

ORIGINAL ARTICLE

Comparison of Effectiveness of Menstrual Blood Sample and Endometrial Biopsy Sample for TB-PCR Test for Detection of Tuberculous Endometritis

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Abstract

Background: Genital tuberculosis is most common cause of infertility in females. The actual incidence of the genital tuberculosis is not correctly known due to asymptomatic presentation and paucity of investigation. With this objective we tried to investigate effectiveness of menstrual blood and endometrial sample for TB PCR in diagnosis of endometrial tuberculosis in infertile patients **Methodology:** In 50 patients presented with infertility in whom the inclusion criteria are met, menstrual blood and endometrial aspiration samples were collected on day 1 and day 21 or 22 respectively in normal saline and sent for Real time TB PCR in the Molecular Biology Department of the Yashoda Super Specialty Hospital Hyderabad. The results were noted and ATT was started in cases with positive results for TB PCR with either of the samples or both. **Results:** The diagnosis of endometrial tuberculosis was confirmed in 12 of 50 (24%) enrolled cases by positive TB PCR (either menstrual blood sample or endometrial biopsy sample or both). Of the 12 positive cases, 10 cases (83.33%) were positive for samples, one case positive for menstrual blood and other case positive for endometrial sample (8.33% each). The sensitivity and specificity of the TB PCR for menstrual blood is 91.67% and 97.33% respectively. These 12 patients were given ATT for 6 months and monitored on monthly basis. 5 of 12 patients (41.66%) conceived. Out of which, 3 are intrauterine viable pregnancies, one is a missed abortion and another one is an ectopic. **Conclusion:** Genital tuberculosis is not uncommonly encountered in our community. The molecular methods should be employed for its rapid diagnosis and menstrual blood is as effective as endometrial sample for the diagnosis of tuberculous endometritis. Treatment with ATT is to be considered before switching to expensive methods like IVF-ET.

Keywords: Tuberculosis, Endometritis, Infertility

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Introduction

Tuberculosis is still an important health problem worldwide. India is a country with one of the highest burden of tuberculosis accounting for one-fifth of global incidence annually. In 2011, out of the estimated global annual incidence of 9 million Tuberculosis cases, 2.3 million were estimated to have occurred in India; prevalence of Tuberculosis is 3.1 million and mortality is 0.32%.^[1] According to WHO report in 2013, India accounted for about 26% of total cases in

2012, where the total estimated cases were 8.6 million.^[2] TB remains a major health problem in many developing countries.^[3] In these areas genital TB is responsible for a significant proportion of females presenting with infertility.^[4] Genital TB is the most common cause of tubal infertility in females (85-90%). Endometrium is involved in 60% cases, ovaries (50%), cervix (5%), vagina and vulva (<1%).^[5] The cause of infertility is mainly tubal block, adhesions in the endometrial cavity and ovulatory dysfunction.^[6] Prevalence of

infertility in general population is around 15-20%. Female factor is responsible for 40-55%. Of this group of infertile patients, the Incidence of Tuberculosis is 5-16% among patients with infertility, though the actual incidence may be under-reported due to asymptomatic presentation of genital tuberculosis and paucity of investigations. Genital tuberculosis accounts for about 15-20% of extra pulmonary tuberculosis and it is the second most common site infected after lungs. [7] Genital tuberculosis is almost always secondary to pulmonary tuberculosis, the genital tract is vulnerable to the disease after puberty and most cases occur during the child bearing period, i.e., 80-90% cases are diagnosed in the patients who are 20 to 40 years old, often during work up for sub fertility. [8] The actual incidence of genital Tuberculosis cannot be determined accurately in any population because it is estimated that at least 11% of the patients are asymptomatic and the disease is discovered incidentally. [9] Incidence varies greatly according to socioeconomic and public health conditions; it usually parallels the incidence of pulmonary and abdominal TB. With this background we tried to determine the presence of Tuberculous Endometritis in Infertile females.

Materials and Methods

The prospective study consists of 50 women attending the infertility clinic at Yashoda Super speciality Hospital, in the Department of Obstetrics and Gynecology, in whom the inclusion criteria are met and other causes of infertility are ruled out. Clearance was obtained from the scientific committee and the ethics committee of our hospital. The study protocol was explained to the patient in detail and informed, written consent was obtained from them.

