



Original Research Article

Evaluation of culture, polymerase chain reaction (PCR) and histopathology for diagnosis of genital tuberculosis in female infertility

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ARTICLE INFO

Article history:

Received 06-03-2020

Accepted 05-06-2020

Available online 26-08-2020

Keywords:

Genital tuberculosis

Culture

Polymerase chain reaction

Histopathology

ABSTRACT

Introduction: Tuberculosis (TB) is a chronic infectious disease and has major health implications. The disease has a global distribution, and the incidence is more in developing countries. The paucibacillary nature of tuberculous bacilli and varied clinical presentation puts the clinician in a quandary for clinical diagnosis of genital tuberculosis. According to some Indian studies, tubercular endometritis and salpingitis accounts for 4-9% cases of infertility.

Objective: The objective of the present study was to evaluate the efficacy of PCR technique in the diagnosis of GTB in female infertility in comparison to culture and histopathological examination.

Materials and Methods: 42 infertile women fulfilling inclusion and exclusion criteria were enrolled over a period of 24 months after Ethics Committee approval and written consent. The women were subjected to routine and specialised investigations of endometrium and ascitic fluid from pouch of douglas as culture, TB, PCR, and histopathology and the data was analysed.

Out of results of 42 women of infertility studied, 19 women had positive findings on hysterosalpingography, 18 women had laparoscopic findings, 11 women had positive TB PCR, 2 cultures and 3 histopathology reports were positive.

There was good agreement when microbiological culture was compared with histopathology whereas the agreement was poor between culture and PCR.

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1. Introduction

Tuberculosis (TB) is a chronic infectious disease and has major health implications. The disease has global spread and the incidences are more in under developed countries.¹ Genital TB is asymptomatic and irretrievably damages the fallopian tube causing infertility which is difficult to treat by medical and surgical methods.^{2,3} Indian studies have shown tuberculous endometritis and salpingitis are as causes of 4-9 per cent of all infertility cases.⁴⁻⁶ The paucibacillary nature of tuberculous bacilli and wide range of clinical results confuse the physician for clinical diagnosis of genital tuberculosis.^{7,8}

Therefore, high value of suspicion is important in the diagnosis of the disease. Common laboratory tests have very

less diagnostic importance. Some examinations, such as, chest X-ray for healed or active pulmonary tuberculosis, contact history, elevated ESR and positive tuberculin test are the contributory investigations.⁴ Typical features on hysterosalpingogram (HSG) or laparoscopy are not diagnostic, but laparoscopy helps in getting tissue sample for culture and histopathological examination.^{5,9,10}

Positive mycobacterial culture, displaying typical histopathological lesion in the sample is diagnostic but it has low sensitivity. Recently, Polymerase Chain Reaction (PCR) technique has been proved as a useful and rapid diagnostic method for pulmonary and extra-pulmonary tuberculosis, but is not done routinely because of non-availability of resources. Diagnostic test for Genital tuberculosis need to be highly sensitive to diagnose the disease in its initial stage so that treatment can initiated on time to save tubes from damage beyond recovery.¹¹

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2. Materials and Methods

This study was conducted in Obstetrics and Gynaecology, at a care hospital. The experiment included 42 infertile women who were included over a period of 24 months (January 2018 to January 2020). The Ethics Committee sanction was obtained to take up the study and concurrence of each patient is taken. Sampling was carried out by convenience sampling technique.

2.1. Inclusion criteria

1. Women with tubal factor infertility proved either by hysterosalpingogram (HSG and/or laparoscopy).
2. Presence of adnexal mass was diagnosed by ultrasound.
3. Women with recurrent pelvic inflammatory ailment refractory to conventional therapy and Women with unexplained infertility.

2.2. Exclusion criteria

Those women where infertility was because of abnormalities of ovulation, male factors endocrine problems, sexual problems, endometriosis and peritoneal adhesions due to previous abdominal surgery were not included.

2.3. Evaluation

Women with history of not able to conceive, visiting gynaecology outpatient department were included in the study on the basis of selection criteria and their concurrence was taken. The details of the women were collected using a preset proforma meeting the requirements of the study by means of personal interview with the patient.

After thorough clinical examination which included per speculum and per vaginal examination, all women have undergone investigations such as Haemoglobin per cent, Total Count (TC), Differential Count (DC), ESR, tuberculin test, pelvic sonogram, hysterosalpingography and laparoscopy.

During laparoscopy, indications suggesting tuberculosis such as frank tubercles, caseation, granulomas and beaded tubes were observed. Evidence of chronic infection in the form of thickened tubes, and terminal hydrosalpinx with retort shaped tubes, tubo-ovarian masses, flimsy adhesions in the Pouch of Douglas (POD) were also looked for.

The sample for the study was gathered from pre-menstrual endometrium and wherever feasible, fluid from pouch of Douglas was aspirated at the time of laparoscopy and was subjected for PCR, culture and histopathology.

The particular diagnostic tests as culture, HPE and PCR were examined using bivariate two by two tables. Sensitivity, specificity and agreement through Kappa test were computed by using SPSS software.

3. Results

42 women with infertility who were fulfilling the inclusion and exclusion criteria were enrolled in the study after taking their written informed consent.

The women were aged between 23 and 33 years with a mean of 26.975 years. 35 women (83.3%) had primary infertility and 7 (16.7%) had secondary infertility.

Oligomenorrhea was the most common menstrual abnormality in 24/42 (57.14%) women.

In five patients (11.9%) there was past history of tuberculosis such as cervical lymphadenopathy, and pulmonary tuberculosis, of these 3 women had history of relative suffering from pulmonary Koch's taking anti tuberculosis treatment.

