Comparison of Two Sample Collection Techniques for Adequacy and Accuracy in Cases of Genital Tuberculosis

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ABSTRACT

Tuberculosis (TB), caused by bacteria of the *Mycobacterium tuberculosis* complex, is one of the oldest diseases known to affect humans and is a major cause of death worldwide. The diagnosis of genital TB is difficult due to lack of reliable confirmatory investigation. Here, we have compared two techniques of sample collection for detection by polymerase chain reaction (PCR). **Material and methods:** We studied 60 cases attending the Gynecology OPD for symptoms suggestive of genital TB. Both endometrial aspirate and biopsy were taken in all cases. The samples were analyzed by PCR and the results were compared. **Results:** Endometrial aspirate had a detection rate of 41% as compared to endometrial biopsy, which had a detection rate of 36.7%. The difference was statistically significant. **Conclusion:** Endometrial aspirate had a better detection rate than endometrial biopsy.

Keywords: Genital tuberculosis, endometrial aspirate, endometrial biopsy, PCR

Tuberculosis (TB), caused by bacteria of the *Mycobacterium tuberculosis* complex, is one of the oldest diseases known to affect humans and is a major cause of death worldwide. The disease most often affects the lungs, although other organs are involved in up to one-third of cases. The morbidity associated with this condition has major health implications. The disease has a worldwide distribution and the incidence is high in developing countries.

Female genital TB is an important cause of significant morbidity with short- and long-term sequelae especially infertility in affected women. The precise incidence of genital TB is difficult to ascertain as it is underreported due to asymptomatic cases and lack of reliable confirmatory investigation. Genital TB is responsible for 1% of all gynecological admissions in India. The frequency of occurrence of genital TB is fallopian tubes (90-100%), endometrium (50-60%), ovaries (20-30%), cervix & vulva and vagina (1%).[1]

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In recent years, polymerase chain reaction (PCR) technique has evolved as a useful and rapid, sensitive and specific molecular biological technique for the diagnosis of pulmonary and extrapulmonary TB. PCR assay target various gene segments including a 65 kD protein encoding gene. TB is diagnosed within 1-2 days with sensitivity and specificity reaching up to 100%.[2]

There are many methods of sample collection out of which endometrial aspirate and endometrial biopsy are mostly used for detection of genital TB. We have carried out this study with an aim to identify the better way of sample collection.

MATERIAL AND METHODS

A total of 60 cases were selected from OPD of Obstetrics and Gynecology, SN Medical College, Agra, Uttar Pradesh. Before conducting the study, the consent of Institutional Ethical Committee was taken. It was a prospective comparative study. Sixty female cases of 20-50 years of age were selected from the OPD in whom genital TB was suspected.

Inclusion criteria were unexplained infertility, infertility cases with tubal pathology, pelvic inflammatory disease (PID) not responding to routine antibiotic treatment, unexplained menorrhagia, unexplained secondary amenorrhea, chronic pelvic pain and oligomenorrhea. Exclusion criteria were pregnant females, suspicion of malignancy and unmarried females. All the 60 cases

enrolled in the study were subjected to a thorough history taking, general, systemic and pelvic examination. The study was evaluated by PCR where samples were taken by endometrial aspiration and endometrial biopsy in all 60 cases. All the women with clinically suspected genital TB were called preferably premenstrual for endometrial biopsy and endometrial aspiration. Endometrial biopsy and endometrial aspiration were done in the same sitting in the OPD. In case woman was amenorrheic or irregular bleeding was present then the procedure was done at the time of presentation. In case of cervical stenosis, where procedure was not possible in OPD then it was undertaken under short general anesthesia. The specimens, extracted by biopsy and aspiration were sent for PCR.

