

Evaluating the utility of menstrual blood versus endometrial biopsy as a clinical sample in the diagnosis of female genital tuberculosis

Bineeta Kashyap*¹, Tripta Kaur¹, Rajat Jhamb², Iqbal R Kaur¹

¹Departments of Microbiology¹ & Medicine², University College of Medical Sciences (UCMS) & Guru Teg Bahadur Hospital (GTBH), Delhi, India.

Abstract

Objective: Nonspecific clinical presentation, inefficacy of laboratory diagnostic tests, and inaccessibility of reproductive clinics have resulted in underreporting of female genital tuberculosis which is an important cause of female infertility, especially in developing country like India. The objective of the present study was to compare the utility of menstrual blood with endometrial biopsy as a sample for the bacteriological diagnosis female genital tuberculosis.

Methods: All endometrial biopsy and menstrual blood specimens received in the Mycobacteriology Lab from clinically suspected cases of genital tuberculosis were screened for acid fast bacilli by Ziehl Neelsen staining and were subjected to culture for Mycobacterium tuberculosis on Lowenstein Jensen medium.

Results: The incidence of genital TB either by microscopy or culture in menstrual blood samples and endometrial biopsy samples in our study was found to be 5/125 (4 %) and 24/1226 (~2%). The correlation between microscopy and culture was found to be 98.04 % and 85.71 % for endometrial biopsy and menstrual blood samples respectively.

Conclusion: More elaborate studies on larger samples size should be taken up to assess the utility of menstrual blood as a potential alternative clinical sample for diagnosis of genital TB.

Key Words: Mycobacterium tuberculosis, Ziehl Neelsen staining, diagnosis, tuberculosis.

INTRODUCTION

Despite decades of research and availability of a number of diagnostic tests and therapeutic regimens tuberculosis (TB) is an increasing public health concern worldwide and more so in developing countries.^[1] TB predominately presents with pulmonary disease, although extra-pulmonary TB is not uncommon; the diagnosis of which is often difficult because of its non-specific clinical and laboratory findings. The most common form of extrapulmonary TB is genitourinary disease, accounting for 27% (range, 14 to 41%) worldwide. The global prevalence of genital tuberculosis (GTB) is estimated to be 8-10 millions cases, with a rising incidence in the developing counties partly as a result of its association with HIV virus infection and emergence of multidrug resistance.^[2] Genital TB in females is by no means uncommon, particularly in communities where pulmonary or other forms of extragenital TB are common. TB is a major socioeconomic burden in India, afflicting mostly the reproductive age group (15–45 years), involved in about 5–16% of cases of infertility among Indian women.^[3,4]

Address for correspondence*

Bineeta Kashyap

Department of Microbiology, University College of Medical Sciences (UCMS) & Guru Teg Bahadur Hospital (GTBH), Delhi, India.

Email :dr_bineetakashyap@yahoo.co.in

though the actual incidence may be under-reported due to asymptomatic presentation of GTB and paucity of investigations. A high degree of suspicion aided by intensive investigations is important in the diagnosis of the disease and routine laboratory values are of little value.

In view of the problems of making a definitive diagnosis of genital TB in females, many physicians tend to adopt the therapeutic test for elimination of any type of TB including the genital type by prompt execution of anti tubercular treatment (ATT) for the requisite period of time. Continuing search is needed for finding simpler and practicable methods for making definitive diagnosis in respect of female genital TB and the use of therapeutic test should be avoided. Moreover since GTB is a paucibacillary disease, and direct mycobacterial demonstration on smear examination and culture is generally negative, hence the diagnosis of GTB for early institution of treatment remains a clinical challenge. Histological detection of typical granulomata is sufficient for diagnosis of pelvic TB, providing that all other causes of granulomatous reactions have been ruled out. Demonstration of mycobacteria on culture is the gold standard for diagnosis of TB, but the rates of positive culture of different specimens from the genital tract are dismally low, even in cases strongly documented on other criteria^[5]. Most experts recommend some form of endometrial sampling for histologic and microbiologic

examination to make the diagnosis of genital TB. Because the endometrium is involved in the majority of cases and is readily accessible to sampling, it is often the first site at which attempts at definitive diagnosis are directed, although its sensitivity suffers from sampling errors. The optimal time for sampling is at the end of the menstrual cycle or within 12 hours after the onset of menstrual flow to allow the endometrial granulomata maximal time to develop and one negative biopsy or curettage does not exclude the diagnosis of genital TB. Previous studies have stated that serial sections needed to be studied because the lesions are frequently patchy and that a positive endometrial culture for TB was found only in about 25% of cases of tuberculous endometritis as the granulomas are often focal and the functionalis layer is shed every four weeks (granulomas take two weeks to develop).^[6] The use of menstrual blood, when available, for bacteriologic or molecular diagnosis has been recommended by some,^[7] while others have reported lower sensitivity of these tests.^[8] The objective of the present study was to compare the utility of menstrual blood with endometrial biopsy as a sample for the bacteriological diagnosis female genital tuberculosis.