Inclusion Criteria

- Age group : 20 to 45 years (married women)
- Previous history of Tuberculosis / Extrapulmonary Tuberculosis
- Positive non-specific / specific tests
- Menstrual disorders (menorrhagia / oligomenorrhoea)
- Secondary amenorrhoea
- Regular / Irregular periods

Exclusion Criteria

- Confirmation of other disease (hormonal / malignancy / acute PID)
- Other causes of infertility

Detailed history of the woman was taken in the pre-designed case proforma and clinical examination done. In these females, previous records of investigations, treatment, coital frequency, occupation, history of recurrent vaginitis, previous history of tuberculosis (pulmonary and extrapulmonary) menstrual pattern, history of dysmenorrhoea and dyspareunia General physical examination was done. Per speculum examination was done to see for cervical lesions suggestive of genital tuberculosis. Per vaginal examination was done to see for any tenderness and palpable masses in the adnexae. Patients were called on the day 1 of the menses (especially within 12 hours of onset of the menstruation). 2-3 ml menstrual blood was collected with the I.U.I cannula in normal saline in sterile tube, under aseptic precautions. The samples were sent to the molecular biology department of our hospital for RT-PCR for mycobacterium test. The results obtained within 2 days and they were noted. The patients on whom menstrual blood samples were taken were called on day 21 or day 22 of the same cycle for endometrial aspiration by pipelle, which was done in the out-patient department. Endometrial biopsy under general anesthesia was undertaken for the patients who were affordable for the procedure. 2-3 ml of the endometrial curettings was collected in the sample manner as mentioned above and was sent to the lab. RT-PCR results were noted. Routine investigations like complete blood picture, blood group, random blood sugar and HIV status were done.

Quantitative Analysis of TB-DNA

A 3ml of menstrual blood and endometrial curetting sample was collected from all the participants and stored at -20C until they were used for DNA isolation. The qualitative detection of Mycobacterium tuberculosis (TB-PCR) was achieved using standard protocol includes, DNA isolation and quantitative analysis of TB-DNA using Real-Time PCR.

Results

A total of 50 infertile women were enrolled in the present study. In these 50 women studied minimum age was 18 years and maximum 41 years with a mean of 29 years and SD 5.04 years. Maximum females were below 35 years. Minimum infertility period in these women was of 1 year in 4 (8 %) cases and maximum 17 years in 1 (2%) case. There were a very high proportion of cases 41 (82%) up to 10 years, amongst these 27 cases (54%) were with 1-5 years of infertility. While only two cases (4%) were with history of infertility period between 16 to 20 years. The diagnosis of endometrial tuberculosis was made in 12 of the 50 cases

(24%) by TB PCR (either menstrual blood or endometrial currettings). Of the 12 TB PCR positive cases, 10 cases (83.33%) were positive by both menstrual blood and endometrial biopsy samples, only one (8.33%) case showed negative for menstrual blood and positive result for endometrial sample; and one case (8.33%) was positive for menstrual blood and negative for endometrial sample. This show the sensitivity and specificity of the TB PCR in menstrual blood is 91.67% and 97.37% respectively. The negative predictive value and the positive predictive values are 97.37% and 91.67% respectively.

Picture 1: TB PCR results and interpretation

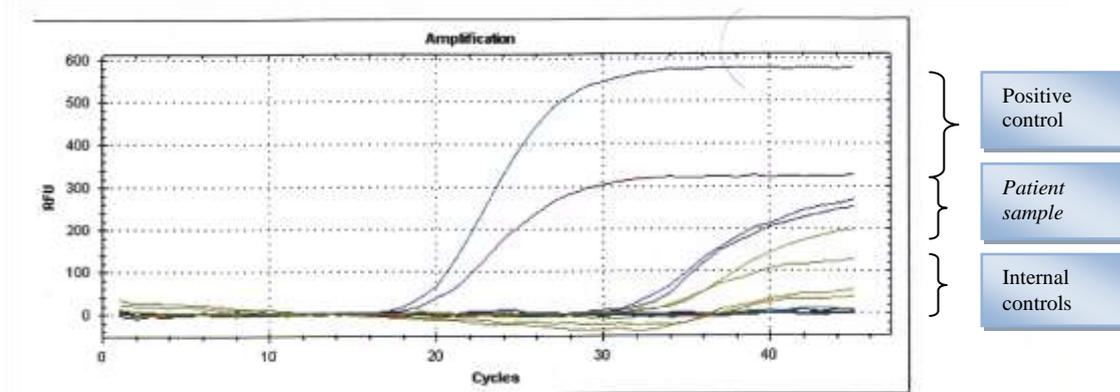


Table 1: Age group and TB-PCR in Endometrial Sample

		TB PCR – EB		Total
		Negative	Positive	
<=28	Count	16	7	23
	% within age	69.6%	30.4%	100.0%
>=29	Count	22	5	27
	% within age	81.5%	18.5%	100.0%
Total	Count	38	12	50
	% within age	76.0%	24.0%	100.0%

This is a positive result of TB PCR in which there is amplification of TB DNA in the patient sample. In the above sample, there is amplification of the internal controls, patient samples and also the positive controls, which shows it is a true positive result.