RESULTS

Genital TB is more common in the reproductive age group. Table 1 shows the distribution of cases according to age, residence, parity and socioeconomic status of the cases. The mean age in this study was 28 ± 6.3 years. Most of the cases, 65% in the study came from rural areas and only 35% belonged to an urban area. Clinical features suggestive of genital TB were more common in nulliparous and primiparous cases, which constituted 38.3% (23/60) and 30% (18/60) of the cases. Distribution of cases according to socioeconomic status in the table well-illustrated genital TB to be the disease of the poor with Class III, IV and V forming 31.7%, 30% and 28.3% cases, respectively.

Table 2 shows that infertility was the most common gynecological symptom in genital TB, out of which primary infertility was 33.3% and secondary infertility was 26.7%. Other complaints were menorrhagia (13.3%), oligomenorrhea (10%), secondary amenorrhea (3.3%), postmenopausal bleeding (3.3%), pelvic pain (33.3%) and vaginal discharge (26.7%). Some women presented with two or more complaints simultaneously.

Table 3 shows the difficulties encountered in sample collection in the two techniques. Endometrial aspiration and biopsy are routine outdoor procedures; 50% of the aspirations and 35% of biopsies were relatively easy. Difficulties were encountered in some cases during sample collection, which were more during biopsy than aspiration. As mentioned in the Table 3, 24 cases had pain during biopsy while only 20 cases complained of pain during aspiration. Rest 5 and 8 cases had bleeding during aspiration and biopsy, respectively. The differences were statistically significant. Procedure was abandoned in favor of general anesthesia in cervical stenosis (2 and 3 in aspiration and biopsy, respectively) and uncooperative cases (3 and 4 in aspiration and biopsy, respectively).

Table 1. Patient Profile

Age		
20-29	36	60
30-39	21	35
40-49	03	05
Locality		
Rural	39	65
Urban	21	35
Parity		
P0	23	38.3
P1	18	30
P2	10	16.7
P3	06	10
>P3	03	5
Socioeconomic status		
I	00	00
II	06	10
III	19	31.7
IV	18	30
V	17	28.3

Table 2. Distribution of Cases According to Presenting Complaints

Chief complaints	No. of patients	Percentage (%)
Primary infertility	20	33.3
Secondary infertility	16	26.7
Menorrhagia	8	13.3
Oligomenorrhea	6	10
Secondary amenorrhea	2	3.3
Postmenopausal bleeding	2	3.3
Pelvic pain	20	33.3
Vaginal discharge	16	26.7
Weight loss	18	30
Low-grade fever	8	13.3
Malaise	12	20
Night sweat	11	18.3

 Echocardiography every three to five years if the mitral valve area (MVA) is > 1.5 cm^2. 2014 American Heart Association/American College of Cardiology (AHA/ACC) Valve Guideline.

Table 3. Distribution of Cases According to Difficulties Encountered During Sample Collection

Difficulties during sample collection	Endometrial aspiration		Endometrial biopsy	
	No.	%	No.	%
Pain	20	33.3	24	40
Bleeding	5	8.3	8	13.3
Uncooperative cases	3	5	4	6.7
Cervical stenosis	2	3.3	3	5
Failure of procedure	4	6.7	10	16.7
No difficulties	30	50	21	35

Table 4. Detection Rate of the Two Techniques

	PCR positive cases	Detection rate
Endometrial aspirate	25/60	41.7%
Endometrial biopsy	22/60	36.7%
Endometrial aspirate and biopsy	18/60	30%

Table 4 shows that out of 60 samples of aspiration, 41.7% were positive and 58.3% were negative by PCR for TB. Similarly out of 60 samples of endometrial biopsy, 36.7% were positive and 63.3% were negative by PCR.

DISCUSSION

The study was undertaken to compare the two techniques of sample collection, endometrial biopsy and endometrial aspirate in detection of *M. tuberculosis* by PCR in suspected cases of genital TB.

