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Full length article

Evaluation of Gene Xpert as compared to conventional methods in diagnosis of Female Genital Tuberculosis

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ABSTRACT

To evaluate Gene Xpert for diagnosis of Female Genital Tuberculosis (FGTB) as compared to conventional methods.

Study Design: It was a prospective study conducted over 167 cases of infertile female genital tuberculosis (FGTB) diagnosed on composite reference standard (CRS) (smear for AFB, histopathological evidence of epithelioid granuloma or definite or possible findings of tuberculosis on laparoscopy). All women underwent endometrial biopsy for AFB microscopy, culture, gene Xpert, PCR and histopathology) and laparoscopy and hysteroscopy for diagnosis and prognostication of disease. The results of Gene Xpert were compared with conventional methods in detection of FGTB. All patients were treated with 6 months course of rifampicin (R), isoniazid (H), pyrazinamide (Z) and ethambutol (E) (RHZE for 2 months, RHE for 4 months) using directly observed treatment short course strategy.

Results: Mean age, parity, body mass index and history of contact was 28.3 years, 0.28, 22.9 Kg/m^2 and 38.92% respectively. Primary infertility was seen in 87.42% cases with mean duration of 2.42 years. Menstrual dysfunctions, abdominal or pelvic pain and lump were seen in 38.92%, 14.37% and 10.77% cases. Abnormal vaginal discharge and adnexal mass were seen in 28.14% and 13.17 % cases. On diagnostic laparoscopy, definite findings of tuberculosis (beaded tubes, tuebrcles and caseous nodules) were seen in 96 (57.48%) women while probable findings of tuberculosis (pelvic or abdominal adhesions, hydrosaplinx, tubo-ovarian mass, pyosalpinx) were seen in 81 (48.50%) women.

On laboratory investigations, positive AFB on microscopy or culture was seen in 2.99% casess, PCR was positive in 47.90% gene Xpert was positive in 18.56% cases while epitheloid granuloma was seen on histopathology in 16 (9.58%) cases. Gene Xpert had sensitivity of 35.63%, specificity of 100%, positive predictive value of 100% and negative predictive value of 58.82% and diagnostic accuracy of 66.47% in the present study.

Conclusion: Gene Xpert is a very useful test to rule in tuberculosis whereas when it is negative it is not a good test to rule out tuberculosis.

Introduction

Female genital tuberculosis (FGTB) accounts for 9 per cent of all extrapulmonary TB (EPTB) cases (1,2). It is an important etiological factor for infertility with its prevalence in infertility varying from 1-19 % and upto 41% in tubal factor infertility(3–10).Fallopian tubes are most commonly involved (90–100%) cases, followed by uterus in 50–80% cases causing intrauterine adhesions (2,3–8).

It manifests as menstrual dysfunction particularly oligomenorrhea and amenorrhea, primary or secondary infertility, lower abdominal pain, chronic pelvic pain or a pelvic mass (3,4,9–12).It also causes pelvic and perihepatic adhesions (Fitz Hugh Curtis Syndrome) (13). It may mimic ovarian cancer necessitating unnecessary surgery (14).

Although gold standard in diagnosis is detection of acid fast bacilli on microscopy or culture of endometrial sampling (biopsy) or demonstration of epithelioid granuloma on histopathology of endometrial or peritoneal biopsy, they are positive in few cases

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only due to paucibacillary nature of disease (3,5,8,15).Polymerase chain reaction has high sensitivity but high false positivity and alone is not sufficient to make the diagnosis (15). Radiology is more useful in adnexal masses but cannot definitely diagnose FGTB (16,17). Laparoscopy is the most reliable tool to diagnose genital tuberculosis especially tubal, ovarian and peritoneal disease and to see tubal patency (18).

In recent times, attention has been devoted to new nucleic acid amplification diagnostic technologies, owing to their rapidity, sensitivity, and specificity (19). In December 2010, the World Health Organization endorsed the use of Gene Xpert MTB/RIF (Xpert; Cepheid, Sunnyvale, CA), an automated nucleic acid amplification test for detection of tuberculosis and Rifampicin resistance for regions with high rates of human immunodeficiency virus (HIV)-tuberculosis co-infection or multidrug-resistant tuberculosis and is useful for both pulmonary and extra pulmonary TB with high specificity(19,20).

We performed a study to evaluate the diagnostic accuracy, sensitivity and specificity of Gene Xpert in diagnosis of FGTB.

Materials and Methods

It was a prospective cross sectional study in women presenting with infertility to a tertiary referral centre between January 2017 to December 2019. A total of 1225 women of infertility between 20 to 41 years were screened by history taking, examination and by endometrial biopsy. Diagnostic laparoscopy and hysteroscopy was done in selected cases with clinical suspicion of TB or with positive polymerase chain reaction (PCR) but negative acid fast bacilli on microscopy or culture or epithelioid granuloma on histopathology. All women presenting with infertility and found to have FGTB on composite reference standard (CRS) (see below) were taken in this study.

The study was approved by the ethical committee of the Institute. Written informed consent was taken from all the subjects. A detailed history was taken from all cases. Detailed physical and gynecological examination was done in all cases. Baseline investigations were done in all cases. Endometrial aspiration was done in all the patients in premenstrual phase for acid fast bacilli (AFB) microscopy or culture, polymerase chain reaction (PCR) Gene Xpert and histopathological examination for epithelioid granuloma. In the present study Cepheid (Sunnyvale, California, USA) Gene Xpert machine was used (Fig. 1) in which separate Gene xpert cartridges (Fig. 2) were used for each patient.

Fig. 1. Cepheid Gene Xpert machine.

Fig. 2. Gene Xpert Cartridge.

Diagnostic laparoscopy with or without hysteroscopy was performed were performed on all 167 patients in post menstrual phase for TB lesions. Diagnostic laparoscopy was performed using Karl Storz laparoscope and whole of abdominal cavity was carefully evaluated for any TB lesions and to rule out any other disease like endometriosis.

Composite Reference Standardwas taken as the gold standard to diagnose FGTB and included smear or culture positive for AFB, histopathology suggestive of epithelioid granuloma, diagnostic laparoscopy with definite findings of FGTB (Presence of tubercles, caseous nodule or beaded tubes) or probable findings of FGTB (Straw colored fluid in POD; extensive dense pelvic, peri-tubal, peri-ovarian adhesions; hydrosalpinx; tubo-ovarian mass; thick fibrosed tubes; mid tubal block and peri hepatic adhesions, hyperemia of tubes). Due to financial and logical constraints testing for Chlamydia trachomatis and Neisseria Gonorrhea was not done as these diseases and endometriosis are known to cause perihepatic adhesions also called Fitz Hugh Curtis Syndrome (FHCS) and pelvic adhesions.

The patients who were found to have FGTB were started on free anti tubercular treatment by the DOTS (Directly Observed Treatment Short course) Centre using Rifampicin (R), Isoniazid (H), Pyrazinamide (Z) and ethambutol (RHZE) for 2 months followed by daily Rifampicin, Isoniazid and ethambutol (RHE) for 4 months, Gene Xpert was then evaluated as diagnostic test in comparison to other methods.

Sample size calculation

Various previous studies have shown about 10% prevalence of female genital tuberculosis (FGTB) in infertility in India was taken for sample size calculation. In pulmonary specimens, the sensitivity of Gene Xpert is reported to be in the ranges of 86-100%. Therefore, for the present study the sensitivity of Gene Xpert for genital TB is assumed to be 90%. Earlier study results showed 10% incidence of infertility with TB and for sample size calculation the prevalence of infertile with TB is assumed to be same 10%. Anticipating that Gene Xpert is a better diagnostic test for diagnosing infertility with TB negative, specificity of test is

assumed to be 90%. Based on the above assumptions with an error margin of absolute 5% (precision), the required sample is 145. Anticipating, 10% prevalence of TB in infertile woman, we needed to screen about 1000 suspected cases. For specificity of 90.0%, all the enrolled patients were studied. To take cases of loss to follow up, we screened 1225 women and took 167 cases.

