THE JOURNAL OF OBSTETRICS AND GYNECOLOGY OF INDIA

The Journal of Obstetrics and Gynecology of India (March–April 2018) 68(2):123–128
https://doi.org/10.1007/s13224-017-1077-1

ORIGINAL ARTICLE

The Role of MGIT 960 Culture Medium in Resolving the Diagnostic Dilemma for Genital Tuberculosis Patients Presenting with Infertility

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Received: 2 September 2017 / Accepted: 4 November 2017 / Published online: 24 November 2017

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Abstract

Background The purpose of this study was to assess the utility of Mycobacteria Growth Indicator Tube (MGIT) 960 culture medium for the diagnosis of genital tuberculosis (GTB) in women presenting with infertility.

Methods The premenstrual endometrial biopsy samples in 300 women presenting with primary and secondary infertility were subjected to AFB smear method, histopathological examination and culture on LJ and MGIT 960 media. Detection rates were compared for diagnostic modalities and their combinations.

Results In total, 30 cases were positive for genital tuberculosis by either of the four tests employed. The detection rates for AFB smear, MGIT culture, LJ culture and HPE were 50, 46.7, 3.3 and 33.3%, respectively. A combination of smear examination for AFB, MGIT 960 culture and histopathological examination was able to detect all the positive cases. A combination of MGIT and LJ media provided no added advantage over MGIT alone since the

only case where LJ culture was positive had been detected by positive MGIT culture. In as many as five positive cases (16.7%), only MGIT culture was positive.
Conclusion The addition of MGIT 960 culture medium to routine battery of investigations in infertility patients significantly improves the diagnosis.

Keywords Genital tuberculosis · Infertility · Culture · MGIT 960 · Diagnosis

Introduction

Tuberculosis (TB) remains a major health concern in most of the developing countries including India. The World Health Organization estimated an incidence of 2.2 million cases of TB for India out of a global incidence of 9.6 million for the year 2015 [1]. Although the major type of prevalent TB is the pulmonary variety, extra-pulmonary types contribute to burden of the disease and present diagnostic and therapeutic challenges. Genital tuberculosis (GTB) a form of extra-pulmonary TB presents itself with myriads of symptoms and incurs significant morbidity by its short- and long-term sequelae [2]. Infertility remains the most frequent clinical presentation of GTB, occurring in 43–74% of the cases [3]. Furthermore, a systematic review has revealed the prevalence of GTB among infertile patients to be as high as 24.2% [4].

Diagnosis of genital TB has profound implications for women seeking infertility treatment. Considering the high prevalence and its adverse effect on fertility, diagnosis and treatment of GTB should be one of the main priorities of health systems, at least in developing countries [4]. The diagnostic dilemma arises because of varied clinical presentations, diverse results on imaging and laparoscopy and a mixed bag of bacteriological and serological tests. Diagnosis depends upon collective evidence from imaging studies, direct visualization by laparoscopy and hysteroscopy, and histopathology of genital tract material, culture and serology [2, 5]. The techniques being used for the detection of *Mycobacterium tuberculosis* are time-consuming and have low sensitivities and specificities. This results in lack of any conclusive evidence in the early course of the disease when disastrous consequences like infertility can be prevented by appropriate measures.

Culture remains the gold standard for diagnosis of GTB [6]. However, conventional culture media like LJ culture take an agonizing long time (6–8 weeks) for the growth of *Mycobacterium tuberculosis*. They frequently yield negative results in cases of paucibacillary disease, contributing to misdiagnosis in several cases. A culture medium which is rapid in identifying mycobacteria, cheap, easily available and confirmative would be an ideal one for diagnosis of GTB, facilitating early and accurate diagnosis.

Newer cultures like Mycobacteria Growth Indicator Tube (MGIT) 960 appear promising in this regard, seemingly fulfilling all the characteristics of an ideal culture medium. However, evidence regarding usefulness of MGIT in routine diagnostic battery and clinical practice is scanty. The purpose of this study, therefore, was to assess the utility of MGIT culture for the diagnosis of GTB in women presenting with infertility.

Materials and Methods

The study was conducted in the infertility clinic run by the Department of Obstetrics and Gynecology in a tertiary-level institute. After approval of ethical committee, a prospective study was conducted on 300 women being investigated for primary and secondary infertility in the age group of 20–40 years. After a detailed clinical history and thorough physical examination, all women were subjected to a battery of diagnostic tests (Fig. 1).

