

Episode Of Endometrial Tuberculosis Among Infertile Patients In 2019 - 2021

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Abstract

Introduction: Tuberculosis (TB) is a massive health concern throughout the world, especially in India. Genital Tuberculosis (GTB) especially endometrial tuberculosis (ETB) is the most prevalent form of extra pulmonary tuberculosis (EPTB) however it is more difficult to diagnose clinically than that pulmonary tuberculosis.

Aim of the study: To analyse the incidence of ETB among infertility cases and their clinical profile.

Materials and Method: A total of 40 clinically suspected Endometrial Biopsy specimens were collected from infertile cases, Ziehl-Neelsen staining, auramine - rhodamine staining, ESR, Mantoux test, HIV screening, and Molecular techniques were done.

Results: Out of 40 cases, 7 (17.5%) showed ETB were infected by *Mycobacterium tuberculosis* using Truenat MTB microchip PCR assay and all were rifampicin resistance. Out of which 6 (85.7%) primary infertility, 4 (57.1%) had regular menstrual cycle and 4 (57.1%) Hypomenorrhea were observed as a menstrual abnormality. Most prevalent age group found to 20-30 years – 5 (71.4%). There were no notable variations in Mantoux test, HIV screening, ESR test.

Conclusion: High specificity and sensitivity were observed by (100%) Truenat MTB microchip PCR assay compared with Ziehl-Neelsen staining and auramine - rhodamine staining. Notably, there was a lack in the diagnosis of ETB because of its asymptomatic nature as these infections were underrated. Hence, a high index of suspicion for ETB is required while investigating infertile cases due to their high frequency.

Keywords: Endometrial Tuberculosis, Infertility, Endometrial Biopsy, *Mycobacterium tuberculosis*

INTRODUCTION:

Tuberculosis (TB) is a massive health concern throughout the world, especially in India, where the disease is more prevalent ^[1]. Globally, 10 million populations (Male - 5.6 million, Female - 3.3 million, children – 1.1 million) were affected, and 1.5 million deaths were encountered by TB (including 214 000 people with HIV) reported to World Health Organization (WHO) 2020. In 2017, according to Global TB Report, India accounted for TB prevalence was 2.8 million cases for almost 1/4th of all TB cases worldwide. In 2020, the 30 nations with an elevated TB burden accounted for new TB cases of 86%. India leads the list, accounting for two-thirds of the total, followed by China and so on ^[2]. During the SARS-CoV-2 pandemic, reported TB infection diagnoses dropped 20% in 2020 and stayed 13% lower in 2021 than before the epidemic. These decreases might indicate a genuine drop in TB disease, as well as missing or delayed TB disease diagnosis owing to the SARS-CoV-2 pandemic ^[3].

Genital Tuberculosis (GTB) especially endometrial tuberculosis (ETB) is the most prevalent form of Extra Pulmonary Tuberculosis (EPTB), accounting for 27% of cases globally (range between 14 to 41%). According to several epidemiological investigations, the worldwide incidence of GTB climbed from 2.2 crores to 186 crores cases between 1995 to 2005, Indian Council of Medical Research reported the incidence of GTB climbed from 19% to 30% between 2011 to 2015 ^[4]. The influence of GTB in ascending order are vagina (1%), uterine myometrium (2.5%), cervix (5-15%), ovaries (20-30%), uterine endometrium, and fallopian tubes (95-100%). Hence ETB is more prevalent ^[5]. ETB results in infertility, menstrual irregularities, chronic pelvic inflammatory disease, and dyspareunia ^[6].

ETB is more difficult to diagnose clinically than that pulmonary tuberculosis because of the low yield of bacilli due to the paucibacillary character ^[7]. To diagnose ETB, many diagnostic modalities were used. In ETB, culture and smear microscopy shows low sensitivity ^[8]. A simple and quick diagnostic device was essential, at least for high-burden countries. Hence Real-Time Quantitative micro-PCR Analyser was developed as a rapid diagnostic approach. It is completely automated and works on the principle of polymerase chain reaction (PCR) that identifies deoxyribonucleic acid (DNA) isolated from the Endometrial Biopsy (EB) and it identifies resistance of rifampicin also with a turnaround time of about 1 hour ^[9]. There is limited data available for the incidence of ETB among infertile cases. Hence this cross-sectional study was undertaken to analyse the incidence of ETB among infertility cases by evaluating smear microscopy

using Ziehl-Neelsen staining, auramine - rhodamine staining, ESR, Mantoux test, HIV screening, and Molecular techniques.