Statistical analysis

- Data Analysis was carried out using STATA software v 12.0. Continuous variables were tested for normality assumption using KOLMOGOROV-SMIRNV test.
- Descriptive statistics such as Mean, Standard deviation, range values were carried for normally distributed dates. Comparison of two groups means were tested using Student's 't' independent test.
- Categorical data were presented as frequency and percentage values. Comparison of categorical values were tested using Chi-Square/ Fischer's exact test.
- To assess the Diagnostic Accuracy between Composite Reference Standard and the Tests like Gene Xpert and Polymerase Chain Reaction, methods such as Sensitivity, Specificity, positive Predictive Value, Negative Predictive Value, Likelihood Ratio (+), Likelihood Ratio (-) were calculated using 95% Confidence Intervals. For overall Diagnostic Accuracy, it was calculated using 95% Confidence Intervals.
- Clinical value of interpreting likelihood ratio (LR) LR + and LR- play a significant role in clinical interpretation of a diagnostic tests. LR + between 1-5 is a useless test to rule in disease, whereas 5-10 it is a moderately useful test and greater than 10 is a very useful test. On the other hand when LR- is less than 0.1 it is a very useful test to rule out disease, between 0.1 - 0.5 a moderately useful test while a LR- between 0.5 - 1 it is a useless test.

Results

A total of 1225 women with infertility coming to a tertiary referral centreover 3 years were screened for female genital tuberculosis, out of which 167 women were found to have FGTB on a composite reference standard (CRS) with incidence of FGTB in infertility patients in the study being 13.63%. The age ranged between 20 -40 years with mean being 28.3 ± 4.8 years while body mass index ranged from 17.9 to 32.1 kg/m^2 with mean being 22.9 ± 2.67 kg/m^2. History of TB contact was seen in 65 (38.92%) women with 136 (81.43%) women being BCG vaccinated. The mean parity was 0.28 ± 0.11. A total of 146 (87.42%) women had primary infertility while 21 (12.57%) women had secondary infertility with mean duration of infertility being 2.42 ± 1.45 years (range 1-7 years). A total of 102 (61.07%) women had normal menstruation while 65 (38.92%) had menstrual abnormalities in the form of heavy periods (1.79%), dysmenorrhea (17, 10.17%), oligomenorrhoea (22, 13.17%), hypomenorrhoea (20, 11.97%) and secondary amenorrhoea (3, 1.79%) cases. On clinical examination, pallor was seen in 18(10.77%) women, lymphadenopathy in 6(3.59%) cases, chest crepitations in 6(3.59%) cases and abdominal distention in 3 (1.79%) cases. On gynecological examination, abnormal vaginal discharge was seen in 47(28.14%) cases while adnexal mass was seen in 22(13.17%) cases. On routine investigations, anemia (Hb<11 g/dl) was seen in 28(16.76%) cases, mean erythrocyte sedimentation rate (ESR) was 32.10 ± 12.78 mm in Ist hour while mean leukocyte count was 5425 ± 2787 per cubic mm and infectious Mantoux test (>10 mm) was seen in 74(44.31%) cases. Chest X ray showed old healed TB lesions in 7(4.19%) cases and mediastinal lymphadenopathy in 8(4.79%) cases.

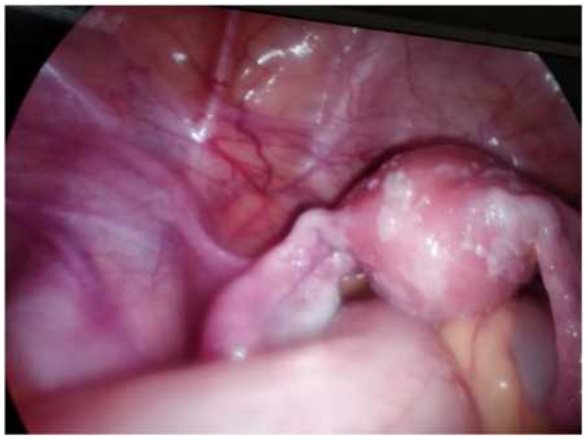

Fig. 3. Laparoscopy showing tubercles on uterus and fallopian tube in a case of FGTB.

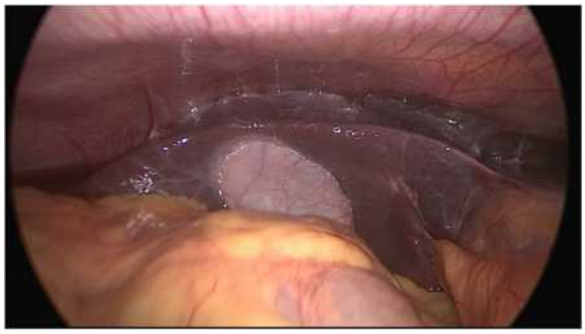

Fig. 4. Laparoscopy showing perihepatic adhesions (Fitz Hugh Curtis Syndrome) with Sharma's hanging gall bladder sign in a case of FGTB.

Various laparoscopic findings were tubercles on uterus (58, 34.73%) cases (Fig. 3), on tubes in 28(16.76%) (Fig. 3) cases and on ovary in (12, 7.18%) cases. Perihepatic adhesions or (Fitz Hugh Curtis Syndrome) (FHCS) was seen in 51(30.53%) cases (Fig. 4).

Bilateral tubal block was seen in 38 (22.75%) cases while unilateral tubal block was seen in 29 (17.36%) cases. Unilateral hydrosalpinx was seen in 29 (17.36%) (Fig. 5) while bilateral hydrosalpinx was seen in 29 (17.36%) cases. Caseous nodules were seen in 16(9.58%) cases (Fig. 5) and congested and hypermeic tubes were seen in 22(13.17%) cases (Fig. 6).

Microbiological and histopathological results in the study are shown in Table 1 with acid fast bacilli (AFB) on microscopy or culture 4 (2.39%) cases. Positive gene Xpert (CBNAAT) was

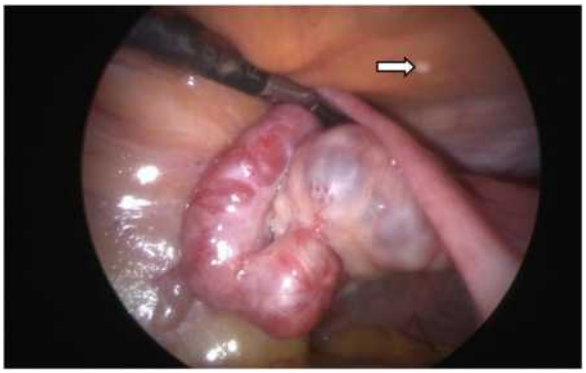

Fig. 5. Laparoscopy showing caseous nodule (arrow) with left sided hydrosalpinx with fimbrial block with enlarged ovary in a case of FGTB.

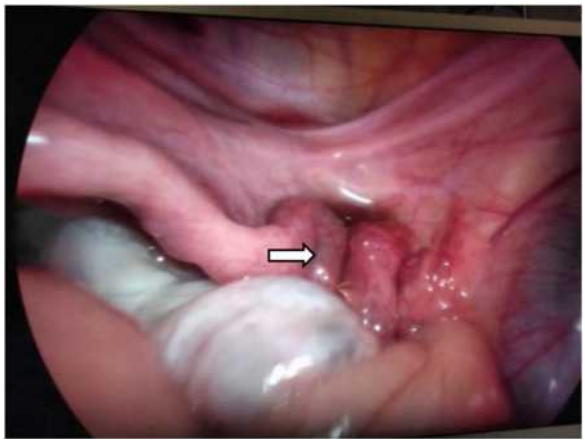

Fig. 6. Diagnostic laparoscopy showing congested and hypermeic tube (arrow) in a case of FGTB.

observed in 31 (18.56%) cases while it was negative in 136 (81.44%) cases. On histopathological findings, epithelioid granuloma was observed in 16(9.58%) cases.

The statistical accuracy of Gene Xpert in detecting TB in relation to composite reference standard (CRS) is shown in Table 2. Thus, sensitivity was 35.63% (CI 26.37-46.11), specificity was 100% (CI 95.42-100). positive predictive value was 100% (CI 88.97-100), diagnostic accuracy was 66.47% (CI 59.01-73.19), negative predictive value was 58.82 (CI 50.42-66.74).

As shown in Table 2, likelihood ratio (LR) of a positive test was undefined which makes the Gene Xpert test a very useful test to rule in disease whereas when it is negative, it is not a good test to rule out the disease. Various findings of other tests in positive gene Xpert cases are shown in Table 3.