MATERIALS AND METHODS

A retrospective study was conducted from January 2011 to December 2011 in the Department of Microbiology, University College of Medical Sciences (U.C.M.S.) & Guru Teg Bahadur (G.T.B.) Hospital, a tertiary care hospital in East Delhi.

All endometrial biopsy and menstrual blood specimens received in the Mycobacteriology Lab from clinically suspected cases of genital tuberculosis during the study period were screened for acid fast bacilli by Zeehl Neelsen (Z-N) staining and were subjected to culture for Mycobacterium tuberculosis on Lowenstein Jensen (LJ) medium. ZN staining was done by the conventional method. The homogenized samples were cultured on LJ medium and were incubated for 3 to 8 weeks. ZN staining was used to identify the acid fast bacilli.

RESULTS

During the study period a total of 125 and 1226 menstrual blood and endometrial biopsy samples were received in the Mycobacteriology laboratory from clinically suspected cases of genital TB; the monthly distribution of which is depicted in Figure 1.

Out of the total 125 menstrual blood samples only 4 (3.2 %) were positive for acid fast bacilli on ZN

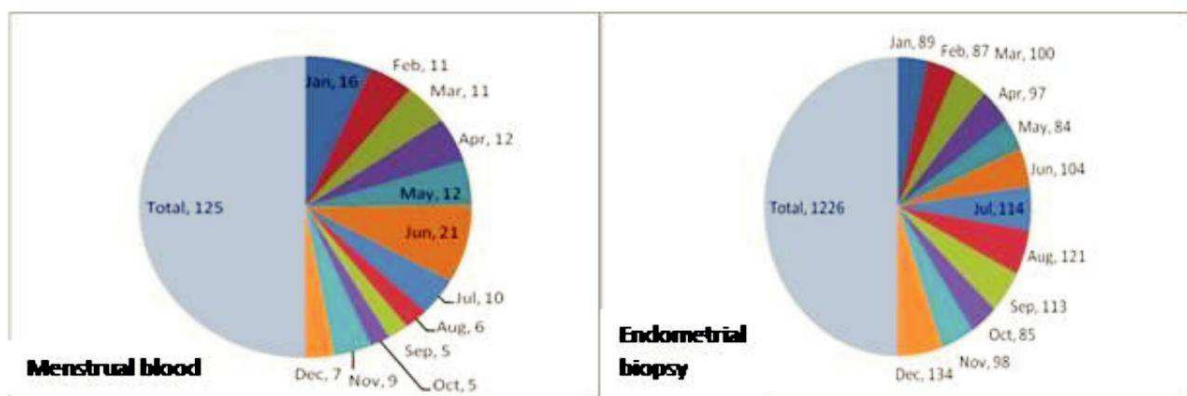


Figure 1: Frequency of menstrual blood and endometrial biopsy samples received during the study period (Jan to Dec 2011)

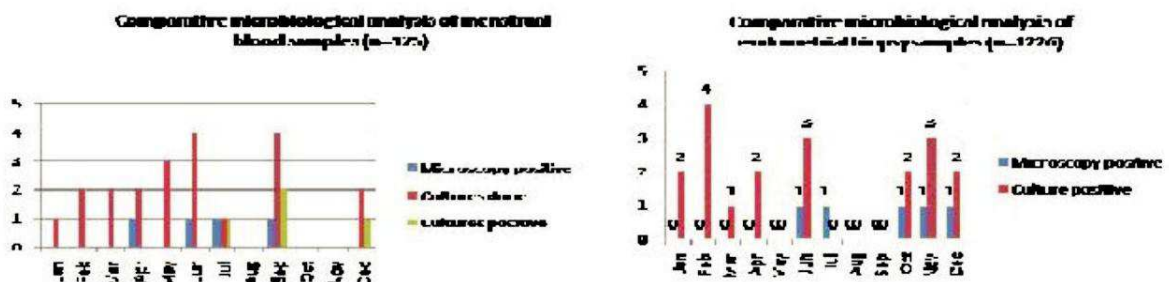


Figure 2: Comparative microbiological analysis of menstrual blood and endometrial biopsy samples

Table 1: Correlation between smear microscopy and culture for endometrial biopsy and menstrual blood samples for diagnosis of genital tuberculosis

Type of sample		Smear microscopy positive	Smear microscopy negative	Percentage correlation
Endometrial Biopsy n=1226	Culture positive	0	19	98.04 %
	Culture negative	5	1202	
Menstrual Blood n=21*	Culture positive	2	1	85.71 %
	Culture negative	2	16	

staining. As per the protocol followed in our laboratory only 21 out of the total 125 menstrual blood samples were subjected to culture depending upon the requisition sent by the clinician out of which 4 (19.05 %) were positive for culture. As regards the endometrial biopsy samples all the 1226 samples received during the study period were subjected to microscopy and culture following the protocol of our laboratory. Out of these total 1226 samples; whereas only 5 samples (0.41 %) were positive for acid fast bacilli on microscopy, 19 samples (1.55 %) for positive for Mycobacterium tuberculosis on culture. (Figure 2)

The correlation between microscopy and culture for Mycobacterium tuberculosis in genital TB is depicted in [Table 1].

