transcription Mediated Amplification (TMA) and hybridization protection assay (HPA). TMA is an RNA transcription amplification system using two enzymes to drive the reaction: RNA polymerase and reverse transcriptase. TMA is isothermal; the entire reaction is performed at the 3 different temperatures (95 °C, 60°C and 42°C) in the heat block. This is in contrast to other amplification reactions such as PCR or LCR that require a thermal cycler instrument to rapidly change the temperature to drive the reaction. TMA can amplify either DNA or RNA, and produces RNA amplicon, in contrast to most other nucleic acid amplification methods that only produce DNA. TMA has very rapid kinetics resulting in a billion fold amplification within 15-30 minutes. TMA has been combined with the GEN-PROBE Hybridization Protection Assay (HPA) detection technique in a single tube format. There are no wash steps, and no amplicon is ever transferred out of the tube, which simplifies the procedure and reduces the potential of contamination. Gen-Probe's Amplified MTD Test uses three core proprietary technologies to identify the presence of *Mycobacterium tuberculosis* with sensitivity and specificity of 95.5 percent and 100 percent, respectively, on smear-positive samples.

The specimen result when tested using the GEN-PROBE AMPLIFIED Mycobacterium Tuberculosis Direct (MTD) Test is interpreted based on an initial negative result (< 30,000 RLU), an initial positive result (500,000 RLU), or an initial equivocal result (30,000 to 499,999 RLU). The MTD test should be repeated from the reserved lysate when an initial test result is equivocal. A repeat result from the lysate greater than 30,000 is considered positive.

Liquid based culture for TB using VersaTREK:

The VersaTREK is the FDA approved machine used for liquid based culture for Tuberculosis. Special bottles called Myco bottles are used for the culture. The myco bottle is capable of detecting mycobacterium from body fluids, urine, respiratory, gastric aspirates, and tissue specimen. The VersaTREK Myco bottle contains Myco modified Middle brook 7H9 broth with cellulose sponge. The cellulose sponge increases the reactive surface area, enhances recovery and signal of the organism. Non sterile specimen requires decontamination prior to inoculation. Mucoid specimens require digestion as well as decontamination treatment. Myco GS (Growth supplement) and Myco AS or Myco PVNA (antibiotic supplement) is aseptically added to each specimen bottle just prior to inoculation. When the bottle is ready for incubation, a VersaTREK connector is attached. The bottle is loaded into the appropriate drawer location. A connector, which

provides a link between bottle and a sensor located on the VersaTREK instrument, is required for the headspace pressure detection. Versatrek has a rapid detection technology which allows for continuous monitoring of pressure changes. Detection is dependent on the microbial growth inside the bottles resulting in to gas consumption, which in turn leads to pressure fall in the headspace, which is detected by the sensor on the VersaTREK. The bottles are incubated at 37°C and the length of incubation is 42 days.

All positive blinked and negative bottles are taken for further confirmation. Further confirmation of bottle is done by final smear preparation which is stained by Zeil Nelson staining method. Depending upon the morphology, growth rate and confirmatory identification by Accuprobe the bacteria are reported as MTB or MOTT.

A smear report is given on the next day of receipt of the sample which is reported as Acid fast bacilli seen / No Acid fast bacilli seen. Two interim Reports are given, first after 10 days and second after 21 days. Final reporting is done after 42 days. It is reported as No mycobacterium species grown on culture / Mycobacterium tuberculosis complex OR MOTT grown in culture. In between, if bottle blinks positive, it is removed and processed in the same way as above.

The advantages of this method are that the Sensors in the drawers are sensitive to detect smallest drop in the gas pressure which indicate consumption of the oxygen by tubercle bacilli and the step of decontamination and addition of PVNL reduces the chances of growth of bacteria other than tuberculosis.