Thus, out of 31 Gene Xpert positive cases, 22 cases (70.96%) had definite findings of FGTB on laparoscopy (tubercles, caseous nodules or beaded tubes) while 9 (29.03%) cases had probable findings of FGTB` on laparoscopy (pelvic or abdominal adhesions, hydrosalpinx etc). Positive PCR was seen in 29(93.54%) cases while epithelioid granuloma was seen in 16 (51.61%) cases and AFB on microscopy or culture was seen in 4 (12.90%) cases. Hence, Gene Xpert when positive was almost certainly a diagnostic of FGTB but its sensitivity was lower (only 35.63%).

Discussion

Female Genital Tuberculosis (FGTB) causes infertility through effects on fallopian tubes and ovaries (2–4). Diagnosis is through detection of acid fast bacilli on microscopy or culture or detecting epithelioid granuloma on histopathology of endometrial aspirate or biopsy and on imaging methods (2,3,15–17,22).

Diagnostic laparoscopy and hysteroscopy are very useful in diagnosis of FGTB due to direct visualization of TB lesions (18,21,23) but should be performed by experienced gynecologists

Table 1
Microbiological and histopathological results of endometrial biopsy (N = 167).

S.No	Test	No	Lower-Upper 95%CIs / Percentage
1.	AFB on microscopy or culture	4	2.39
	Sensitivity	4.598%	1.802%-11.23
	Specificity	100%	95.42%-100
	Positive Predictive value	100%	51.01%-100
	Negative predictive value	49.08%	41.52%-56.69
2.	Positive PCR	80	47.90
3.	Negative PCR	87	52.09
4.	Positive Gene Xpert	31	18.56
5.	Negative Gene Xpert	136	81.44
6.	Histopathology		
	i) Normal secretory endometrium	128	76.64
	ii) Proliferative endometrium	23	13.77
	iii) Epithelioid granuloma	16	9.58
	Sensitivity	18.39%	11.65-27.81
	Specificity	100%	95.42-100
	Positive Predictive value	100%	80.64-100
	Negative predictive value	52.98%	45.04-60.77

Table 2
Observations of Gene Xpert vs Composite Reference Standard in FGTB Patients.

Single Table Analysis			
Positive Negative Total			
Positive	31	0	31
Negative	56	80	136
	87	80	167
Parameter	**Number**	**Lower-Upper 95%CIs / Percentage**	
Positive	31	18.56%	Wilson Score
Negative	56	33.53%	Wilson Score
Sensitivity (%)	35.63%	26.37-46.11%	Wilson Score
Specificity (%)	100%	95.42-100%	Wilson Score
Positive Predictive Value (%)	100%	88.97-100%	Wilson Score
Negative Predictive Value (%)	58.82%	50.42-66.74%	Wilson Score
Diagnostic Accuracy	66.47	59.01-73.19	Wilson Score
Likelihood ratio of a Positive Test	Undefined	Undefined	Wilson Score
Likelihood ratio of a Negative Test	0.6437	(0.6215 - 0.6666)	Wilson Score

Likelihood ratio (+) is undefined as there were no false positives for Gene Xpert.

Table 3
Various Findings in positive Gene Xpert samples N = 31.

Findings	Percentage % (n = 31)	
	No	%
Definitive findings of FGTB on laparoscopy	22	70.06
Probable findings of FGTB on laparoscopy	9	29.03
Positive PCR	29	93.54
Epithelioid Granuloma on histopathology	16	51.61
AFB on microscopy	4	12.90

due to more difficulty and risk of higher complications (24,25). Composite reference standard is usually taken for diagnosis of FGTB which takes into consideration positive AFB smear or culture, histopathological evidence of epithelioid granuloma and definite or probable findings of TB on laparoscopy (26).

PCR though sensitive and specific test has high false positive rate and is not recommended by National Tuberculosis Elimination Program of India (NTEP) (27,28). Newer molecular methods like Gene Xpert MTB/RIF assay and loop isothermal mediated amplification method (LAMP) assay have been used on endometrial biopsy to diagnose FGTB with varying accuracy (29–31).

In the present study with CRS as gold standard for diagnosis of FGTB, Gene Xpert was positive in only 18.56% cases with sensitivity of only 35.63% (CI 26.37-46.11) but a very high specificity of 100% with very high positive predictive value of 100% and diagnostic accuracy of 66.47%. Most gene xpert positive cases had other definite tests also positive like positive AFB on microscopy, positive histopathology and definite or probable findings of TB on laparoscopy. Considering the high sensitivity (almost 100%) of Gene xpert, invasive diagnostic laparoscopy can be avoided in patients with positive Gene Xpert report and anti-tuberculous treatment can be commenced as a positive test is almost certainly diagnostic of FGTB. However, due to its low sensitivity (35-63%) in the present study, it may miss cases of FGTB. Hence, if gene Xpert is negative but there is suspicion of FGTB on history, clinical examination and non specific tests like PCR, then laparoscopy should be performed to rule out FGTB.

In lieu of almost 100 % specificity of Gene Xpert, we postulate that Gene Xpert should be a part of composite reference standard for diagnosis of FGTB in clinical practice.

Although multi drug resistant female genital tuberculosis (MDR TB) has been reported by us before (32), none of the cases on gene Xpert had rifampicin resistance in the present study. All women with FGTB in the present study were treated with 6 months course of anti-tubercular therapy as per recommendations of National Tuberculosis Elimination program (NTEP) of India and results of our previous study(28,33).

The strength of the study is that it is the first large study on role of Gene Xpert for diagnosis of FGTB in which 1225 women with infertility were screened of which 167 women were found to have female genital TB on compose reference standard (CRS). The study clearly demonstrates low sensitivity (35.63%) but very high specificity (100%) of Gene Xpert as compared to conventional methods of diagnosis.

The limitation of the study are that we couldn't perform testing for Chlamydia trachomatis and Neisseria gonorrhea due to financial and logical constraints. It may be possible that some of the findings like FHCS, pelvic adhesions and hydrosalpinx etc could have been due to non-tuberculous infections as we dependend upon composite reference standard and laparoscopic findings for diagnosis of FGTB.

To conclude gene Xpert appears to be highly specific but less sensitive test for diagnosis of female genital TB. The LR + of the study makes the Gene Xpert test a very useful test to rule in disease, whereas the LR- result suggests that it is not a good test to rule out the disease. However large multi-centre studies are recommended before its routine recommendation in clinical practice.

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Declaration of Competing Interest

The authors reported no declarations of interest.

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Six months versus nine months anti-tuberculous therapy for female genital tuberculosis: a randomized controlled trial

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ABSTRACT

Objective: To compare six months versus nine months anti-tuberculous therapy in patients of female genital tuberculosis.
Study design: It was a randomized controlled trial in a tertiary referral center teaching institute on 175 women presenting with infertility and found to have female genital tuberculosis on clinical examination and investigations. Group I women (86 women) were given 9 months of intermitted anti-tuberculous therapy under directly observed treatment short course (DOTS) strategy while Group II (89 women) were given 6 months of anti-tuberculous therapy under DOTS. Patients were evaluated for primary end points (complete cure, partial response, no response) and secondary end points (recurrence rate, pregnancy rate) during treatment. All patients were followed up further for one year after completion of therapy to assess recurrence of disease and further pregnancies.
Results: Baseline characteristics were similar between two randomized groups. There was no difference in the complete clinical response rate (95.3% vs 97.7%, $p = 0.441$) between 9-months and 6-months groups. Four patients in 9-months group and two patients in 6-months group had recurrence of disease and required category II anti tuberculous therapy ($p = 0.441$). Pregnancy rate during treatment and up to one year follow up was also similar in the two groups (23.2% vs 21.3%, $p = 0.762$). Side effects occurred in 27(31.4%) and 29(32.6%) in 9-months and 6-months of therapy and were similar ($p = 0.866$).
Conclusions: There was no difference in complete cure rate, recurrent rate and pregnancy rate for either 6-months or 9-months of intermittent directly observed treatment short course anti-tuberculous therapy in female genital tuberculosis.
Clinical trial registration: The trial was registered in clinicaltrials.gov with registration no: **CTRI/2009/091/001088**.

Introduction

World Health Organization declared tuberculosis a global emergency in 1993 and promoted a strategy of providing anti-tuberculous therapy under direct observation 'Directly Observed Treatment Short Course' (DOTS) [1]. As per global tuberculosis report 2015, 3.2 million TB cases occur in women in a year with 480,000 deaths amongst them [2]. DOTS has also been adopted by the Government of India under the Revised National Tuberculosis Control Programme and is now routinely available for both pulmonary and extra-pulmonary tuberculosis throughout India with almost 86% cure rate [3].

