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COMPARATIVE STUDY OF GENITAL TUBERCULOSIS DIAGNOSIS IN WOMEN WITH INFERTILITY

Microbiology

Riden saxena — Ph.D. Scholar, Department of Obsts & Gynecology, Institute of Medical Science, Banaras Hindu University, Varanasi.

Kriti Shrinet — Ph.D. Scholar, School of Biotechnology, Institute of Science, Banaras Hindu University, Varanasi.

Madhu Jain — Professor, Department of Obsts & Gynecology, Institute of Medical Science, Banaras Hindu University, Varanasi. - CORRESPONDING AUTHOR

ABSTRACT

Background- Genital tuberculosis (GTB) is one of the important factors that cause infertility in women. GTB is mostly asymptomatic in nature and infertility generates high suspicion for GTB in reproductive age. The object of this study was to compare the different methods to diagnose GTB in Infertile patients.

Material and method- Women patients seeking diagnosis of infertility in the OPD of Obstetrics & Gynecology department during April 2016 to March2017 were included in this study. A total of 62 endometrial samples were processed by AFB smear microscopy, *L-J* culture, BACTEC culture and Genexpert to diagnose the tuberculosis. Results- Out of 62 patients with infertility suspected of GTB, 26 were found to be positive either by AFB smear, L-J culture, BACTEC culture and Genexpert. Only one sample was found to be positive by all methods. Fourteen samples to be found positive with both *L-J* culture and BACTEC culture methods.

Conclusion- The study indicated that the old method of AFB smear is less sensitive when compared with the *L-J* culture and BACTEC culture. Although Genexpert have a high specificity and sensitivity but still there are limitations in the diagnosis of endometrial biopsy samples. This study suggest both liquid and solid culture have an important role in the diagnosis of genital tuberculosis.

KEYWORDS:

Genital Tuberculosis, Culture, Infertility, Genexpert, Diagnosis

INTRODUCTION

Female genital tuberculosis (FGTB) is still a major problem in the low resources countries like India which causes significant morbidity specifically infertility in reproductive age[1-3]. Sometime FGTB have varied presentation and mostly asymptomatic so that it is difficult to diagnosis. Diagnostic dilemma of FGTB is still a major problem due to paucibacillary nature. FGTB is secondary to the pulmonary Tuberculosis [4-9]. Lack of specified diagnostic modalities and reports in the literatures might elevate the prevalence of FGTB higher than the imagination.

MATERIALS & METHODS

The study was conducted in the Department of Obstetrics and Gynecology and Department of Microbiology of Sir Sunderlal Hospital, Banaras Hindu University, Varanasi.

A total of 62 endometrial tissue samples were collected from the women who were going through diagnosis for infertility.

On the basis of the clinical presentation, women with infertility were selected and lined up. On the day between 20 and 25th day of menstruation, endometrial biopsies were taken by using endometrial biopsy cannula. Samples were collected in normal saline in a sterile container.

Inclusion criteria: Women visited Out Patient Department of Obstetrics & Gynecology with the primary or secondary infertility suspected for the Genital Tuberculosis were included in this study.

Exclusion criteria: Women who have taken or on the regimen of Anti tuberculosis drug and HIV positive women were excluded from the study.

METHOD

Sample tissues were primarily homogenized by the glass bead homogenizer. A homogenized tissue sample was distributed in to four parts, one ml for Genexpert, 50µl for *L-J* culture, 500µl for BACTEC culture and 50µl sample for AFB Smear in separate vials.

Ziehl-Neelson's (Z-N) staining: About 50 µl of homogenized sample was spread on the glass slide, air dried for 10 minutes than heat fixed again for 10 minutes. Heat fixed slides were stained with carbofuchsin. Destained with Acid alcohol then slides were counterstained by methylene blue and rinsed under water to remove excess counter stain. Stained slide were examined under oil emersion microscope. Pink stained portion on the pale blue background was noted to count the bacilli [10].

Gene*xpert* MTB/RIF assay: One ml of homogenized sample was added to 2.0 ml of Gene*xpert* sample reagent. Mixture was vortexed for 30 seconds. The sample was left to stand for 15 minutes at room temperature and then 2 ml of mixture sample was transferred to the test cartridge. Cartridge was loaded onto the *Xpert* instrument. Results were reported as positive or negative and sensitivity by the RIF resistance determining region of the *rpo*B gene with molecular beacons within 2 hours (Cephid,Inc, Sunnyvale, CA)[11].