In the present study of 60 cases, it was found that majority of the cases were in the age group of 20-29 years (60%). Mean age was 28.6 years. Similar results were reported by Abdul Hakim et al, where the mean age was 29.4 years. Majority of the cases, 65% resided in the rural area. These findings were similar to those by Nezar et al (2009) and Abdul Hakim Ali Aleryani et al (2014).[3] Our study shows that 31.3%, 30% and 28.3% cases were of low socioeconomic status i.e., Class III, IV and V, respectively, according to Modified BG Prasad Classification. Other studies done by Shaheen et al (2006) and Shahzad et al (2012)[4] also reported majority of cases belonging to low socioeconomic status. Genital TB usually presents with infertility among women of reproductive age group. Our study also found that most common presenting complaint was infertility. Those cases where other factors including male infertility were excluded, genital TB was implicated as a causative factor in majority of cases. Whether primary or secondary, infertility followed by pelvic pain, vaginal discharge, menorrhagia, oligomenorrhea, secondary amenorrhea and postmenopausal bleeding were other complaints. Other constitutional symptoms like weight loss, low-grade fever, night sweat and malaise were also present.

In our study, endometrial aspiration and biopsy were taken in all 60 cases and send for PCR. It was seen that 41.7% cases were positive by PCR in endometrial aspirate. Other authors reported similar results. Sharma et al in a study of 28 cases of endometrial aspirate reported a PCR positivity rate of 46.4%. Jindal et al[5] studied 443 cases of endometrial aspirate reported a PCR positivity rate of 38.15%. Endometrial biopsy was positive by PCR in 36.7%. Kumar et al[6] reported a positivity result on endometrial biopsy in 31.3% cases. Thangappah et al also reported 36.7% PCR positive cases (Table 5).[7,8]

Comparative studies however have reported conflicting results (Table 6).[9,10]

It could be explained by the fact that endometrial aspirate involves instillation of normal saline into the endometrial cavity followed by its aspiration. This implies washing off entire endometrium of its surface cells while in endometrial biopsy we take out tissue only from the cornual site. Tubercular pathology at other sites in the endometrium can be missed out resulting in false negative findings.

Table 5. Comparative Results of Other Studies

Authors	Year	No. of cases (endometrial aspirate and biopsy)	Results of endometrial aspirate	Result of endometrial biopsy
Bhanu et al[8]	2005	21 endometrial aspiration and 15 biopsy	47% positive by PCR	53.3% positive by PCR
Thangappah et al[7]	2011	49 endometrial aspirate and biopsy	44% positive by PCR	36.7% positive by PCR
Our study	2015	60 endometrial aspiration and biopsy	41.7% positive by PCR	36.7% positive by PCR

Table 6. Comparative Results Reported Conflicting Results

Authors	Year	Results						
		Infertility	Pelvic pain	Menorrhagia	Oligomenorrhea	Amenorrhea	Dysmenorrhea	Vaginal discharge
Gatongi et al[9]	2005	43-74%,	42.5%	19%	54%	14%	12-30%	
Bhanothu et al[10]	2014	100%	15.34%	4.45%	12.87%	8.91%	46.53	
Our study	2015	60%	33.3%	13.3%	10%	3.3%		26.7%

Also, in most of the studies, sample collection was hysteroscopic-guided, whereas in our study this was a blind procedure carried out in the OPD. This could explain the low detection rate of endometrial biopsy in our study.

Several studies have taken separate groups for aspiration and biopsy, whereas we carried out both the procedures in the same case. This could be a reason for the discrepancy in the results.

CONCLUSION

Based on this study, we can thus say that genital TB, in present scenario is one of the common causes of infertility, so genital TB should always be considered as a probable cause in the diagnostic work-up of an infertile couple, especially in a population with high prevalence. The key to optimal outcome lies in early diagnosis and treatment of TB.

There are a lot of investigations for TB but they take more time with low sensitivity and accuracy. PCR is the most sensitive test to diagnose TB in a short period of time.

We found that endometrial aspiration has a better detection rate of genital TB in clinically suspected cases than endometrial biopsy. Aspiration is also technically easier as it is performed on an outpatient basis and is a more effective procedure as it involves the surface of the entire endometrial cavity. Biopsy if carried out under hysteroscopic guidance can result in improved detection rate but this requires admission and administration of anesthesia.

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