Female genital tuberculosis is an important variety of extra-pulmonary TB causing significant morbidity and short and long term sequelae especially infertility in infected women [4,5]. Female genital TB is an important cause of infertility and recurrent implantation failure in developing countries [6,7]. It causes infertility due to blockage of fallopian tubes or through involvement of endometrium with Asherman's syndrome or through effect on ovarian function [8,9]. It also causes pelvic and

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perihepatic adhesions [10]. It may mimic ovarian cancer necessitating unnecessary surgery [11]. It is also an important cause of ectopic pregnancy in India [12].

Although gold standard in diagnosis is detection of acid fast bacilli on microscopy or culture on endometrial sampling (biopsy) or on demonstration of epithelioid granuloma on histopathology on endometrial or peritoneal biopsy, they are positive in few cases only due to paucibacillary nature of disease [13]. Polymerase chain reaction has high sensitivity but high false positivity and alone is not sufficient to make the diagnosis [14,15]. Imaging methods like ultrasound, computerized axial tomography, magnetic resonance imaging, positron emission tomography are more useful in tuberculous tubo-ovarian masses but cannot make definite diagnosis of female genital tuberculosis. Laparoscopy is the most reliable tool to diagnose genital tuberculosis especially tubal, ovarian and peritoneal disease and to see tubal patency [16,18]. However, dye test should be avoided in case of frank female genital tuberculosis for the fear of risk of further dissemination of disease. Hysteroscopy can also diagnose female genital tuberculosis by detecting pale cavity, tubercles and intrauterine adhesions. Treatment is medical with combination chemotherapy with surgical treatment being rarely required only as drainage of abscess [4,23]. American Thoracic Society [23] and National Institute of Clinical Excellence Guidelines recommended standard daily regimen of anti-tuberculous medicines. However, World Health Organization has recommended directly observed treatment short course (DOTS) treatment in which drugs are given intermittently thrice a week under direct supervision [1]. A 6-month anti-tuberculous drugs regimen using a combination of rifampicin, isoniazid, pyrazinamide and ethambutol for 2 months, followed by rifampicin and isoniazid for 4 months cures about 90% human immunodeficiency virus negative patients is universally accepted treatment for drug-susceptible, active tuberculosis especially pulmonary TB [1,2]. Poor compliance and irrational prescription of anti-tuberculous drugs enhances the emergence of drug resistant and multi drug resistant tuberculosis which is more difficult to treat necessitating toxic and expensive medicines for longer duration [25].

The duration of treatment and compliance are important issues in managing female genital tuberculosis. The duration of treatment of female genital tuberculosis, whether six months or longer continues to be controversial and debatable as there are no proper randomized controlled trials on treatment of female genital tuberculosis for 6 months or longer treatment [4,10]. The old studies performed in 1990 and 1992 recommended short course chemotherapy for 9 months to 1 year for female genital tuberculosis [26,27] but there is no direct comparison of 6 months versus 9 months therapy in literature. Although most guidelines recommend six-months treatment for pulmonary and extra pulmonary tuberculosis, evidences to support such recommendations are weak [1,2]. Furthermore, in spite of recommended 6-months treatment duration, many gynecologists continue to treat such patients for longer duration of 9 months or even up to 1 year [28].

Although the efficacy of therapy for pulmonary tuberculosis using DOTS strategy is well established there is lack of data on its efficacy in treatment of extra-pulmonary tuberculosis especially female genital tuberculosis [29].

We, therefore, conducted a randomized controlled trial to determine the efficacy of intermittent short-course anti-tuberculous drugs regimen for 9-months and 6-months under DOTS strategy for treatment of female genital tuberculosis with infertility. A secondary objective was to determine the pregnancy rate and differences in the recurrence rate at one year follow up after completion of primary treatment in the two groups.

Materials and methods

Study design and over sight

It was a prospective randomized controlled trial (between May 2010 and April 2014) conducted in a tertiary referral center. The study was approved by the Ethics Committee of the Institute and was conducted in accordance with the International Conference on Harmonization Good Clinical Practice Guidelines. The study was designed by the principal investigator (first author) with the involvement of academic investigators and a biostatistician in consultation with the funding agency. The first author authenticates the data accuracy and analysis and the fidelity of the study in accordance to the protocol. The randomized control trial was registered in clinical trials.gov with registered number **CTRI/2009/091/001088**.

The enrollment was done as per consort flow diagram.

Study population

Consecutive newly diagnosed patients (age between 20 years and 40 years) with female genital tuberculosis with infertility were recruited after obtaining their informed written consent. Women who took anti-tuberculous therapy during last 5-years; those with human immunodeficiency virus infection, malignancies or significant co-morbidities; those allergic to drugs and those not willing to participate and those who were pregnant or lactating at the time of enrollment were excluded. Patients who had received any investigational agents during past 6 months, were also excluded.

Clinical data collection

All patients underwent a detailed clinical and laboratory evaluation including hematological, biochemical tests, Mantoux test, chest radiograph and ultrasound of pelvis. Hysterosalpingography was not routinely performed but its findings were collected whenever it was already done from outside. Diagnostic video laparoscopy and video hysteroscopy (using glycine as distended medium) were performed whenever possible.

During laparoscopy, a careful inspection was performed of whole pelvic and abdominal cavity especially uterus, fallopian tubes, ovaries, uterovesical pouch, pouch of Douglas, intestines, peritoneum, liver and gall bladder for any tuberculous lesions like tubercles, shaggy areas, hydrosalpinx, pyosalpinx, beading of tubes, pelvic, abdominal or perihepatic adhesions, patency of tubes, tuberculosis of ovaries and all the findings were carefully recorded.

During hysteroscopy uterine cavity and both ostia were carefully inspected for color of endometrium, endometrial glands opening and for any tuberculous findings like tubercles, shaggy areas and intra-uterine adhesions. Endometrial biopsy was performed in all women in premenstrual phase and the specimens were sent both for histological and microbiological tests. For histology, biopsies were fixed in 10% buffered formaldehyde (Formaline) and for microbiological tests [culture, staining for acid fast bacilli and polymerase chain reaction were collected in sterile normal saline].

Diagnostic criteria for female genital tuberculosis

A '*definite*' diagnosis of female genital tuberculosis was made in presence of the followings: (i) acid fast bacilli on smear or culture of endometrial biopsies; (ii) presence of epithelioid granuloma on histopathological examination of endometrial biopsy; (iii) definite findings of tuberculosis on laparoscopy and hysteroscopy.

Presumptive (probable) diagnosis of female genital tuberculosis – positive polymerase chain reaction alone was not taken for diagnosis of female genital tuberculosis but these women if had positive findings on ultrasound (tubo-ovarain mass), hysterosalpingography (tuberculosis findings), laparoscopy (presumptive findings of tuberculosis) were taken as presumptive female genital tuberculosis and were treated.

Randomization

The randomization was done using computer-generated table in 1:1 ratio by a person not involved in the study.

Concealment of randomization

The randomized treatment allocation (i.e. 9- or 6-months) were printed and concealed in sealed envelopes bearing the serial number of the patients.

Intervention

Patients fulfilling inclusion and exclusion criteria were randomized into two groups: Group I and Group II received Revised National Tuberculosis Control Program of India category I treatment for 9-months and 6-months, respectively. In this regimen, the intensive phase included four drugs (rifampicin 450 mg [600 mg with weight >60 kg] isoniazid 300 mg, pyrazinamide 1500 mg and ethambutol 800 mg) three times a week for 2-months. The continuation phase included rifampicin and isoniazid for further 4-months in those randomized to 6-months and for 7-months to those randomized to receive 9-months of therapy.

Patients were registered with the DOTS center at near their residence areas and drugs were administered to them under supervision of health care provider at DOTS center. All the patients were followed up at clinics of respective centers and at the institute at regular intervals.

Follow-up and management of side effects

All the side effects were recorded. liver function tests were done at 2 months intervals. Drug-induced hepatitis was managed by substituting hepatotoxic drugs (isoniazid, rifampicin, pyrazinamide) by quinolones and streptomycin. While monitoring liver function tests closely first-line drugs were gradually reintroduced after resolution of hepatitis. The total duration of interruption due to hepatitis was compensated by prolongation of the treatment duration.

Adherence to treatment

All the women received treatment under direct supervision through the network of DOTS centers. They were asked to report to the enrolling center at 2-monthly intervals. Nodal officer and medical social workers kept track of all the patients for compliance. The drug intake was recorded in a diary and a drug intake for more than 80% of days was considered compliant. Poorly compliant patients were counseled again. Non-compliant patients were excluded from study.