Liquid culture by BACTEC MGIT 960: Homogenized samples were cultured using the BACTEC MGIT 960 system. 500µl of the sample was inoculated in a MGIT tube containing 0.8ml PANTA antibiotics and growth supplements. MGIT tubes were incubated in BACTEC960 instrument. This system automatically identifies positive samples[12].

Solid culture on Lowenstein-Jensen (L-J) egg media: About 100 ml of homogenized sample was inoculated on the L-J medium slant in bottle and left in a horizontal plain until the inoculums was absorbed. The culture bottles were incubated at 37°C in BOD incubator. The inoculated bottles were examined after 24 hours, 48 hours and then every week till 8 weeks. Growth colony were smeared and stained by Z-N staining then examined under microscope for presence of *M. tuberculosis*[13].

RESULTS

A total of 62 patients were suspected of suffering from Genital tuberculosis. 26 were found to be positive by any of the three methods. Total 36 samples were subjected to AFB smear, 48 samples to cultures and 62 were subjected to Gene*xpert.*

Out of 48 processed samples, L-J culture and BACTEC culture were positive in 8 samples. L-J culture and Gene*xpert* were positive in one sample. Only L-J culture was positive in 3 samples. Out of 36, L-J culture with AFB smear was positive in only 1 case.

Out of 48 samples, only BACTEC culture was positive in 5 samples, BACTEC culture with Gene*xpert* was positive in only one sample. Out of 36 BACTEC culture with AFB smear was positive in one case only.

Out of 68, Gene*xpert* was positive in only one sample and that was positive in all methods.That sample was found to be resistant for Rifampicin. No any sample was positive alone by Gene*xpert*.

TABLE 1: Distribution of samples according to L-J culture

Findings (Positive)	Total samples	No. of positive samples	Percentage (%)
Only *L-J* culture	48	3	6.2
L-J culture+AFB smear	36	1	2.7
L-J culture+ BACTEC culture	48	8	16.6
L-J culture+ Genexpert	48	1	2
Total *L-J* culture positive	48	11	22.9

Table 2: Distribution of samples according to BACTEC culture

Findings (Positive)	Total samples	No. of positive samples	Percentage (%)
Only BACTEC culture	48	5	10.4
BACTEC culture+AFB smear	36	1	2.7
BACTAC culture+Genexpert	48	1	2
Total BACTEC positive	48	13	27

Table 3: Distribution of samples according to Genexpert

Findings (Positive)	Total samples	No. of positive samples	Percentage (%)
Only Gene*xpert*	62	0	0
Gene*xpert*+AFB smear	36	1	2.7
L-J culture+ BACTEC culture+ Gene*xpert*+AFB smear	36	1	2.7
Total Gene*xpert*	62	1 (Rif.resistant)	1.6

DISCUSSION

Due to the lack of specific test and diagnostic modalities it is difficult to diagnose and conclude the presence of genital tuberculosis. On the basis of clinical presentation a women cannot be diagnosed with FGTB. Multiple imaging techniques are not specific for tuberculosis confirmation but hysteroscopy and laparoscopy are useful to diagnose the FGTB by presence of intrauterine adhesion, pale endometrium and tubercles that can help to sort the highly suspicious FGTB patients [8,9].

Endometrial biopsy should be taken in premenstrual phase for good results on AFB smear, culture, Genexpert, PCR and other tests[4,5]. Polymerase Chain Reaction (PCR) is not conclusive for FGTB due to false positive and false negative[5,6]. We applied conventional methods for the detection.

In our study, 22.9% of women had L-J culture positive suggestive for FGTB which is higher as compare to the studies by Goel et.al Thangappah et.al. and Kumar et.al. where they found 1.83%,5.6% and 4.6% respectively[14-16]. With the help of BACTEC culture we found 27% samples positive where as it was 3.3% & 8.8% positive in the study of Prasad et. al. and Goel et.al[14,17]. respectively. Genexpert scored 1.6% of positive samples which is lesser then the study by Sharma et.al. but the sample was found to be resistant for Rifampicin[18].

CONCLUSION

In our study we have compared the performance of various diagnostic methods for FGTB diagnosis. However conventional methods i.e. liquid-solid culture have their specific place for the diagnosis of FGTB. Along with the clinical diagnosis, conventional gold standard culture should be collaborated with the rapid, appropriate and cost effective test for tuberculosis diagnosis. This study also reveals the relatively high burden of female genital tuberculosis that causes infertility in the region of North India.

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