Endometrial biopsy was taken in premenstrual phase using paracervical block, after obtaining informed consent and duly explaining the procedure. The sample was processed for preparation of smear, histopathological examination and culture (conventional and rapid).

The MGIT 960 media contained 4 ml of Middlebrook 7H9 broth with an oxygen-sensitive fluorescent sensor embedded in silicon at the bottom of the tube which fluoresces under ultraviolet light when oxygen was depleted indicating mycobacterial growth. Uninoculated tubes served as negative control and tubes inoculated with H37RV as positive control. The MGIT-positive cultures were then subjected to PNB (para-nitro benzoic acid) test to differentiate it from non-tubercular mycobacteria (NTM).

Statistical Analysis

Based on a p value of 0.05 and power of 90%, the minimum sample size for this study was calculated to be 300, considering the prevalence of GTB in infertility patients to be around 10% (by the most conservative estimates).

The endometrial biopsy samples positive by any of the four methods were labeled as positive. The baseline characteristics of the GTB and non-GTB groups were compared using the two-tailed Fischer's exact test. The diagnostic accuracy of various tests and their combinations was evaluated based on their sensitivity. The statistical analysis was carried out using SPSS version 19 (SPSS *Inc*., Chicago Illinois).

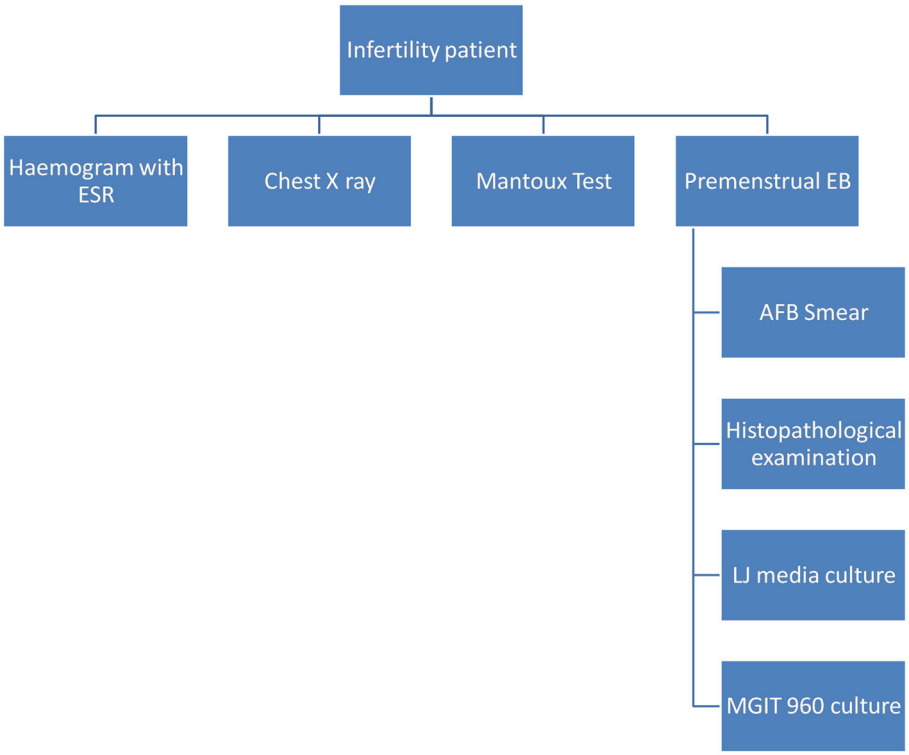

Fig. 1 Diagnostic battery employed

Results

The average age of 300 women in our series was 28.35 ± 4.29 years, and maximum patients of infertility were aged 26–30 years. In our series, 76% (228/300) had primary infertility, while 24% had conceived earlier.

Analysis of the social profile of women in our series revealed that the maximum cases of infertility (45.7%) belonged to lower-middle socioeconomic strata of the society. The mean Kuppuswamy socioeconomic score was 15.26. Demographic data of the study population are summarized in Table 1.

The endometrial biopsy sample was positive for GTB in 30 cases by either of the four tests employed in the diagnostic battery. The detection rates for AFB smear, MGIT culture, LJ culture and HPE were 50, 46.7, 3.3 and 33.3%, respectively. The mean time for growth on MGIT 960 culture was 12.79 ± 4.6 days. In as high as 77% cases that were positive by MGIT 960 culture, the growth on the tube was evident within 15 days of inoculation. LJ culture was positive in only one case, and the growth in that case was evident after a time period of 29 days. In that case, MGIT had already clinched the diagnosis after 14 days of culture. A graphic comparison of the growth time of LJ and MGIT 960 culture is depicted in Fig. 2.