Assessment at end of treatment

Randomized patients were clinically evaluated for symptoms resolution. All the women were subjected to the same investigations by which their initial diagnosis was made. Then all the women (except those who fell pregnant or lost to follow up or declined the second investigation) were subjected to ultrasound scan, relook laparoscopy, hysteroscopy and repeat endometrial biopsy (for acid fast bacilli microscopy, acid fast bacilli culture, polymerase chain reaction and histopathological examination. During repeat laparoscopy, adhesiolysis was performed for peritubal, pelvic adhesions to free tubes in selected cases (not for deep and dense adhesions with blocked tubes). Similarly hysteroscopic adhesiolysis was performed in grades I–III adhesions at repeat hysteroscopy.

Follow-up for one year for recurrence of tuberculosis

Patients were followed up three-monthly for one year after completion of the primary treatment. Those who failed to visit the clinics were contacted telephonically and interviewed for recurrence of disease and pregnancy outcome.

Outcome measures

Complete response was defined as resolution of symptoms, disappearance of acid fast bacilli on microscopy or culture, disappearance of tuberculous granuloma on histopathology and disappearance or healing of active tuberculous lesions on relook laparoscopy and hysteroscopy. A *partial response* was defined as resolution of clinical manifestations and partial disappearance of tuberculous lesions at end of therapy. *Non-response* was defined persistent clinical symptoms, persistence of acid fast bacilli on microscopy or culture, persistence of tuberculosis granuloma on histopathology or persistence of active tuberculosis lesion on relook laparoscopy or hysteroscopy at end of therapy. In patients not agreeing for end of treatment laparoscopy and hysteroscopy, endometrial biopsy or where repeat tests could not be performed due to pregnancy, the response in them was defined as 'Complete clinical response' if there was complete symptomatic response with normalization of biochemical and hematological tests.

Statistical analysis

The study was designed to test the hypothesis that 9-months of anti-tuberculosis drugs is more efficacious than 6-month regimen using DOTS strategy. To demonstrate a difference in the complete clinical response rate of 15% between 9-months and 6-months treatment and considering a power of 80% and 5% significance, 152 patients (76 patients in each group) were required. With an expected loss of 10% of patients on follow-up and some women conceive during treatment, 175 women were recorded. Statistical analysis was conducted using STATA software, version 12.0. Continuous variables were tested for normality assumption using Kolmogrov–Smirnov tests. Mean values of variables found to be approximate normal were compared using Student's '*t*' independent tests. Non parametric category of variables was compared using Chi Square/Fischer's Exact test. For all statistical tests, 2 tabled probability of $p < 0.05$ was considered for statistical significance.

Results

Study cohort

Of 285 patients who were screened, 110 were excluded and 175 were included in this study (Fig. 1). Of 175 eligible patients, 86 and 89 patients were randomized to 9-months (Group I) and to 6-months regimen (Group II), respectively.

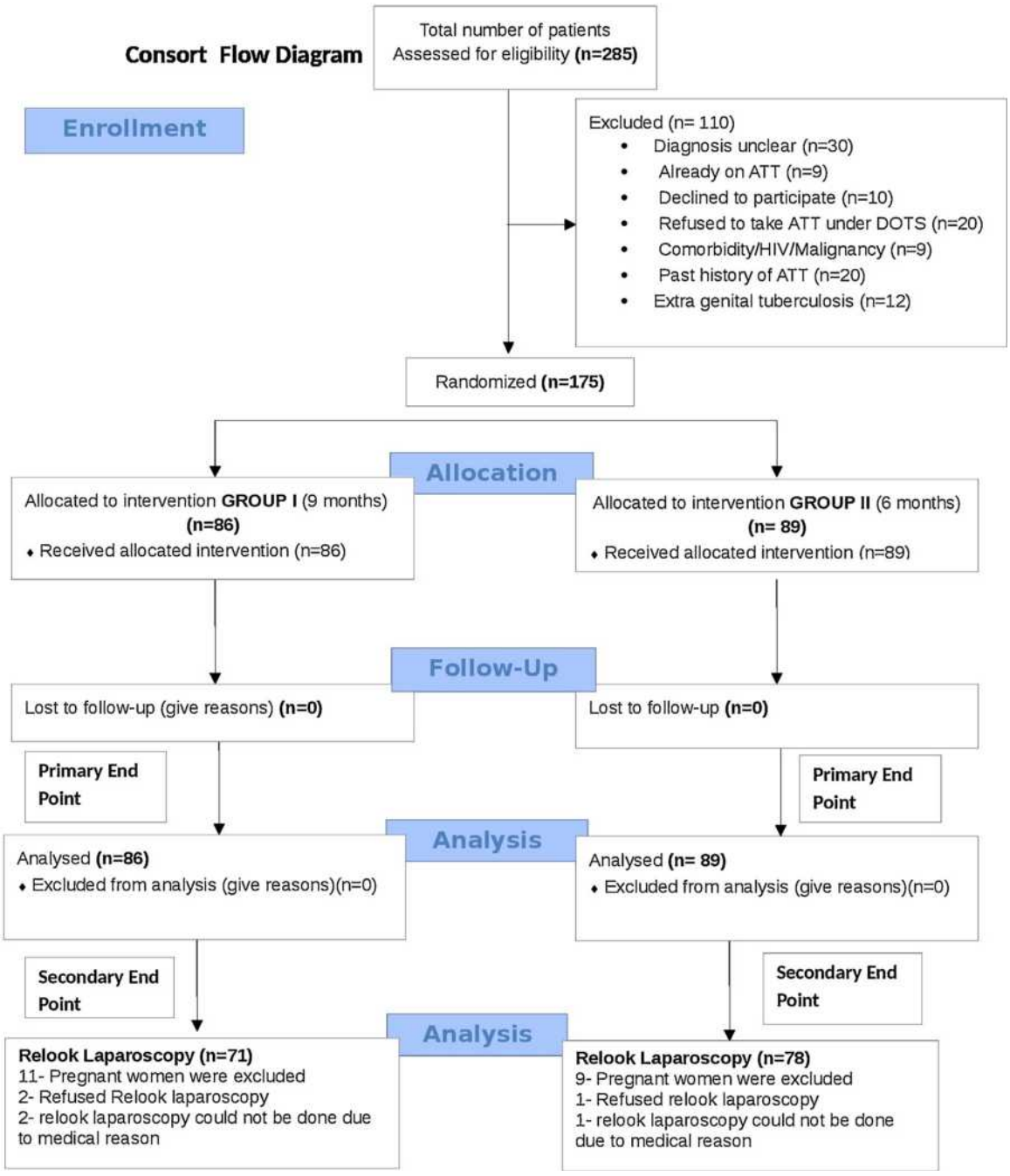

Fig. 1. Consort chart.

Characteristics of the patients and the disease at baseline

There was no difference in the demographic, clinical, hematological and biochemical parameters at baseline between the two groups (Table 1).

Endometrial biopsy findings

Endometrial biopsy findings in the two groups are shown in Table 1. The definitive findings of female genital tuberculosis were presence of acid fast bacilli on microscopy, positive acid fast bacilli culture and tuberculous granuloma on histopathology, positive polymerase chain reaction alone was not taken sufficient for diagnosis of female genital tuberculosis. An equal number of cases had definitive diagnosis of female genital tuberculosis in the two groups.

Radiological (ultrasound and hysterosalpingography findings)

Transvaginal ultrasound was performed in all the cases. The various abnormal ultrasound findings were hydrosalpinx, pelvic inflammatory disease, enlarged uterus, tubo-ovarian mass, enlarged cystic ovary which were equal in two groups. Only in some cases findings included beaded tubes, tubal block, hydrosalpinx, bicornuate uterus. The findings are given in Table 2. Thus, an equal number of findings of FGTB were present in the two groups.

Endoscopic (laparoscopic and hysteroscopic findings)

Various laparoscopic and hysteroscopic findings in the two groups are shown in Table 3. The various findings of FGTB like presence of tubercles, shaggy areas, caseous nodules, hydrosalpinx,

Table 1
Baseline demographic, clinical and laboratory characteristics of patients randomized in Group I and Group Ii.