MATERIALS AND METHODS:

A cross-sectional study was done at a tertiary care hospital, Chengalpattu, during the period of August 2019 to July 2021. A total of 40 clinically suspected EB specimens were collected from infertile cases. Institutional Ethical Committee – 1842/IEC/2019 has cleared before the commencement of the study. All clinically suspected EB among infertile cases irrespective of the past history of Anti-Tubercular Treatment (ATT) and whose ATT treatment failed for the recent course were included in this study. Specimens of pulmonary and EPTB from the patients who have a successive course of ATT were excluded. As per standard precautions, EB specimens were collected from clinically suspected ETB among infertile cases with the proper consent from the patients. In all 40 cases, Erythrocyte sedimentation rate (ESR) and Mantoux test were performed.

Microscopy: Direct smears were prepared for identification of acid-fast bacilli from EB specimens and stained using Ziehl-Neelsen staining and auramine - rhodamine staining as per the standard protocol and screened. Results were interpreted as per National Tuberculosis Eliminating Program guidelines.

Truenat MTB microchip PCR Method:

Decontamination: Specimens were centrifuged in a sterile tube at 5000 revolutions per minute for 5 minutes. EB specimens were washed with sterile water, and they were amalgamated using a tissue grinder. EB specimens were treated as per the manufacturer's pre-treatment protocol. The supernatant was discarded from a centrifuged specimen. In a Lysis buffer tube (LBT), 500µl of sediment was transferred. The amalgamated tissue samples were treated with Liquefaction buffer solution for 5 minutes and transferred to LBT and tubes are vortexed.

Extraction procedure: By using Molbio True Prep Auto Universal Cartridge, Deoxyribonucleic acid (DNA) was extracted. In the cartridge containing sample chamber (SC) and elution chamber (EC), in SC the pre-treated samples were transferred and loaded into the device. In EC, entire elute were aspirated after 20 minutes of extraction and collected in an Elute Collection Tube (ECT).

PCR procedure: A 6 µl of Elute (purified DNA) was transferred to freeze-dried PCR reagents in a micro tube and added to the lyophilized master mix present in the Truenat MTB microchip and loaded in the PCR machine. Results were obtained after 40 cycles (40 minutes).

RESULT:

A total of 40 clinically suspected EB specimens from infertile cases were enrolled in this study. Out of which, 29 (72.5%) cases had primary infertility and 11 (17.5%) cases had secondary infertility. Out of 40 cases, 28 (70%) had their regular menstrual cycle and 12 (30%) had an irregular menstrual cycle. All 40 cases were having certain menses disturbances which involves Hypomenorrhea 21 (52.5%), Oligomenorrhea 10 (25%), and menorrhagia 9 (22.5%). Out of these 40 cases, 14 (35%) were aged between 20 – 30 years, 17 (42.5%) were between 31 – 40 years, and 9 (22.5%) were between 41-45 years. Among them, 37 (92.5%) had chronic pelvic pain and the rest had no pain [Table - 1].

Table 1: Socio-Demographic And Clinical Profile Among Infertility Patients.

