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Comparison of Molecular Test with Hystero-Laparoscopy in Detection of Genital Tuberculosis amongst Women with Infertility

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Abstract

The study aimed to compare molecular methods (Gene Xpert and TB-PCR) with laparoscopy as a diagnostic modality in the early diagnosis of female genital tuberculosis causing infertility. Premenstrual endometrial biopsy specimens were collected from 176 infertile women of the reproductive age group suspected of having genital tuberculosis. Samples were processed for molecular diagnostic tests: TB-PCR and Gene Xpert. Patients detected positive on Gene Xpert and TB-PCR were subjected to hystero-laparoscopy as a part of the routine protocol to look for affirmative findings of genital tuberculosis. The results were analyzed and compared between laparoscopy findings and molecular diagnostic tests. Hystero-Laparoscopy was performed in 16 patients who were tested positive with either Gene Xpert or TB-PCR. Out of them, 15 patients had affirmative findings on laparoscopy. No patient was detected with rifampicin resistance. Genital tuberculosis is a paucibacillary disease hence multiple diagnostic modalities are needed for diagnosis. Both Hystero-laparoscopy and molecular tests are complementary tests and together can effectively confirm the diagnosis of Genital TB.

Keywords: Female, Tuberculosis, Infertility, Polymerase chain reaction

Introduction

Female genital tuberculosis (T.B.) continues to be major gynecological morbidity in Indian women. In India, 15 to 20% of TB cases are estimated to be cases of Extra Pulmonary TB (1). Tuberculosis is a public health problem throughout the world affecting about 10.4 million people of which 3.5 million cases are from India and causing about 1.4 million deaths annually (2). The exact prevalence of female genital tuberculosis in India is not known but varies from 1-19 % amongst gynecological patients and it is responsible for 10 % of cases of infertility (3-5). Its presentation is very vague and may present as infertility, uterine adhesions, pelvic pain, abdominal mass, menstrual dysfunction (oligomenorrhoea, hypomenorrhea, menorrhagia, and amenorrhoea), vaginal discharge, and poor general health. (3, 6, 7).

The gold standard for diagnosis of tuberculosis is culture but it requires a minimum of 10-100 bacilli/ml for diagnosis and a long incubation period which leads to delay in diagnosis and treatment (5). Microscopy offers lower sensitivity in the detection of genital tuberculosis as it requires 10^4 bacilli/ml and genital tuberculosis being a paucibacillary disease, yield is very low. Laparoscopy is also considered the gold standard for diagnosis of genital tuberculosis but it is associated with higher complication rates in patients with genital TB because of the basic underlying pathology and adhesions (8).

Recently the trend has been shifted to molecular methods as an efficient tool for the detection of genital tuberculosis. Endometrial TB-PCR targets 65 kDa protein-encoding gene and its results have been validated in various studies as a highly sensitive tool. False PCR positivity and negativity remain a concern. Gene Xpert has emerged in the form of nucleic acid amplification as a newer molecular diagnostic method, owing to their rapidity, sensitivity, and specificity. (9) It is capable of detecting 80-130 M. tuberculosis/ml in the infected material and also detects rifampicin resistance within 2 hours (10, 11). Its sensitivity was well compared to culture (12). Most of the studies available in the literature are on sputum samples of pulmonary tuberculosis patients and have shown high sensitivity up to 98% and 90% in smear-positive and negative patients respectively (13, 14).

So, our present study aimed to test the efficacy of molecular tests in comparison to diagnostic hystero-laparoscopy to confirm the diagnosis of genital tuberculosis.

Materials and methods

This was a prospective analytical cross-sectional study conducted at the Fertility and IVF Clinic in a University Teaching Hospital for six months. Institutional ethical committee permission was taken.

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The primary outcome measure of the study was to compare the effectiveness of molecular methods (Gene Xpert and TB-PCR) with hystero-laparoscopy as a diagnostic modality in the diagnosis of female genital tuberculosis causing infertility and the secondary outcome was the detection of rifampicin resistance in confirmed Female Genital Tuberculosis.

The inclusion criteria were infertile patients with age groups between 20 to 40 years only having female factor as the cause of infertility. A total of 176 patients were recruited in the study after taking written informed consent. Patients were excluded if any gynecological malignancy is detected, tested positive for HIV, and/or the previous history of Anti-Tubercular Treatment intake was present.

A detailed history comprising of the duration of infertility, presence or absence of constitutional symptoms like chest pain, fever, and cough with sputum, loss of appetite, weight loss, and detailed menstrual history were noted. Baseline investigations including ESR and Mantoux test were done in all women. Chest radiography P-A view and ultrasonography were done in all women for any evidence of tuberculosis.