Characteristics		Patients Randomized Group I (9 Months ATT) ($n=86$)	Patients Randomized Group II (6 Months ATT) ($n=89$)	p value
Mean age(±SD)		28.8 ± 4.4	29.1 ± 4.7	0.612
Body mass index (±SD)		22.4 ± 3.8	23.2 ± 3.6	0.527
Clinical features, n (%)	Weight loss	66 (76.7)	68 (76.4)	0.958
	Anorexia	58 (67.4)	61 (68.5)	0.875
	Fever	52 (60.5)	56 (62.9)	0.738
	Chronic cough	12 (13.9)	13 (14.6)	0.902
	Abdominal or pelvic pain	34 (39.5)	37 (41.6)	0.784
	Abdominal or pelvic lump	4 (4.6)	5 (5.6)	0.990
	Menstrual dysfunction	68 (79.1)	70 (78.6)	0.946
	Oligomenorrhea	34 (39.5)	37 (41.6)	0.784
	Hypo menorrhea	26 (30.2)	25 (28.1)	0.755
	Amenorrhea	5 (5.8)	6 (6.7)	0.999
	Menorrhagia	3 (3.5)	2 (2.2)	0.679
	Infertility	76 (88.4)	80 (89.9)	0.747
	Primary	64 (74.4)	66 (74.2)	0.968
	Secondary	22 (26.6)	23 (24.7)	0.902
Investigations	Anemia (Hb≤11) (n%)	11 (12.8)	12 (13.5)	0.892
	Mean (±SD) leukocyte count	5615 ± 2932.0	5522.2 ± 3041.2	0.854
	ESR infectious (>20) (n%)	45 (52.3)	41 (46.1)	0.438
	Infectious (≥10 mm) by Mantoux test (n %)	43 (50.0)	56 (62.9)	0.085
Endometrial biopsy	AFB on microscopy	10 (11.6)	11 (12.4)	0.882
	Positive BACTEC culture	9 (10.5)	10 (11.2)	0.870
	Positive gene Xpert	1 (1.2)	1 (1.1)	0.995
	Positive PCR	82 (95.3)	84 (94.4)	0.995
	Negative PCR	4 (4.7)	5 (5.6)	
Histopathology evidence, n (%)	Granulomatous endometritis	9 (10.5)	6 (6.7)	0.379
	Multiple epitheloid granuloma/Ill-defined granuloma	5 (5.8)	11 (12.4)	0.1333
	Isthmic endometrium	3 (3.5)	2 (2.2)	0.679
	Secretory endometrium	56 (65.1)	59 (66.3)	0.870
	Proliferative endometrium	12 (13.9)	9 (10.1)	0.434
	Inadequate specimen	1 (1.2)	2 (2.2)	0.990

Some patients had more than one findings.

Table 2
Details of radiological (ultrasound and hysterosalphinography) investigations among the patients in Group I And Group Ii.

Clinical investigations		Patients randomized Group I (9 months ATT) ($n=86$)	Patients randomized Group II (6 months ATT) ($n=89$)	p value
Ultrasonography,[a] n (%)	Normal USG	42 (48.8)	40 (44.9)	0.606
	Enlarged Uterus	6 (7.0)	8 (9.0)	0.624
	Hydrosalphinx	10 (11.6)	12 (13.5)	0.711
	PID	3 (3.5)	3 (3.4)	0.999
	Tubo-ovarian mass	12 (13.9)	9 (10.1)	0.434
	Enlarged ovary	11 (12.8)	10 (11.2)	0.752
		Patients randomized Group I (9 months ATT) ($n=60$)	Patients randomized Group II (6 months ATT) ($n=47$)	p value
Hysterosalpingography (HSG),[a] n (%)	Beaded tubes	4 (6.7)	4 (8.5)	0.728
	B/L tubal block	29 (48.3)	19 (40.4)	0.414
	B/L hydrosalpinx	6 (10.0)	3 (6.4)	0.728
	Right hydrosalpinx	2 (3.3)	1 (2.1)	0.999
	Left hydrosalpinx	1 (1.7)	0 (0.0)	0.999
	Right cornual block	7 (11.7)	5 (10.6)	0.995
	Left cornual block	4 (6.7)	4 (8.5)	0.728
	Normal	13 (21.7)	15 (31.9)	0.231
	Bicornuate uterus	0 (0.0)	1 (2.1)	0.439

[a] Some patients had more than one findings.
HSG was not done in all cases.

pyosalpinx, beaded tubes, pelvic or perihepatic adhesions were equal in two groups (Figs. 2–4). The various hysteroscopic findings of FGTB like tubercles, shaggy areas, pale endometrium and intrauterine adhesions were also equal in the two groups as shown in Table 3 and Figs. 5 and 6.

Post anti tuberculous therapy outcome measures and laboratory and endoscopic findings

At the end of treatment all women were evaluated for outcome by repeating endometrial biopsy, laparoscopy, hysteroscopy

Table 3
Details of endoscopic (laparoscopic and hysteroscopic findings) among the patients in Group I and Group Ii.

Clinical investigations		Patients randomized Group I (9 months ATT) (*N* = 86)	Patients randomized Group II (6 months ATT) (*N* = 89)	*p* value
Laparoscopic Findings,[a] *n* (%)	Fitz hugh curtis syndrome/perihepatic adhesions	18 (20.9)	20 (22.5)	0.805
	Abdominal adhesions	30 (34.9)	25 (28.1)	0.333
	Hydrosalpinx	11 (12.7)	13 (14.6)	0.727
	Pyosalpinx	4 (4.6)	2 (2.2)	0.438
	Convulated tubes/tortuous tubes	8 (9.3)	5 (5.6)	0.353
	Beaded tubes	6 (7.0)	7 (7.8)	0.823
	Congested tubes	8 (9.3)	8 (9.0)	0.943
	Tubo-ovarian mass	14 (16.3)	9 (10.1)	0.227
	B/L free spill absent	8 (9.3)	8 (9.0)	0.943
	B/L free spill present	15 (17.4)	21 (23.6)	0.314
	B/L cornual block	11 (12.8)	3 (3.4)	0.022
	Shaggy areas	16 (18.6)	14 (15.7)	0.614
	Tubercles	36 (41.9)	28 (31.5)	0.153
	Pelvic adhesions	17 (19.8)	23 (25.8)	0.339
	B/L tubes, ovaries, uterus normal	4 (4.6)	2 (2.2)	0.438
	Blebs	5 (5.8)	1 (1.1)	0.113
	Absence of one tube due to past Salpingectomy	2 (2.3)	0 (0.0)	0.240
	Fibroids	1 (1.1)	2 (2.2)	0.995
	Spill at one end of tube	8 (9.3)	3 (3.4)	0.128
	Cyst/bulky ovary	13 (15.1)	13 (14.6)	0.925
	Bicornuate uterus	0 (0.0)	1 (1.1)	0.999
	Bulky uterus	0 (0.0)	1 (1.1)	0.999
		Patients randomized Group I (9 months ATT) (*N* = 86)	Patients randomized Group II (6 months ATT) (*N* = 89)	*p* value
	Normal	32 (37.2)	33 (37.1)	0.986
	Adhesions	29 (33.7)	30 (33.7)	0.999
Hysteroscopy,[a] *n* (%)	Grade I adhesions	15 (17.4)	14 (15.7)	0.761
	Grade II adhesions	7 (8.1)	8 (9.0)	0.841
	Grade III adhesions	3 (3.5)	5 (5.6)	0.720
	Grade IV adhesions	4 (4.6)	3 (3.3)	0.717
	Both ostia not seen	14 (16.3)	16 (18.0)	0.766
	One ostium seen	18 (20.9)	20 (22.5)	0.805
	Tubercles	12 (14)	13 (14.6)	0.902
	Pale endometrium	28 (32.6)	26 (29.2)	0.632
	Shaggy areas	14 (16.3)	13 (14.6)	0.759

[a] More than one findings was recorded in majority of cases.

wherever possible. Repeated laparoscopy, hysteroscopy and endometrial biopsy could only be done on 71 women in Group I and 78 women in Group II (could not be done in women who become pregnant, refused repeat testing or due to medical reasons). The repeat findings are shown in Table 4 and were equal in two groups. Repeat laparoscopic findings are shown in Fig. 7.