In these five cases laparoscopy was suggestive of tuberculosis, Mantoux and ESR were elevated in 3 cases.

Table 1: Showing the results of various tests (N=42)

Test	Positive result(%)	Negative result(%)
PCR(Polymerase chain reaction)	26.2	73.80
Laparoscopy	42.8	57.20
Culture	4.76	95.23
Histopathology	7.14	92.85
Hysterosalpingography	45.23	54.76
Mantoux test	23.20	76.20
ESR	50	50

ESR was high in 21 cases (50%), Positive tuberculin test was seen in 10 cases (23.2%) with induration of 13 mm (range 11-16 mm). In these 10 cases, there was firm evidence of tuberculosis in nine (90%) (laparoscopy was positive in all nine and ESR was high in five).

Hysterosalpingography was taken in 38/42 cases. Typical features of genital tuberculosis as distorted endometrial cavity, beaded appearance of the tubes, retort shaped hydrosalpinx, calcified areas and cornual blocks were seen in 19/42 (45.2%). Of these, laparoscopy was positive in 18 cases (94.2%), ESR was positive in 10 and Mantoux was positive in six cases. Laparoscopy was carried out in all 42 cases. Microbiological culture of the endometrium was positive in two samples (4.76%).

On histopathological examination three of 42 (7.1%) endometrial samples were positive for tuberculosis. one was positive by culture alone, one by histology alone and one had both histology and culture positive.

PCR of endometrium was done in 42 women. PCR was positive in 11 endometrial samples. In seven women, at the time of laparoscopy, fluid from pouch of Douglas was also aspirated and sent for culture and PCR study. Four of these POD aspirate samples were positive by PCR.

There was good agreement when microbiological culture was compared with histopathology whereas the agreement was poor between culture and PCR.

Table 2: Showing comparison of microbiological culture with Histopathology and PCR

	HPE(%)	PCR(%)
Sensitivity	100	50
Specificity	97.44	74
Correctly classified	97.56	73.17
Area under the curve	0.98	0.62
Kappa	0.78	0.07
P value	0	0.22

Table 3: Showing comparison of TB PCR with Microbiological culture with Histopathology

	Culture (%)	HPE(%)
Sensitivity	9.09	18.18
Specificity	96.67	96.67
Correctly classified	73.17	75.61
Area under the curve	0.52	30.57
Kappa	0.07	0.19
P value	0.22	0.05

There was poor agreement when TB PCR was compared with microbiological culture but the agreement was better with histopathology.

Table 4: Showing comparison of Histopathology with TB PCR and Microbiological culture

	Culture (%)	PCR(%)
Sensitivity	66.67	68.67
Specificity	100	76.32
Correctly classified	97.56	75.61
Area under the curve	0.823	0.71
Kappa	0.78	0.19
P value	0	0.05

There was poor agreement when histopathology was compared with TB PCR and good agreement with Microbiological culture.

Out of 42 women of infertility studied, 19 women had positive findings on hysterosalpingography, 18 women had laparoscopic findings, 11 women had positive TB PCR, 2 cultures and 3 histopathology reports were positive.

4. Discussion

Female genital tuberculosis poses a challenge due to varied presentation, lack of sensitive and specific tests for diagnosis.

Culture on Lowenstein Jenson medium is the gold standard. Accurate identification of *M.tuberculosis* through culture is the need of the hour for diagnosis but inspite of

inoculation on different media, the yield is very low may be due to Paucibacillary nature and a substantial number of TB lesions of the genital tract are bacteriologically mute or bacteriostatic nature of substance which inhibits the growth of the bacilli. Histopathological examination is easy, quick and cheap and helps diagnosis of *M.tuberculosis*. The secondary nature of the genital tuberculosis, results in sparse number of organisms which may not be picked by sample.

Table 5: howing comparison of results of various studies

Author	Culture sensitivity	Histopathology sensitivity	TB PCR sensitivity
Seema Chopra et al ¹²	8.5%	21.4%	72.8%
RBP Thanappah et al ¹³	Low 5.6%	6.9%	36.7%
Kohli et al ¹⁴	-	4%	13%
Kumar et al	1.83%	3.2%	38.9%
Mani et al	13.6%	3.6%	-
Our study	4.76%	7.14%	26.2%

From the above table it is seen that as compared to culture and histopathology the sensitivity of TB PCR is better as per all the studies. In our study PCR was positive in 11 cases out of 42. It was found that the positivity was more in cases with clinically suspicious findings. The false positivity was low.

There was poor agreement when TB PCR was compared with microbiological culture and histopathology may be because it detects both live and dead bacilli.

Of the 18 women with positive laparoscopic findings, 11 women had PCR positive and 7 cases with positive findings were missed. So anti tubercular treatment cannot be started just on the basis of positive PCR though favourable outcomes with ATT only on positive PCR was shown, but later was criticised.

5. Conclusion

Conventional diagnostic methods though have low sensitivity, definitely have a place in diagnosis of genital tuberculosis in low resource settings.

TB PCR has better sensitivity but has false negative results too which makes it reliable only when added with culture to decrease false negative results during different steps of diagnosis. For early diagnosis and preventing the distortion of fallopian tubes by tuberculosis it is wise to take into consideration clinical and investigative findings of laparoscopy, TB PCR, and culture to start anti koch treatment.

Large case control studies are required to make our own guidelines for treatment of genital tuberculosis as the search for a reliable diagnostic test is still on.

6. Source of Funding

None.

7. Conflict of Interest

None.

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Cite this article: Bhalerao A, Sarma S. Evaluation of culture, polymerase chain reaction (PCR) and histopathology for diagnosis of genital tuberculosis in female infertility. *Panacea J Med Sci* 2020;10(2):158-161.