Premenstrual endometrial biopsies were taken from all women. The endometrial samples were sent for processing was for TB-PCR & Gene Xpert (1 ml each in Normal Saline).

TB-PCR was done for the β-globin gene to assess the quality of extracted DNA and its suitability for amplification. The DNA was extracted using a Qiagen DNA extraction kit (Qiagen, GmbH, 40724 Hilden, Germany) following protocol as proposed by the manufacturer.PCR was carried out to amplify the 165bp region of the 65kDA HSP gene of Mycobacterium tuberculosis. Electrophoresis was done on the amplified products on 1.5% agarose gel and stained with ethidium bromide to look for the presence of specified bands (5, 15).

Gene Xpert purifies M. tuberculosis bacilli from the sample, isolates genomic material from the captured bacteria by sonication, and subsequently amplifies the genomic DNA by PCR. It utilizes a real-time hemi-nested automated PCR and gives results within 2 hours. Besides, it identifies all the clinically relevant Rifampicin resistance-inducing mutations in the RNA polymerase beta (rpoB) gene in the M. tuberculosis genome in a real-time format using fluorescent probes called molecular beacons.

All women who tested positive with Gene Xpert and TB PCR further underwent hystero-laparoscopic evaluation as a part of the routine protocol.

Affirmative findings suggestive for TB observed under direct visualization through laparoscope included the presence of tubercles, presence of caseation, Grade II adhesions**, Tubo-Ovarian masses, beaded tubes, and Cornual blockage (16).

Grading of adhesions covering adnexa was done as follows:

*Grade I- localized covering one-third of the adnexa.

**Grade II- moderate, covering one-third to two-thirds of the adnexa

Affirmative findings suggestive for TB observed under direct visualization through hysteroscope included fibrosis ostia, presence of tubercles, cervical stenosis, intrauterine adhesions, and small uterine cavity.

A case was labeled as TB positive if there were affirmative findings of tuberculosis on hystero-laparoscopy. All cases having evidence of Genital TB were put on Anti Tuberculosis Treatment according to Revised National Tuberculosis Control Programme guidelines - Category I regimen which comprised of daily 2(HRZE) for 2 months followed by 4(HR)3 thrice weekly for 4months.

Statistical analysis of differences between the two groups was done using Statistical Package for Social Sciences (SPSS) version 17. Quantitative variables were determined by unpaired t-test or Mann-Whitney U tests. Qualitative variables were determined by the Chi-square test or Fischer exact test. The level of significance ($p \leq 0.05$) was used.

Observations and results

Demographic profiles showed the mean age of women was 28.95 ± 4.7 years. Most of the women (54.6%) were in the age group of 20-30 years. The mean BMI was calculated as 25.72±4.61. The majority of the women were Hindu by religion 134 (76.1%) followed by Muslim women 42 (23.9%).

The majority of the women were vaccinated with the BCG vaccine (92.6%) in their childhood. Constitutional symptoms such as fever in 18 (10.2%), cough in 3 (1.7%), chest pain in 1

(0.6%), anorexia in 11 (6.2%) and weight loss in 21 (11.9%) women were present respectively. 81.2% of women were diagnosed with primary infertility and 18.8% had secondary infertility. The mean duration of infertility was 7.3±3.6 years. Irregular cycles were present in 123 (69.9%) women. Oligomenorrhoea was found to be common, present in 76 women (43.2%). Menorrhagia was seen in 35 (19.9%) whereas hypomenorrhea was present in 10 (5.7%) women. Two (1.1%) women were amenorrheic for the past 6 months. Dysmenorrhea was seen in 99 (56.2%), dyspareunia in 138 (78.4%) women and a history of chronic pelvic pain was found in 21 (11.9%) women.

On local examination, vaginal discharge was present in 69 out of 176 women (39.2%). Fornical fullness, adnexal masses, bulky uterus, restricted mobility of uterus was found in 19 (10.8%) women. Anemia was present in 20 (11.4%) women. Mantoux test (more than 10mm) was seen in 104 (59.1%) women. Erythrocyte sedimentation rate (ESR) (more than 20) was raised in 126 (71.6%) women. Both Mantoux and ESR were raised in 77 women. Positive findings of old pulmonary tuberculosis were present in 11 (6.2%) women on chest X-Ray.