The cure rate was 95.5% in Group I and 97.7% in Group II and was similar in the two groups (Table 5). The failure rate was equal in the two groups (4.7% vs 2.3%) and these women were given category II anti tuberculous therapy. They were diagnosed by persistent tubo-ovarian mass with positive polymerase chain reaction and presence of tubercles or other active lesion on repeat

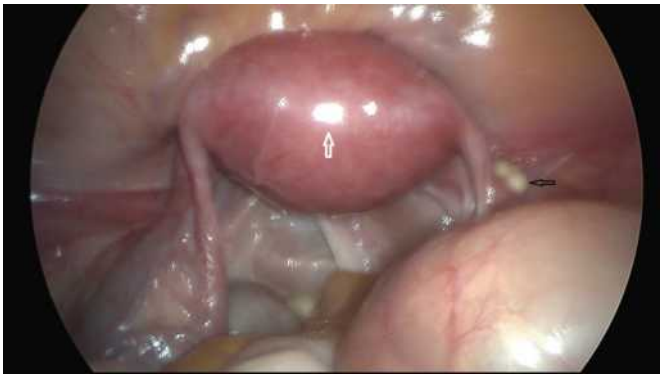

Fig. 2. Laparoscopic picture showing tubercles (black arrow) and shaggy areas (white arrow) in a proven case of female genital tuberculosis.

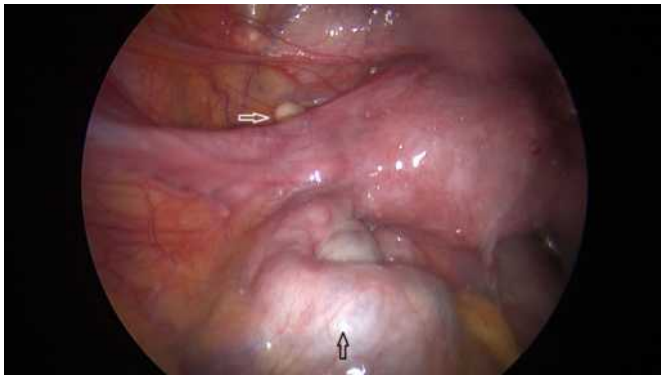

Fig. 3. Laparoscopic picture showing caseous nodules (white arrows) and hydrosalpinx (black arrows) in a case female genital tuberculosis.

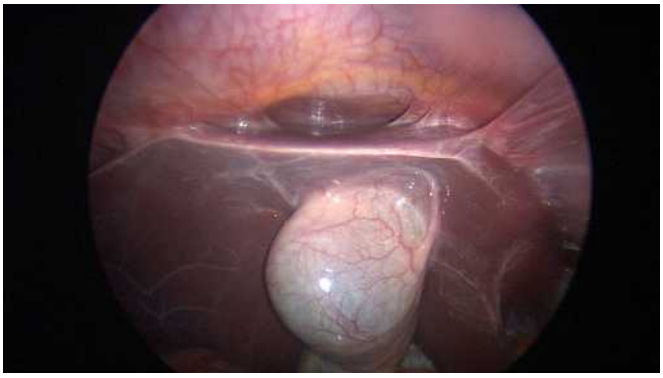

Fig. 4. Laparoscopic picture showing perihepatic adhesions with hanging gall bladder in a proven case of female genital tuberculosis.

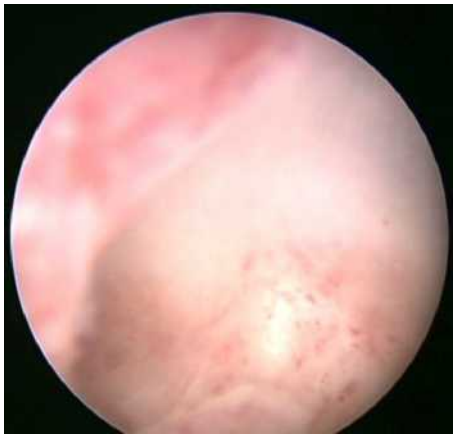

Fig. 5. Hysteroscopic picture showing pale endometrium in a case of female genital tuberculosis.

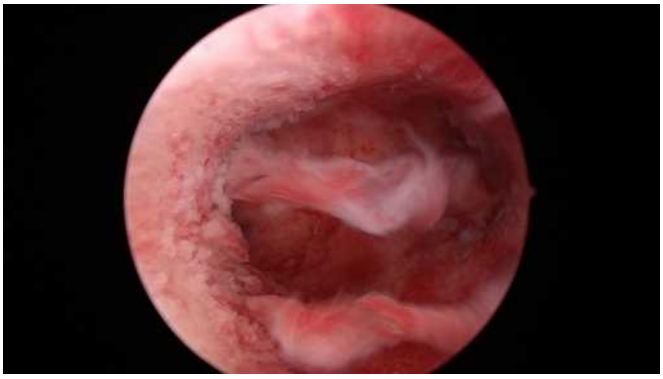

Fig. 6. Hysteroscopic picture showing grade III adhesions in a case of female Genital tuberculosis.

laparoscopy or hysteroscopy tuberculosis activity. They underwent another relook laparoscopy, hysteroscopy and endometrial biopsy after completion of category II treatment with disappearance of TB activity and tubo-ovarian masses. The pregnancy rate at completion of therapy (12.8% vs 10%) and up to 1 year after completion of therapy (10.5% vs 11.2%) which are equal in the two groups.

Mortality, morbidity and side effects

There was no death in any women in either groups. The various side effects in two groups are shown in Table 6. The side effects were nausea, vomiting, anorexia, epigastric pain, hepatitis. Overall

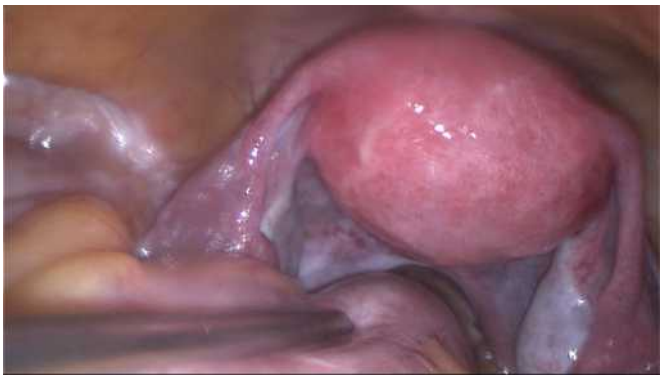

Fig. 7. Laparoscopic picture showing disappearance of tubercles and caseous nodules after completion of antituberculous therapy.

side effects were seen in 31.4% and 32.8% in Group I and Group II respectively and was equal ($p = 0.866$). Hepatitis was seen in 1(1.2%) and 2(2.2%) cases in Group I and Group II and they were managed as described above. All of them were followed closely and after resolution of hepatitis, the first line of drugs was reintroduced to them. All of them recovered and completed the treatment.

Discussion

Female genital tuberculosis is a common disease in developing countries causing significant morbidity especially infertility [4–7]. It affects fallopian tubes in 90–100% cases, uterus in 50–80% cases (causing intrauterine adhesions), ovaries in 20–30% cases, cervix in 5–15%, vagina and vulva in 1% of cases [4,13–25]. It also causes pelvic and perihepatic adhesions (Fitz Hugh Curtis Syndrome) [10]. It may mimic ovarian cancer necessitating unnecessary surgery [11].

Being paucibacillary disease, diagnosis of female genital tuberculosis is difficult. Endometrial biopsy in premenstrual phase for acid fast bacilli on microscopy, acid fast bacilli on culture, polymerase chain reaction, Gene Xpert and for epithelioid granuloma on histopathology is performed [4,13,14]. Ultrasound scan, computerised topographic scan, magnetic resonance imaging and positron emission tomography scan are only useful in tuberculous tubo-ovarian masses but are not specific for tuberculosis [4].

Polymerase chain reaction alone is not diagnostic of female genital tuberculosis due to false positivity and negativity [4,14,15]. Laparoscopy can be used to diagnose female genital tuberculosis by findings like tubercles, pelvic and perihepatic adhesions, caseous nodules, hydrosalpinx and pyosalpinx, beaded and convulated tubes [16–18]. Dye test should not be performed in suspected case of female genital tuberculosis due to risk of further dissemination of disease. In the present study, dye test was not performed in frank case of tuberculosis but in only those cases who were negative for acid fast bacilli on microscopy, culture and histopathology where chances of dissemination is much less.

However, one has to be very careful while performing laparoscopy in a case of abdomino-pelvic and female genital tuberculosis due to increased risk of complications like difficulty in creating pneumoperitoneum and insertion of trocal and cannula, excessive bleeding, risk of injury to intestines and post- operative complications as observed by us and other authors [25,27].