Table 2: Age group and TB PCR in Menstrual Blood Sample

		TB PCR – MB		Total
		Negative	Positive	
<=28	Count	15	8	23
	% within age	65.2%	34.8%	100.0%
>=29	Count	23	4	27
	% within age	85.2%	14.8%	100.0%
Total	Count	38	12	50
	% within age	76.0%	24.0%	100.0%

According to chi-square statistics, age group and TB PCR test has been compared. Age is divided into two groups as less than 29 and more than or equal to 29, as the mean of the age presented to us with infertility is 29 years. On endometrial biopsy sample 30.4% of the cases are positive for TB PCR are of age group less than 29 years, while 18.5% cases are of age group above 29 years (square =0.967, $p = 0.325$) and is not statistically significant. For the menstrual blood sample, these values are 34.8% and 14.8%, for the age group less than and more than 28 years respectively (square =2.715, $p= 0.99$). In positive cases for endometrial samples, the primary infertility accounts for 27.8% and secondary infertility in 14.3% with square of 1.006 and p value of 0.316. The values are similar for menstrual blood sample too.

Discussion

The reported prevalence of genital tuberculosis varies widely world over. This due to the differences in the population group studied, social stigma, sensitivity and specificity of the tests used for its diagnosis and the timing of the sample with respect to the menstrual cycle. Endometrial tuberculosis often goes undiagnosed because it is asymptomatic in majority cases and requires high index of suspicion.^[10] The typical presentation of genital TB includes infertility (58-60%). Therefore genital TB should always be considered as a probable cause in diagnostic work up of infertile couple especially in population with high prevalence of tuberculosis as in India. As PCR which is the method of DNA amplification, is proved to be a rapid and sensitive method, useful diagnostic test in early disease and requires less than 10 bacilli /ml for diagnosis. When compared to culture, smear and histology, which have low detection rates and delayed reporting, PCR is highly reliable, especially in the endemic areas.

In our study, diagnosis of endometrial TB was made in 24% of the cases by TB PCR test, which was positive either in menstrual blood or endometrial sample or both. These results are comparable to the study conducted by Bharathi et al^[11] where they diagnosed 24.61% cases of genital TB by TB PCR (Real time) in endometrial biopsy sample. In their study 11.07% of menstrual blood samples were found to be PCR positive and they suggested for

further studies for comparing both the samples. Khanna et al, diagnosed 26 out of 100 infertility patients with genital TB with positive TB PCR results.^[12] In our study, the average age of women presenting with infertility is [29 + 5.04 years]. The mean age among the TB PCR positive cases was 24.59 years. Though tuberculosis is prevalent in all age groups, our maximum patients were in the age group of 20-25 years as reported by Nagpal et al^[13] 54% of TB PCR positive cases had infertility duration of 1 to 5 years and the results are similar to those of Patil et al (106). Majority of the patients had primary infertility (72% cases) and this is almost same when compared to other recent studies.^[14, 15] It is said that approximately 85% of the patients with genital TB have never conceived in the remaining 15%, symptoms of genital TB develop in one third to one half within 1 year after their last pregnancy was completed. Past history and contact history of tuberculosis is present in 13.3% of the cases^[15] and other study showed no such history in the past.^[16] In the present study it is 33.33% and 25% respectively. Out of the 4 cases with previous history of tuberculosis, 3 cases had history of pulmonary tuberculosis and one case had history of tubercular peritonitis for which she had taken ATT 10 years back. The low incidence of past history pulmonary tuberculosis may be related to gastrointestinal sources of the infective organisms, illiteracy and delay in seeking medical advice. PCR is proved in many studies in literature as a rapid diagnostic test for genital TB with high sensitivity, specificity and clinical correlation of TB PCR with genital TB.^[17] The sensitivity and specificity of PCR in genital TB is 96.4% and 100%. The sensitivity is 100% when PCR is combined with culture of mycobacteria.^[18] Taking this into consideration we have compared TB PCR test of menstrual blood sample with endometrial biopsy sample. The most widely used primers to detect *M.tuberculosis* in clinical specimens by PCR are from the insertion element IS6110. We have used the same in our study.

In the total of 12 positive TB PCR cases, 10 cases showed positive for both the samples and rest two cases are positive for each of the sample. We have also compared the age distribution pattern, years of infertility, type of infertility and menstrual abnormalities with TB

PCR results, using chi-square test, p value is < 0.05 in every case, which showed it is statistically not significant. Genital TB necessitates early diagnosis with timely and complete short course ATT before IVF-ET is initiated to optimize the chance of achieving successful pregnancy. According to a recent study ^[19] hysteroscopic visualization of the endometrium after 6 months of ATT showed an improvement in the mucosal morphology. Other study ^[20] a total of 70 women with genital TB, who were treated with ATT for 6 months underwent second look hysteroscopy, showed mean endometrial thickness on day 21 was improved from 6.8mm to 8.1mm after treatment. There are no adverse reactions with ATT except for one patient who developed fever with vomiting, for which symptomatic treatment was given and two patients had asymptomatic elevation of liver enzymes.