A total of 16 patients were positive with Gene Xpert (2/16) and TB-PCR (15/16). One patient was positive with both Gene-Xpert and TB-PCR. Laparoscopy was performed in all 16 women who tested positive with either Gene Xpert or TB PCR. Out of 16 women, affirmative findings suggestive of tuberculosis were seen in 15 patients. One woman, who was positive for TB-PCR, had normal hystero-laparoscopic findings. There was one woman who was negative for TB-PCR but was positive with Gene Xpert; underwent hystero- laparoscopy and was found to have affirmative findings of tuberculosis. (Table 1) No woman was detected with Rifampcin Resistance in our study group.

Comparison of TB-PCR and Gene Xpert with laparoscopy is depicted in (Table 2).

Discussion

Genital Tuberculosis poses a major diagnostic challenge as it is a paucibacillary disease. Diagnosis is often delayed and is made presumptively based on clinical features, radiological and

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investigations, endometrial biopsy sample evaluation, laparoscopic evaluation. None of the tests is 100% sensitive.

Table 1. Affirmative findings saw in Laparoscopy and Hysteroscopy

Laparoscopy	Number of patients
Normal findings	1
Presence of tubercles	8
Grade 2 adhesions in pelvis	4
Caseation	3
Bilateral cornual block	6
Hysteroscopy	**Number of patients**
Normal findings	14
Intrauterine synechae & Small Uterine Cavity	2
Fibrosed Osteia	**0**

Table 2. Comparison of TB-PCR and Gene Xpert with laparoscopy. (N1+ N2= 16)*

Parameters (N1 + N2 =16)*		TB-PCR + ve cases (N1)	Gene xpert +ve cases (N2)
		15	2
Laparoscopy Findings	Normal	1	0
	Abnormal	14	2

*One patient was positive with both TB-PCR and Gene Xpert

Prognosis is directly related to the time delay in starting treatment. Mortality further increases in patients having multi-drug resistant Genital Tuberculosis (GTB). Thus an early diagnosis, information regarding multidrug resistance, and institution of appropriate anti-tubercular treatment are critical for better outcome. (2, 17) Some reports have been published citing the importance of the molecular tests in the early detection of Genital TB. This study aims to correlate Gene Xpert and TB-PCR with hystero- laparoscopic findings.

The detection rate of TB-PCR was more as compared to Gene Xpert in our study. A.G.Radhika et al had shown the higher sensitivity of TB-PCR in the detection of genital tuberculosis similar to our study but authors had raised concern regarding the high false-positive rate of TB-PCR. (18) Similar results have also been given by S.Sethi et al and S.Prasad et al. (5, 19) On the contrary; the rate of false-positive TB-PCR was low in our study as compared to the published studies which may be attributed to strict hygiene method of sample collection; avoiding cross-contamination by interspersing several negative controls between the samples.

In the present study, Gene Xpert was able to detect genital tuberculosis in 2 patients out of 176 patients. Garg et al were not able to detect any patient positive with Gene Xpert amongst 81 infertile women who are suspected of having genital tuberculosis as the cause of infertility. (20) J.B.Sharma in their study found the sensitivity and specificity of the test as 46.6% and 100% respectively. (21) The poor detection rate of Gene Xpert could be attributed to blood acting as an inhibitor for this test. J.B Sharma et al had found the role of Gene Xpert in the detection of rifampicin resistance especially in MDR-TB in high-risk patients and were able to detect it in 5 patients but in our study, none of the patients were detected with rifampicin resistance. (22) Our study had also shown one case in which laparoscopy was positive and TB-PCR was negative which was attributed to the advanced stage of the diseased endometrium with little tissue available for diagnosis. Considering the paucibacillary nature of the disease, false-negative remains an area of concern. However, the Gene Xpert was positive in this case suggesting the role of this test in the advanced stage of the disease. U.N Jindal et al had shown that both TB-PCR and Laparoscopy are required for detection of genital tuberculosis; since many cases may give the false-negative result of TB-PCR due to destroyed endometrium but hysteroscopy can pick up these cases. (23)

Gene Xpert has few limitations such as the limited shelf-life of the diagnostic cartridges, operating temperature, and humidity restrictions. (24) It can detect resistance to only rifampicin not to other ATT drugs. The major limitation of the study was the small sample size due to time constraints and being a cross-sectional study; no treatment response was monitored. Hence, further, adequately powered randomized controlled trials are required to arrive at a conclusion and support the hypothesis of our study.

Conclusion

Both laparoscopy and molecular tests are complementary tests and together can effectively confirm the diagnosis of Genital TB.

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Conflict of interests

The authors declare that there is no conflict of interest.

Authors contributions

Sudha conceived the idea of the study. Renu performed all the surgeries. Kriti planned the manuscript. Sahil carried out data analysis.

Ethical issue

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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