The higher detection rates afforded by MGIT medium over LJ culture came out to be significant. Since the individual methods were not able to detect more than 50% of the cases, sensitivities of combinations of tests were then evaluated (Fig. 3). It was found that a combination of smear and MGIT culture detected around 70% of the cases as positive. MGIT and LJ media provided no added advantage over MGIT alone since the only case where LJ culture was positive had been detected by positive MGIT culture. A combination of smear examination for AFB, MGIT 960 and histopathological examination was able to detect all the positive cases.

Analyzing the diagnostic value of MGIT culture, it was found that in as many as five positive cases (16.7%) only MGIT culture was positive. In smear-negative cases (15/30), MGIT was positive in seven cases. Similarly in smear-positive cases, MGIT was positive in same number of cases. Thus, no difference in MGIT positivity was detected in smear-positive and smear-negative endometrial samplings.

A comparison of the symptomatology, demographics and clinical findings was undertaken between the GTB group of infertile women and the whole study population. The findings are summarized in Table 2.

Discussion

At present, the diagnosis of GTB is at best a collective one, employing multiple modalities like clinical picture, imaging, laparoscopy, bacteriological and serological tests.

Table 1 Demographic profile of the study population

	Number	Percentage
Age (years)		
20–25	77	25.7
26–30	148	49.3
31–35	47	15.7
36–40	20	6.7
> 40	8	2.7
Type of infertility		
Primary	228	76
Secondary	72	24
Kuppuswamy's socioeconomic score		
< 5	02	0.67
5–10	36	12
11–15	137	45.67
16–25	118	39.33
26–29	7	2.33

Although culture remains the gold standard for laboratory diagnosis, it does not yield accurate and speedy results. The disease meanwhile continues to simmer inflicting more and more damage leading to complications, which can be avoided if the problem is diagnosed and treated in its early course.

In the current study, out of three hundred women screened for genital tuberculosis thirty women (10%) were diagnosed to have genital tuberculosis on the basis of diagnostic tests carried out on endometrial samples. In a review by Sharma, the incidence of GTB in infertility patients was reported to be between 3 and 16% [2]. A metaanalysis conducted by Chaman-Ara et al. [4] showed that the overall prevalence of GTB in infertile women is 24.2%. The differences in the findings of various studies stem from the differences in study populations since the prevalence of TB in general depends on several factors like the socioeconomic levels and the degree of congestion. Even in the metaanalysis mentioned above, the percentage prevalence of GTB in infertile women ranged from 2.9 to 75.6% in different studies.

In our study, the average duration of infertility in GTB patients was 87.2 months which was much longer than the mean infertile period of the whole cohort (58.8 months). This revelation underlines the chronic and persistent nature of genital tuberculosis and its contribution to unremitting infertility. Average age of patients with GTB was a little higher than those in the whole study population. Around two thirds of entire GTB patients had age less than 30 years. In a review conducted by Neonakis et al. [7], it was found the disease inflicted younger age group in developing countries when compared to developed ones. Same findings of age trend in GTB patients have been quoted by Gupta et al. [8] and other studies [2] in the literature.

On evaluation of socioeconomic status with Kuppuswamy's socioeconomic status scale (SES) 2007 as a tool, we found out that around three fourth patients of GTB (73.3%) belonged to lower and lower-middle socioeconomic strata of the society. A study was conducted by Valsangkar et al. [9] in which 42.4% women belonged to lower socioeconomic scale. As a corollary, improvement in socioeconomic conditions of the society might help to decrease the prevalence of infertility secondary to genital tuberculosis. This is one among many reasons for the decreased infertility rates in developed nations.

Among the patients diagnosed with GTB, history of past affliction with tuberculosis was present in 20% of the cases diagnosed to have genital tuberculosis, pulmonary tuberculosis being the commonest one. Other studies [8] have also found similar figures in respect of past history of tuberculosis.