RESULTS

The patients were aged between 20 and 42 years with median age of 29 years. All the women who were tested had come to seek treatment for infertility. Of these, 76.5% were women with primary infertility and 23.5% were women with secondary infertility. The duration of infertility was from 2 years to 11 years. Menstrual cycles were irregular in 31.2% of women. Menstrual irregularities such as oligomenorrhoea (82.9%), hypomenorrhoea (14.6%) and menorrhagia (2.4%) were seen. Oligomenorrhoea was the commonest menstrual abnormality. A history of previous abortion was present in 13% (16/123) of the women. There was history of close contact with tuberculosis patients in 16.2 % (20/123) of the women.

The mean endometrial thickness was 8.8mm (minimum 5 and maximum 14 mm). Hysterosalpingogram was carried out in 115 women. Characteristic features suggesting genital tuberculosis such as distorted endometrial cavity, beaded appearance of the tubes, hydrosalpinx, calcified areas and cornual blocks were seen in 17.3% (20/115).

Among these, diagnostic laparoscopy report was available in 46 women (46/115). Laparoscopic evidence of tuberculosis was positive in 26.6% (12/46) of women.

Table 1: Results of tests in Group 1 (n=81)

Menstrual blood		Positive	Negative
	Smear	0	81
	MTD	0	81
	Final culture	1 MTB 1MOTT	79

Table 2: Results of tests in Group 2 (n=42)

Visit1 Menstrual Blood		Positive	Negative
	Smear	0	42
	MTD	0	42
	Final Culture	2MOTT	40
Visit2 Menstrual Aspirate		Positive	Negative
	Smear	0	42
	MTD	0	42
	Final Culture	0	42

The prevalence of endometrial tuberculosis in our study population was 0.8%.

Out of the total 123 menstrual blood samples tested, none of the samples tested positive by the MTD test. Only one menstrual blood sample tested positive for culture for *M. tuberculosis*. Mycobacterium other than Tuberculosis (MOTT) was found in 3 samples of menstrual blood on culture reports (2.43 per cent). On species identification, one of the MOTT sample was identified to be *Mycobacterium inter medium*.

DISCUSSION

There is a high incidence of involvement of the endometrium in genital tuberculosis^{12,13,14,15}. Differences in the prevalence of endometrial tuberculosis in infertile women in different studies could be due to variation in the endemicity of tuberculosis. Histo-pathological examination has limited utility in diagnosing endometrial tuberculosis. Culture has sensitivity of 91.6% and specificity of 88% while PCR has sensitivity of 96% and specificity of 100% for detection of *M. tuberculosis* in endometrial samples⁸. NAA techniques allow for detection of *M. tuberculosis* from samples containing relatively few *M. tuberculosis* bacilli. When used for AFB smear-positive respiratory samples, the Gen-Probe MTD test is quite reliable, with high sensitivity (83% to 100%), positive predictive value (94–100%), and negative predictive value (96–100%) reported in the literature^{16,17,18}. However when used in AFB smear-negative respiratory samples, early studies suggested diminished reliability of this test with sensitivity of 50% and positive predictive value of 3–

50%, although specificity remained high¹⁹. Many studies suggest that the Gen-Probe MTD test is also useful in assessing extra pulmonary specimens with high sensitivity (84–100%), specificity (98–100%), and positive predictive value (90–100%)^{16, 17, 18, 20, 21}.

Most published studies in area of female genital tuberculosis which have compared different diagnostic tests are on endometrial curettage samples and very few studies with small sample size are on menstrual blood samples hence we assessed the utility of the MTD test and culture on menstrual blood samples. The prevalence of endometrial tuberculosis in our study population is 0.8%. Bineeta *et al* have reported the incidence of genital TB either by microscopy or culture in menstrual blood samples and endometrial biopsy samples to be 5/125 (4%) and 24/1226 (~2%)⁹. To our knowledge there are no published studies which have compared the results of molecular diagnostic tests and culture on menstrual blood samples of infertile women to detect *M. tuberculosis*.

Out of 123 samples only one sample tested positive for *M. tuberculosis* by culture. The possible reasons for the low incidence of culture positivity in endometrial tissue could be due to paucibacillary nature and a substantial number of TB lesions of the genital tract are bacteriologically mute²². The low rate of positivity in culture may also be due to the presence of a bacteriostatic substance which inhibits the growth of the bacilli²³.