Characteristics	Frequency (n = 40)	Positive (n=7)	Negative (n=33)
Infertility			
<i>Primary infertility n (%)</i>	29 (72.5%)	6 (85.7%)	23 (69.7%)
<i>Secondary infertility n (%)</i>	11 (17.5%)	1 (14.3%)	10 (30.3%)
Menstrual Cycle			
<i>Regular Menstrual cycle n (%)</i>	28 (70%)	4 (57.1%)	24 (72.7%)
<i>Irregular Menstrual cycle n (%)</i>	12 (20%)	3 (42.9%)	9 (27.3%)
Age (in years)			
<i>20-30 years n (%)</i>	14 (35%)	5 (71.4%)	9 (27.3%)
<i>31-40 years n (%)</i>	17 (42.5%)	1 (14.3%)	16 (48.4%)
<i>41-45 years n (%)</i>	9 (22.5%)	1 (14.3%)	8 (24.3%)
Menses disturbance			
<i>Hypomenorrhoea n (%)</i>	21 (52.5%)	4 (57.1%)	17 (51.4%)
<i>Oligomenorrhoea n (%)</i>	10 (25%)	2 (28.6%)	8 (24.3%)
<i>Menorrhagia n (%)</i>	9 (22.5%)	1 (14.3%)	8 (24.3%)
Pain			
<i>Chronic Pelvic Pain n (%)</i>	37 (92.5%)	4 (57.1%)	33 (100%)
<i>No Pain n (%)</i>	3 (7.5%)	3 (42.9%)	0 (0%)

In all 40 cases, ESR and Mantoux tests were performed, and also HIV tests were screened with proper consent [Table - 2]. Smear Microscopy both Ziehl-Neelsen staining and auramine-rhodamine staining and molecular techniques were done in all 40 EB specimens.

Table 2: Clinical Investigations Among Infertility Patients

<i>Investigations</i>			
<i>Characteristics</i>	<i>Frequency (n = 40)</i>	<i>Positive</i>	<i>Negative</i>
<i>ESR Test n (%)</i>	40 (100%)	40 (100%)	0 (0%)
<i>Mantoux Test n (%)</i>	40 (100%)	0 (0%)	40 (100%)
<i>HIV Test n (%)</i>	40 (100%)	0 (0%)	40 (100%)

Out of which, 7 (17.5%) were positive for *Mycobacterium tuberculosis* by either of the methods, 6 (85.7%) had primary infertility whereas 1 (14.3%) had secondary infertility. Out of 7 cases, 4 (57.1%) had regular menstrual cycles and 3 (42.9%) had an irregular menstrual cycle. Among 7 positive cases, 4 (57.1%) - Hypomenorrhea was the predominant menstrual abnormality followed by 2 (28.5%) - Oligomenorrhea and 1 (14.4%) had menorrhagia. Of 7 positive cases, 6 (85.7%) had chronic pelvic pain. Out of 7 cases, 5 (71.4%) were aged 20 – 30 years, 1 (14.3%) were aged 31 – 40 years, 1 (14.3%) and was aged between 41 – 45 years [Table/Fig-1]. None of them were positive for the Mantoux test. In all 7 cases, ESR was raised (> 20 mm in an hour). None of them were reactive to the HIV test [Table - 2]. Out of 7 positive cases, the Ziehl-Neelsen staining technique shows 3 (42.9%) positives, auramine - rhodamine staining techniques show 5 (71.4%) positives and Truenat MTB microchip PCR assay shows all 7 (100%) positives, and also sensitive to rifampicin [Table - 3].

Table 3: Various Diagnostic Methods In Detecting Etb

<i>Diagnostic Methods</i>	<i>Total Samples Tested (n=40)</i>	<i>Positive (n=7)</i>
<i>Ziehl-Neelsen staining n (%)</i>	40 (100%)	3 (42.9%)
<i>Auramine - rhodamine staining n (%)</i>	40 (100%)	5 (71.4%)
<i>Truenat MTB microchip PCR assay n (%)</i>	40 (100%)	7 (100%)

DISCUSSION:

Infertility is described as a sexually active, non-contracepting couple's failure to conceive within a year and they could be primary or secondary [10]. Primary infertility is a condition when a person has not become pregnant without using birth control, and the latter occurs as a result of the incapability to become pregnant after successful parturition [11]. In developing countries, infertility was significantly contributed by GTB which affects endometrium and fallopian tubes [12]. *Mycobacterium tuberculosis* is a predominant pathogen that damages the endometrium and fallopian tubes which may not get cured by surgical and medical methods [13]. To improve fertility outcomes, early detection and treatment of GTB play a major role. Hence this cross-sectional study was undertaken to analyze the incidence of ETB among infertility cases.