Conclusion

It was concluded in this study that genital TB is not uncommonly encountered during gynaecological practice and should consider in the diagnosis more frequently than is in the current scenario. Evaluation of every infertile patient for genital TB in the endemic areas is important. The possibility of this disease should be considered in an infertile patient for whom no etiology can be discovered. Molecular diagnosis of genital tuberculosis by real-time PCR has a great potential to improve the clinician's ability to initiate proper treatment which could cure infertility leading to successful pregnancies and positive change in the psychology of the patient. Menstrual blood sample s as effective as the endometrial biopsy sample in diagnosing endometrial tuberculosis by TB PCR technique and it is cost effective method, non invasive, not painful and simple outpatient based procedure.

Conflict of Interest: None declared

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Ethical Permission: Obtained

References

1. Revised National Tuberculosis Program 2013. Annual status report. [Online]. 2013; Available from www.tbcindia.org. Accessed on 20/6/2016.
2. World Health Organization. Global tuberculosis report 2013. Available from www.apps.who.int. Accessed on 30/6/2016.
3. 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: McGraw - Hill 2008;1006-36
4. Muir DG; Belsey MA. Pelvic inflammatory disease and its consequences in the developing world. Am J ObstetGynecol 1980; 1(138):913-28.
5. Arora VK, Gupta R, Arora R. Female genital tuberculosis: Need for more research. Ind J Tub 2003; 50(9): 36-38.
6. Roy H, Roy S. Use of polymerase chain reaction for the diagnosis of endometrial tuberculosis in high risk infertile women in an epidemic zone. J ObstetGynaecol India 2003;53:260-3
7. Tripathy SN, Tripathy SN: Endometrial tuberculosis. J Indian Med Assoc. 1987; 85:136-9.
8. Schaefer G. Female genital tuberculosis. ClinObstetGynecol 1976;19: 223-39.
9. Goldin AG, Baker WT. Tuberculosis of the female genital tract. J Ky Med Assoc 1985; 83:75.
10. Baxi A, Neema H, Kaushal M, Sahu P. Genital tuberculosis in infertile women : Assessment of endotrial TB PCR results with laparoscopic and hysteroscopic features. Ind j ObstetGynecol 2011;61(3):301-306.
11. Bharti M, Parul S, Saroj H, Leela V. Rapid diagnosis of genital tuberculosis by Real-time polymerase chain reaction. J South Asia Fed ObstetGynecol 2012;4(1):39-42.
12. Khanna A, Agarwal A. Markers of genital tuberculosis in infertility. Singapore Med J 2011;52(12):864-67.
13. Sankar MM, Kumar P, Munawar A, Kumar M, Singh J, Singh A, Parashar D. Usefulness of Multiplex PCR in diagnosis of genital tuberculosis in females infertility. Eur J Clin Microbial infect Dis. 2013;32(3):399-405
14. Sharma JB, Pushparaj M, Roy KK, Neyaz Z, Gupta N, Jain SK, Mittal S. Hysterosalpingographic findings in infertile women with genital tuberculosis. Int J gynecolObstet 2008; 101:150-55.
15. Sughra S. Investigation of the prevalence of female genital tract tuberculosis and its relation to female infertility An observational analytical study. Iran J Reprod Med. 2012; 10:581-8.
16. Gurgan T, Zeyneloglu H, Urman B, Develioglu O, Yarali H. Pelvic peritoneal tuberculosis with elevated serum and peritoneal fluid CA-125 levels: A report of two cases. GynecolObstet Invest 1993; 35:60-61.
17. Bhanu NV, Singh BU, Chakraborty M, Naga S, Arora J, Rana T. Improved diagnostic value of PCR in the diagnosis of female genital tuberculosis leading to infertility. J Medical Microbiology. 2005; 927-31.
18. Thangappah RB, Paramasivan CN, Narayanan S. Evaluating PCR, culture and histopathology in the diagnosis of female genital tuberculosis. Ind J Med Res 2011; 134:40-46.
19. Atul K, Alka K. Relook hysteroscopy after anti-tubercular therapy. Ind J ObstetGynecol 2007;07:1346.
20. Bahadur A, Malhotra N, S Mittal, S Gurunath. Second look hysteroscopy after anti tuberculous therapy in infertile women with genital tuberculosis undergoing IVF. 2009;08:31.