None of the samples tested positive by MTD test. The MTD results were negative in the patients who had positive clinical, radiological and laparoscopic features of genital tuberculosis. This could indicate the possibility of false negative MTD results. MTD has failed to detect 1 case which was positive by culture. The possible explanation could be due to paucibacillary nature of the specimen, and the portion of the specimen taken for MTD would not have had any *M. tuberculosis*. The analyzed specimen may also contain inhibitors of PCR. Restrepo *et al*²⁴ have shown that mycobacterial DNA amplification was compromised when the human: bacterial genome ratio was at least 190:1. As menstrual blood samples are always mixed with blood, this could possibly explain the negative results in this study.

Mycobacterium inter medium, is a new type of slow growing Mycobacterium, which is found to coexist in water and soil. On literature search for the pathological significance of *Mycobacterium inter medium*, we found one case reported of isolation from Granulomatous Dermatitis²⁵ from hot tub exposure in immune-competent host and other from sputum sample in patient suffering with pulmonary disease. We did not come across any case report of *Mycobacterium inter medium* in menstrual blood. *Mycobacterium inter medium* could be a possible

contaminant but the presence of MOTT in menstrual blood and endometrial samples and its association with infertility needs further research.

The high false negative result is an important limitation in this study. However results of this study have a very important clinical practice application since results of the tests on menstrual blood samples are heavily relied upon by practitioners for further treatment. A negative MTD test may result in missing the diagnosis in a few cases. In clinically suspected cases, in the presence of positive MTD and culture results, an infertile woman should be considered as having endometrial tuberculosis and should be treated, however when the MTD results are negative, it indicates the need for further evaluation using other diagnostic tests and repeat testing to confirm/exclude diagnosis and empirical treatment based on clinical judgment.

INTERPRETATION AND CONCLUSIONS

In our study we did not find menstrual blood to be a potential alternative clinical sample for diagnosis of endometrial tuberculosis with existing tests. Conventional microbiological techniques as well as highly sensitive molecular techniques for the diagnosis of endometrial tuberculosis when applied on menstrual blood samples have limitations. The growing practice of testing menstrual blood samples with commercially available molecular diagnostic tests is a matter of concern. Results of available tests on menstrual blood samples for diagnosis and management of endometrial tuberculosis (as is widely used in infertility clinics) should be done with caution in the light of clinical findings. Pathological significance of *Mycobacterium inter medium*, though a possible contaminant, needs further research.

REFERENCES

1. Deepjyoti VG, Kiran M, Neera A, Sunil G. Isolation of mycobacteria from cases of infertility in women. *J Obstet Gynaecol India* 1990;40:803-5.
2. Deshmukh K, Lopez J, Naidu AK, Gaurkhede MD, Kasbawala MV. Place of laparoscopy in pelvic tuberculosis in infertile women. *J Obstet Gynecol India* 1987;37:289-91.
3. Javeri CL, Javeri DD, Limbachiya MM. Genetal tuberculosis. *Proceedings of XVIIth All India Obstetrics and Gynecological Congress*. New Delhi; 1972. p. 32.
4. Sathe AV, Vaidya PR, Deshmukh MA, Motashaw ND. Genital tuberculosis in an endocrine clinic. *J Obstet Gynaecol India* 1979;29 :199-202.
5. Muir DG, Belsey MA: Pelvic inflammatory disease and its consequences in the developing world. *Am J Obstet Gynecol* 1980;138:913-28.
6. Ellner JJ: Tuberculosis. In Kelley A (ed): *Textbook of Internal Medicine*. Philadelphia, JB Lippincott, 1989. p. 1569.
7. Dhillon SS, Gosewehr JA, Julian TM, *et al*: Genital tuberculosis: Case report and literature review. *WMJ* 89:14, 1990.
8. Roya Rozati, Sreenivasagari Roopa, Cheruvu Naga Rajeshwari. Evaluation of women with infertility and