Most research in Africa and other Asian nations has focused only on infertility women in order to determine the role of GTB in infertility, as well as the clinical manifestations associated with it, and the effectiveness of therapy on conceiving [14]. This present cross-sectional study indicated an overall incidence of ETB among infertility cases accounted for 17.5%. A slight variation was observed in the study of Gupta et al and their incidence showed 26.66% [15]. In several other studies, the prevalence was reported as less than 1% to 20%. It's probable that the exact incidence of ETB in our context is greater because the majority of cases are asymptomatic or present with non-specific symptoms and hence go unnoticed. Due to a lack of sensitive and specific investigations, a larger proportion of occurrences go unreported [16].

In this study, out of 7 cases, 4 (57.1%) had regular menstrual cycles and 3 (42.9%) had an irregular menstrual cycle. Similar results were correlated as per Arphitha et al study findings wherein 57.9% possessed normal menstrual cycles and others were having an irregular menstrual cycle [17]. Among 7 positive cases, 4 (57.1%) were suffering from Hypomenorrhea, which was found to be the predominant menstrual abnormality followed by 2 (28.5%) Oligomenorrhea and 1 (14.4%) menorrhagia cases. On the contrary, Thangappah et al, resulted that a predominant menstrual abnormality was observed among Oligo amenorrhea cases (59.5%) [18]. In our study, out of 7 positive cases, 6 (85.7%) were having primary infertility whereas 1 (14.3%) had secondary infertility. Identical study findings have been found in Gupta N et al (Primary infertility - 84.5% and Secondary infertility - 15.5%) [15] but the primary and secondary infertility ratio was slightly higher in Hooja et al (Primary infertility - 92.21% and Secondary infertility - 7.79%) [19]. In the present study, none of them were positive for the Mantoux test. Conversely, the study results of Raut et al [20] and Khanna A et al [21] showed 50.7% and 61.50% positivity respectively. ESR was raised (> 20 mm per hour) for all positive cases and our results were correlated with the study findings of Thangappah et al (90.9%) [18].

Many works of literature reveal that *Mycobacterium tuberculosis* is a predominant pathogen that damages the Endometrium [14]. To reduce ETB morbidity and mortality, rapid identification and treatment of ETB are necessary. In order to effectively manage the disease, it is critical to have a precise and specific approach to diagnosing ETB in EB. PCR has been compared with the smear microscopy approach in the diagnosis of ETB from EB by a different researcher. In our study findings, out of 40 suspected cases, 7 (17.5%) isolates were *Mycobacterium tuberculosis*. Out of which, 100% (7/7) showed positive Truenat MTB microchip PCR assay these results were correlated by Goel et al. which shows (100%)

high sensitivity and specificity in molecular techniques [22]. About 71.4% (5/7) were positive for auramine - rhodamine staining, and 42.9% (3/7) were positive for the Ziehl-Neelsen staining technique. On the contrary, Bhanu NV et al resulted that all were negative for both smear microscopy [23].

CONCLUSION:

This cross-sectional study indicates that the incidence of ETB in infertility cases was obtained to be 17.5% by either method. Notably, there was a lack in the diagnosis of ETB because of its asymptomatic nature as these infections were underrated. To improve the fertility outcomes, timely therapy, and diagnosis of ETB at early-stage are fortunate in pregnancies. Hence, a high index of suspicion for ETB is required while investigating infertility cases due to their high frequency.

ABBREVIATION

ATT	-	Anti-Tubercular Treatment
EB	-	Endometrial Biopsy
EPTB	-	Extra Pulmonary Tuberculosis
ESR	-	Erythrocyte sedimentation rate
ETB	-	Endometrial Tuberculosis
GTB	-	Genital Tuberculosis
MTB	-	<i>Mycobacterium tuberculosis</i>
TB	-	Tuberculosis

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Conflict of Interest

The authors declare that there is no conflict of interest.

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