DISCUSSION

Although in advanced countries the incidence of tuberculosis has shown a downward trend, unfortunately due to co-infection with HIV, tuberculosis still claims many victims in all age groups throughout the world. The discovery of genital TB, with few symptoms in its mild or moderate phase, is usually made unexpectedly, most often via an endometrial biopsy in the course of investigative studies into infertility. This can render a large percentage of women in the reproductive age group incapable of conception or of achieving a successful pregnancy unless detected and treated adequately in its earliest phases.

Since the involvement of the genital tract in TB could be generalized or localized, and the technique of sample collection is blind, there is a possibility of missing the infected area. The technique of sampling and the kind to sample collected plays an important role in

the accuracy of detection of GTB. Histological findings of caseation in endometrial TB is reported to be rare in women of reproductive age group as it depends on the cycle stage when the biopsy was taken and also on the site in the uterus in which it occurs.^[9] The disease process has to regenerate after menstrual shedding from the basal layer of endometrium with start of each menstrual cycle and the granulomas become well developed and numerous as the cycle progresses. So, biopsy is recommended immediately before the menstrual phase as the granulomas get longest possible time to develop and greater chance of providing accurate diagnosis. False-negative histology can occur, especially if samples are inadequate or non-representative. In a study by Reys and Maheshwari involving 500 endometrial biopsies no characteristic endometrial pattern was found to be specific for a tuberculous lesion.^[10] The present study assesses and compares the utility of menstrual blood and endometrial biopsy samples for diagnosis of Mycobacterium tuberculosis in clinically suspected cases of genital TB and attempts to reemphasize on the need of a validated diagnostic algorithm for genital TB.

The incidence of genital TB either by microscopy or culture in menstrual blood samples and endometrial biopsy samples in our study was found to be 5/125 (4 %) and 24/1226 (~2%) respectively which compares fairly well with other studies from this geographical region.^[11] However actual frequency of female genital tuberculosis is unknown despite different published data from various countries as it is usually an indolent infection and takes years to manifest clinically after initial seeding and is often discovered incidentally or remain 'undetected' in symptomless patients.

ZN staining of acid fast bacilli requires a large number of

bacteria, at least more than 10^5 /ml, to be present in the specimen.^[12] Thus in practical terms, it is a presumptive test with low sensitivity. This is also evident in our study by the fact that smear positivity was found in only 3.2 and 0.41 % of menstrual blood and endometrial biopsy samples respectively when compared to 19.05 and 1.55

% of culture positivity in the same samples. On the other hand bacterial culture being more sensitive (requiring ~ 100 microorganisms/milliliter) has 100% specificity but a major problem is that, in addition to high cost, weeks and months are required for precise identification of the species. Recent Indian study showing comparison of the percentages of sensitivity for detection of mycobacteria showed that the PCR assay has the highest sensitivity (31.3%) followed by detection by microscopy and isolation of the pathogens by the culture technique with sensitivities of 5.1% and 4.2% respectively with the least sensitive (2.4%) technique being histopathological examination for granulomatous tissue reactions compatible with tuberculosis infection.^[13] The new automated culture techniques have appreciably reduced the time for detection and antimicrobial testing.^[12]

However, most of these techniques are too expensive and sophisticated to be of any practical benefit to the vast majority of TB patients living in developing countries like India for whom an early and inexpensive diagnosis remains as elusive as ever and a more judicious use of the conventional diagnostic techniques is the only available alternative.

The gold standard remains the proof of acid-fast bacilli in biological specimens or culture. In patients presenting with sub-fertility and/or abnormal bleeding, a culture of menstrual fluid may be the most useful strategy^[14] Obtaining menstrual fluid for culture need not be a difficult procedure. The patient is invited to attend the out-patient clinic during the second day of her normal menstruation, when she will be put in the lithotomy position and a sterile speculum is passed. About 10 to 20 ml of normal saline is instilled into the vagina with a sterile syringe and the normal saline is mixed with the menstrual blood. It is then aspirated and sent for culture. This approach has a good yield for positive cultures as is evident in our study also with a high culture positivity of 19.05 % when compared to only 1.55% positivity by culture on endometrial biopsy samples. Similar lower detection rates of *M. tuberculosis* by culture have been reported earlier, the possible reasons for which could be due to paucibacillary nature of the infection, presence of bacteriostatic substance or the fact that a substantial number of TB lesions of the genital tract are bacteriologically mute.^[15-17]

The importance of Extra Pulmonary TB among all forms of tuberculosis has not yet been ascertained in developing countries due to the difficulty of the clinical and laboratory diagnosis by culture confirmation.