- genital tuberculosis. *J Obstet Gynaecol India* 2006;56:423-426.
9. Jindal UN, Jindal SK, Dhall GI. Short course chemotherapy for endometrial tuberculosis in women. *Int J Gynecol Obstet* 1990; 32 :75-6.
 10. Bineeta Kashyap, Tripta Kaur, Rajat Jhamb, Iqbal R Kaur. Evaluating the utility of menstrual blood versus endometrial biopsy as a clinical sample in the diagnosis of female genital tuberculosis. *Asian J Med Res.* 2012;1(2):45-49.
 11. Kumar.P, Shah NP, Singhal A, Chauhan DS, Katoch VM, Mittal S, Kumar S, Singh MK, Gupta SD, Prasad. Association of Tuberculosis endometritis with infertility and other gynaecological complaints of women in India. *J.Clin.Microbiol* 2008;46 (12):4068-70.
 12. Schaefer G. Tuberculosis of the female genital tract. *Clin Obstet Gynecol* 1970;13: 965-98.
 13. Onuigbo WIB. Genital tuberculosis and reproductive function. *J Reprod Med* 1978;21:249-50.
 14. Nogales-Ortiz F, Tarancion I, Nogales FF Jr. The pathology of female genital tuberculosis: A 31-year study of 1436 cases. *Obstet Gynecol* 1979;53:422.
 15. Sutherland AM. Genital tuberculosis in women. *Am J Obstet Gynecol* 1960;79:486.
 16. Gamboa F *et al.* Comparative evaluation of initial and new versions of the Gen-Probe Amplified *Mycobacterium Tuberculosis* direct test for direct detection of *M. tuberculosis* in respiratory and nonrespiratory specimens. *J Clin Microbiol* 1998;33:2699.
 17. Vlaspolder F, Singer P, Roggeveen C. Diagnostic value of an amplification method (Gen-Probe) compared with that of culture for diagnosis of tuberculosis. *J Clin Microbiol* 1995;33:2699-2703.
 18. Chedore Pn, Jamieson FB. Routine use of the Gen-probe MTD2 Amplification Test for detection of *M. tuberculosis* in clinical specimens in a large public mycobacteriology laboratory. *Diagn Microbiol Infect Dis* 1999;35:185-191.
 19. Rapid diagnostic tests for tuberculosis: What is the appropriate use? American Thoracic Society Workshop *Am J Respir Crit Care Med* 1997;155:1804-14.
 20. Gamboa F, Manterola JM, Vinado B, Matas L, Gimenez M, Lonca J, Manzano JR, Rodrigo C, Cardona PJ, Padilla E. Direct detection of *Mycobacterium tuberculosis* complex in nonrespiratory specimens by Gen-Probe Amplified *Mycobacterium Tuberculosis* Direct Test. *J Clin Microbiol* 1997;35:307-31.
 21. Ehlers S, Ignatius R, Regnath T, Hahn H. Diagnosis of extrapulmonary tuberculosis by Gen-Probe amplified *Mycobacterium tuberculosis* direct test. *J Clin Microbiol* 1996;34:2275-9.
 22. Bhanu NV, Singh UB, Chakraborty M, Suresh N, Arora J, Rana T, *et al.* Improved diagnostic value of PCR in the diagnosis of female genital tuberculosis leading to infertility. *J Med Microbiol* 2005;54:927-31.
 23. Soltys MA. An anti-tuberculous substance in tuberculous organs. *J Comp Pathol* 1953;63:147-52.
 24. Restrepo BI, Gomez DI, Shipley GL, McCormick JB, Fisher -Hoch SP. Selective enrichment and detection of mycobacterial DNA in paucibacillary specimens. *J Microbiol Methods* 2006;67:220-9.
 25. Randall S. Edson, Christine L, Terrell W, Mark Brutinel, Nancy L Wengenack. *Mycobacterium intermedium* Granulomatous Dermatitis from Hot Tub Exposure. *Emerg Infect Dis* 2006;12(5):821-23.