The menstrual profile found in GTB patients presented a stark contrast to the one found in total infertility cohort. In

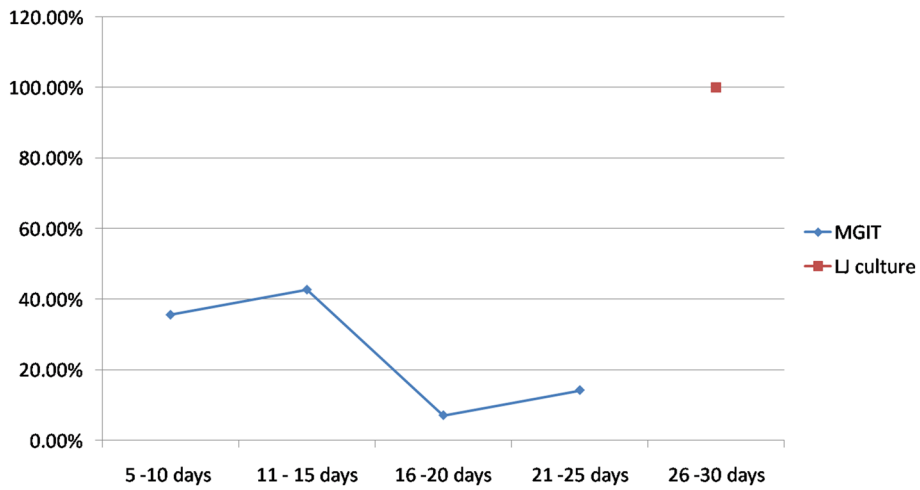

Fig. 2 Graphic comparison of the growth time of LJ and MGIT 960 culture

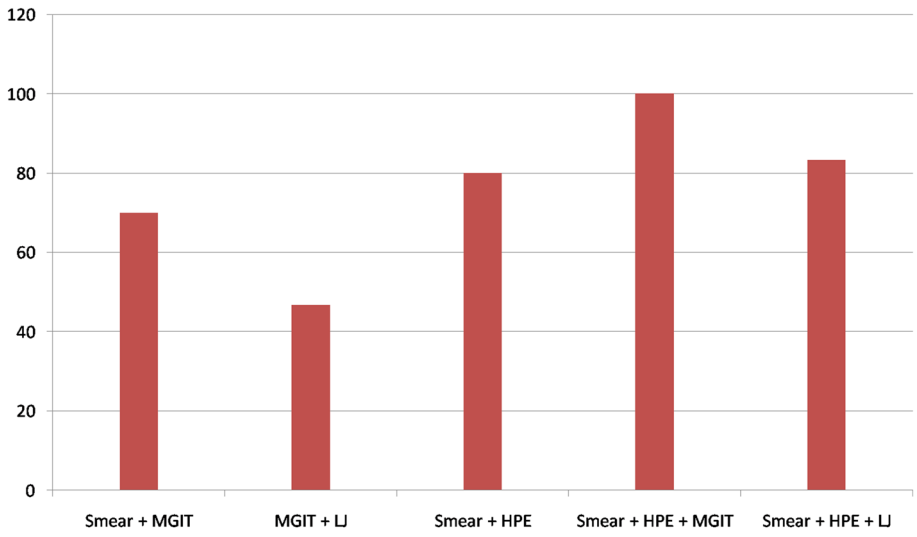

Fig. 3 Detection rates of combination of various methods (in percentage)

Table 2 Tabulated comparison of baseline characteristics of GTB infertile women from study cohort

	Total infertility cohort ($n = 300$)	GTB cohort ($n = 30$)	Statistical analysis
Average age	28.39 ± 4.41 years	29.6 ± 3.67 years	–
Duration of infertility	58.8 months	87.2 months	$p < 0.001$
Primary infertility	76%	66%	–
Kuppuswamy's socioeconomic score	15.28 ± 4.45	13.3 ± 3.61	$p < 0.01$
Average menstrual cycle length	30.8 days	36.17 days	$p < 0.001$
Scanty periods	16.29%	56.67%	$p < 0.001$
Positive past history of TB	6.29%	20%	$p < 0.01$
Ectopic pregnancy history	14.51%	50%	$p < 0.02$
ESR (> 15 mm)	35.5%	80%	$p < 0.001$

as many as 50% of the cases, the length of menstrual cycle was more than 35 days. More than half of GTB cases (56.7%) had scanty flow during their menstrual periods. The comparison of the menstrual duration and flow in GTB and total study population was statistically significant ($p < 0.001$). Also the incidence of hypomenorrhea among infertile women was found to be extremely high in GTB cases than in non-GTB ones. This finding underlines the pathogenesis of tubercular infertility by causing endometrial destruction.