Nonspecific clinical presentation, inefficacy of laboratory diagnostic tests, and inaccessibility of reproductive clinics have resulted in underreporting of female GTB which is an important cause of female infertility, especially in developing country like India. The possibilities of genital TB should always be considered in the evaluation of every infertile patient, especially with increasing number of TB cases in our environment. Taking into consideration the low detection rates for genital TB on endometrial biopsy samples, more elaborate studies on larger samples size should be taken up to assess the utility of menstrual blood as a potential alternative clinical sample for diagnosis of genital TB.

REFERENCES

1. Raviglione MC, O'Brien RJ. Tuberculosis. In: Fauci AS, Braunwal E, Isselbacher KJ, Wilson JD, Martin JB, Kasper DL, et al, editors. Harrison's principles of internal medicine 17th ed. New York: Mc Graw - Hill; 2008. p. 1006-36.
2. Marjorie PG, Holenarasipur RV. Extrapulmonary tuberculosis: An overview. *Am Fam Physician*. 2005;72:1761-8.
3. Parikh FR, Naik N, Nadkarni SG, Soonawala SB, Kamat SA, Parikh RM. Genital tuberculosis – a major pelvic factor causing infertility in Indian women. *Fertil Steril* 1997; 67: 497-500.
4. Roy A, Mukherjee S, Bhattacharya S, Adhya S, Chakraborty P. Tuberculous endometritis in hills of Darjeeling : a clinico pathological and bacteriological study. *Indian J Pathol Microbiol* 1993; 36, 361-369.
5. Baum SE, Dooley DP, Wright J, Kost ER, Storey DF. Diagnosis of culture-negative female genital tract tuberculosis with involvement of polymerase chain reaction. *J Reprod Med* 2001; 46: 929-932.
6. Falk V, Ludviksson K, Agren G. Genital tuberculosis in women: Analysis of 187 newly diagnosed cases from 47 Swedish hospitals during the ten-year period 1968 to 1977. *Am J Obstet Gynecol* 1980; 138:933.
7. Abebe M, Lakew M, Kidane D, Lakew Z, Kiros K, Harboe M. Female genital tuberculosis in Ethiopia. *Int J Gynaecol Obstet* 2004; 84:241-246.
8. Simon HB, Weinstein AJ, Pasternak MS, et al. Genitourinary tuberculosis: Clinical features in a general hospital population. *Am J Med* 1977;63:410.
9. Roy A, Mukherjee S, Bhattacharya S, Adhya S, Chakraborty P. Tuberculous endometritis in hills of Darjeeling : a clinico pathological and bacteriological study. *Indian J Pathol Microbiol*

-
- 1993; 36:361-369.
10. Reys H, Maheshwari HB. Tuberculosis of the endometrium: a histopathology of 500 biopsy cases. *Indian J Tubercul* 1971; 18: 27-33.
11. Jindal UN. An algorithmic approach to female genital tuberculosis causing infertility. *Int J Tuberc Lung Dis* 2006; 10: 1045-50.
12. Butt T, Ahmad RN, Kazmi SU, Raja KA, Mahmood A. An Update on the diagnosis of Tuberculosis. *J Coll Physicians Surg Pak* 2003; 13:728-34.
13. Kumar P, Shah NP, Singhal A, Chauhan DS, Katoch VM, Mittal S, Kumar S, Singh M K, Datta Gupta S, Prasad HK. Association of Tuberculous Endometritis with Infertility and Other Gynecological Complaints of Women . *India. J Clin Microbiol* 2008; 46: 4068-4070.
14. De Vynck WE, Kruger TF, Joubert JJ, et al. Genital tuberculosis associated with female infertility in the western Cape. *S Afr Med J* 1990; 77:630–1.
15. Bhanu NV, Singh UB, Chakraborty M, Suresh N, Arora J, Rana T, et al. Improved diagnostic value of PCR in the diagnosis if female genital tuberculosis leading to infertility. *J Med Microbiol* 2005; 54: 927-31.
16. Soltys MA. An anti-tuberculous substance in tuberculous organs. *J Comp Pathol* 1953; 63: 147-52.
17. Srivastava N, Manaktala U, Baveja CP. Role of ELISA (enzyme-linked immunosorbent assay) in genital tuberculosis. *Int J Gynaecol Obstet* 1997; 57: 205-6.
-