Hysteroscopy can also diagnose female genital tuberculosis by intrauterine adhesions, pale endometrium and tubercles [8,19]. Thus diagnosis of female genital tuberculosis is made by combination of tests [17].

This randomized controlled trial which was adequately powered demonstrates equal cure rates in 9 months (95.3%) and

Table 4
Comparative findings of post att outcome measures (laparoscopic, hysteroscopic, PCR and endometrial biopsies findings) between both groups.

Characteristics		Patients in Group I (9 months ATT) ($n=71$)[a]	Patients in Group I (6 months ATT) ($n=78$)[b]	p value
Laparoscopic findings, n (%)	Normal findings	29 (40.8)	37 (47.4)	0.419
	Tubercles	4 (5.6)	2 (2.6)	0.425
	Pelvic adhesions	25 (35.2)	22 (28.2)	0.358
	Hydrosalpinx	0 (0.0)	1 (1.3)	0.999
	Perihepatic adhesions	12 (16.6)	16 (20.5)	0.302
	Non-visualization of tubes	7 (9.9)	2 (2.6)	0.087
	Persistence to mass with TB activity	4 (5.4)	2 (2.6)	0.425
	Pelvic adhesiolysis	10 (14.1)	11 (14.1)	0.997
	Normal findings	60 (84.5)	68 (87.2)	0.640
Hysteroscopic findings, n (%)	Adhesions	11 (15.5)	10 (12.8)	0.640
	Grade I adhesions	2 (2.8)	2 (2.6)	0.999
	Grade II adhesions	2 (2.8)	1 (1.3)	0.605
	Grade III adhesions	3 (4.2)	4 (5.1)	0.999
	Grade IV adhesions	4 (5.6)	3 (3.8)	0.709
	Hysteroscopic adhesiolysis	7 (9.9)	5 (6.4)	0.551
Endometrial biopsy, n (%)	AFB microscopy	0 (0.0)	0 (0.0)	0.999
	AFB BACTEC culture	0 (0.0)	0 (0.0)	0.999
	PCR	63 (88.8)	51 (65.4)	0.001
	Positive			
	Negative	8 (11.2)	27 (34.6)	
	Histopathology evidence	61 (85.9)	71 (91.0)	0.327
	Secretory endometrium			
	Proliferative endometrium	10 (14.1)	7 (9.0)	0.327
	Tuberculous granuloma	0 (0.0)	0 (0.0)	0.999

[a] In Group I, 11 Patients got Pregnant and 2 refused for relook Laparoscopy, in another 2 women laparoscopy could not be done due to medical reasons.
[b] In Group II, 9 Patients got pregnant, 1 patient had lost to follow up and 1 patient relook laparoscopy could not be done due to medical reasons.
Few patients showed more than one findings.

Table 5
Details of treatment outcome measures between Group I and Group II.

Treatment outcome, n (%)	Patients randomized Group I (9 months ATT), ($n=86$)	Patients randomized Group II (6 months ATT), ($n=89$)	p value
1. Cured	82 (95.3)	86 (97.7)	0.441
2. Not cured	4 (4.7)	2 (2.3)	
3. Cat II ATT[a]	4 (4.6)	2 (2.3)	0.441
4. Refused laparoscopy	2 (2.3)	2 (2.3)	1.000
5. Pregnant at completion of therapy	11 (12.8)	9 (10.1)	0.578
6. Treatment for infertility advised			
i. OVI/IUI[b]	28 (32.6)	41 (46.1)	0.068
Pregnancies	4 (4.7)	6 (6.7)	0.747
ii. IVF (In Vitro Fertilization)	30 (34.9)	22 (24.7)	0.141
Pregnancies	5 (5.8)	4 (4.5)	0.744
iii. Adoption/Surrogacy	9 (10.5)	13 (14.6)	0.409
Pregnancies	0	0	NA
Total further pregnancies during 1 year follow up	9 (10.5)	10 (11.2)	0.870
Total pregnancies during treatment and up to 1 year follow up	20 (23.2)	19 (21.3)	0.762
Outcome			
• Spontaneous abortion	3 (3.4)	2 (2.2)	0.679
• Pre term intrauterine death	2 (2.3)	3 (3.4)	0.995
• Preterm delivery with live infant	4 (4.6)	4 (4.5)	0.995
• Full term pregnancy with live infant	11 (12.8)	10 (11.2)	0.752
• Take home baby rate	15 (17.4)	14 (15.7)	0.761
Recurrence at 1 year follow up	0 (0.0)	0 (0.0)	0.999

[a] Positive findings of active TB.
[b] Ovulation induction; intra-uterine insemination.

6 months (97.7%) of anti-tuberculous therapy in female genital tuberculosis. It also confirms the efficacy of intermittently given drugs under DOTS strategy. It also demonstrates no difference in the recurrence rate at 1 year follow up after completion of antituberculous therapy with no recurrence in either group. There was equal number of pregnancies during treatment (12.8% vs 10%) and up to one year of follow up after anti-tuberculous therapy (10.5% vs 11.2%) in the two groups. The study also confirms the safety of the new regimens while no significant difference in the side effects profile in the two groups (31.4% vs 32.6%) (Table 6).

Another finding was that pregnancy rate during antituberculous therapy and up to 1 year of follow up was similar in the two groups (23.2% vs 21.3%). A recent study by Makharia et al., [28] for abdominal tuberculosis found 6 months intermitted DOTS therapy to be as effective as 9 months intermittent DOTS therapy with equal success and recurrence rate. Jindal et al., [29] observed favorable fertility outcome following antituberculous treatment solely on assess of positive polymerase chain reaction.

Most women in both the groups tolerated drugs very well with overall side effects rates being equal in the two groups (31.4% vs

Table 6
Side effects of antituberculous therapy in the two groups.

Side effects	Patients randomized Group I (9 months ATT) (n = 86)	Patients randomized Group II (6 months ATT) (n = 89)	p value
Nausea	12 (13.9%)	11 (12.4%)	0.755
Anorexia	3 (3.5%)	4 (4.5%)	0.995
Epigastric discomfort or pain	7 (8.1%)	8 (9.0%)	0.841
Hepatitis	1 (1.2%)	2 (2.2%)	0.995
Vomiting	5 (5.8%)	6 (6.7%)	0.995
Overall Single Side Effects	27 (31.4%)	29 (32.6%)	0.866

32.6%) (Table 6). Most adverse events were mild. One and two patients in 9-months and 6-months therapy developed drug induced hepatitis, which resolved with modification of drugs and prolongation of the treatment duration.

The strengths of the study include it being adequately powered randomized controlled trail with significant observations including cure rates, recurrence rates and pregnancy outcome. The sample size was calculated by the statistician and was well powered to note the differences. We feel that the study has significant observations that six months therapy is equally effective as nine months therapy. To the best of our knowledge, this is the first randomized controlled trial comparing six months versus nine months antituberculous therapy for female genital tuberculosis from India. The trial has proven efficacy of six months antituberculous therapy to be equal to nine months therapy which appears to be useful addition to the knowledge. However, there are some limitations of this study. One limitation was that definite diagnosis of female genital tuberculosis could only be made in some cases, while in others diagnosis was presumptive (laparoscopic and hysteroscopic findings) which is an inherent problem in all the types of extra pulmonary tuberculosis including female genital tuberculosis due to its paucibacillary nature.

It was intended to perform repeat laparoscopy, hysteroscopy, endometrial sampling in all the patients at the end of antituberculous therapy, However, repeat testing could only be done in 71 out of 86 (82.5%) in Group I and in 78 out of 89 (87.6%) in Group II. It could not be done in others due to pregnancy, refusal for relook laparoscopy and testing or medical reasons. Another limitation was that late recurrence of the disease after one year was not evaluated due to financial and logistics constraints. There may have been some recurrence after one year in the study patients.

Conclusions

The current randomized control trial confirms that antituberculous therapy using DOTS for 6 months duration was as effective as 9 months duration. The cure rate, treatment, failure rates and recurrence rates were similar in the two groups. There was no significant difference in adverse effects in the two groups. The pregnancy rate was similar in two groups. The results of this study therefore demonstrate the efficacy and safety of 6 months duration of directly observed treatment short course (DOTS) for female genital tuberculosis to be equal to 9 months. The results provide evidence to policy makers for recommendation of intermitted DOTS therapy for 6 months for patients with female genital tuberculosis especially in developing nations where disease is more prevalent and where compliance to treatment is a barrier for effective treatment of tuberculosis.

Conflict of interest

We declare no conflict of interest.

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