Erythrocyte sedimentation rate was 23.1 ± 11.37 in positive cases and 15 ± 5.12 in negative cases, and the difference was highly significant ($p < 0.0001$). In other studies [9] also, ESR was found to be raised in as many as 90–98% cases of genital tuberculosis. Although a positive correlation between ESR and the presence of tuberculosis was found, the low specificity precludes its use as an indicator for start of antitubercular therapy in clinical usage.

Detection rates for endometrial biopsy AFB smear, MGIT culture, LJ culture and HPE were 50, 46.7, 3.3 and 33.3%, respectively. The detection rate of the conventional LJ medium was very poor as compared to other modalities. Sorlozano et al. [10] found out the sensitivity of MGIT 960 to be the best (86.5%) among a comparison of MGIT 960, MB/BacT ALERT 3D and LJ medium. They, however, found that LJ medium was best to detect non-tuberculous mycobacteria with a sensitivity of 76.2%. Rodrigues et al. [11] determined that MGIT culture was able to detect 97.9% of isolate containing *Mycobacterium tuberculosis,* while LJ medium was able to detect 57.4% of isolates. The mean growth time in culture for smear-positive cases was nine days for MGIT 960 and 38 days for LJ medium. For smear-negative cases, it was 16 days for MGIT versus 48 days for LJ medium. In our study, the mean time for growth was 12.79 ± 4.6 days. In as high as 77% cases positive by MGIT method, the growth on the tube was evident within 15 days of inoculation. LJ culture was positive in only one case and that too after a time period of 29 days.

The relative difference between the detection rates of MGIT and LJ medium encountered in our study as

compared to those determined by various authors might be explained by the difference in clinical samples submitted for analysis. The studies described above have used all kinds of clinical samples for analysis. The samples ranged from respiratory to body fluids, biopsies, exudates from various sources. We conducted an extensive review of the literature and found that no study has taken endometrial biopsies from infertility patients as clinical samples for evaluation of detection rates of MGIT and LJ media. The low detection rates determined by the present study as compared to the literature reflect upon the relative difficulty to grow isolates from endometrial samples since the endometrium is shed off cyclically every month.

Since the pathological samples are subjected to a battery of tests in routine clinical practice, sensitivities of a combination of tests were assessed. It was found that a combination of smear and MGIT culture detected around 70% of the cases as positive. MGIT and LJ media provided no added advantage over MGIT alone since the only case where LJ culture was positive had been detected by a positive MGIT culture. A combination of smear and histopathological examination excluding all kinds of culture yielded a detection rate of 80%, whereas a combination of smear examination for AFB, MGIT 960 culture and histopathological examination was able to detect all the positive cases. Thus, analyzing the diagnostic value of tests in the determination of positive cases in our study, it was found that LJ medium offered no help in diagnosis.

On the contrary, in as many as five cases out of 30 (16.7%), only MGIT culture was positive. Had MGIT 960 culture not been included in the diagnostic tests, these five cases would not have been detected by any routine method. Another important finding that is derived from the current study is no difference in MGIT positivity detected in smear-positive and smear-negative endometrial samplings. This is in contrast to other clinical samples where it has been determined that the culture failure was commoner in smear-negative samples than in smear-positive ones [12]. This also represents another probable difference between the results of endometrial samples and other materials analyzed. It can be easily concluded that addition of MGIT 960 culture media to the routine diagnostic battery in infertility patients for detection of genital tuberculosis will ensure accurate and an early diagnosis.

The absence of a gold standard test for diagnosis of tuberculosis as cause of infertility remains the biggest drawback of our study. The number of positive cases was taken to be those where any one test came out to be positive. We were not able to compute and evaluate differences between specificities of various methods since there were no false positives derived from our study. The option of using PCR as a gold standard was lucrative during the setting up of the trial protocol, but a deeper analysis revealed a large number of false positive cases by using that method. Although this may be perceived as a potential limitation, the literature reveals that no single test can be used as gold standard for detection of tuberculosis.

Authors' Contributions Study was conceptualized and designed by LKD and SS. The data were collected, analyzed and interpreted by NJ and SG. The drafting of the manuscript was done by NJ.

Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical Approval 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.

Informed Consent Informed consent was obtained from all individual participants included in the study.

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