BJOG An International Journal of Obstetrics and Gynaecology

DOI: 10.1111/1471-0528.15814
www.bjog.org

Original article

Significance of isolating non-tuberculous mycobacterial organisms in infertile women with tubal disease: an observational study

T Radha Bai Prabhu,[a] N Pandiyan,[b] N Sujatha,[c] MS Jawahar[d]

[a]Department of Obstetrics and Gynaecology, Meenakshi Medical College and RI, Kancheepuram and Govt Hospital for Women and Children, Chennai, Tamil Nadu, India [b]Department of Reproductive Medicine, Chettinad Hospital and Research Institute, Kelambakkam, Tamil Nadu, India [c]Tuberculosis Research Centre, Chetput, Chennai, India [d]National Institute of Research in Tuberculosis and Indian Council of Medical Research, Chennai, Tamil Nadu, India

Correspondence: T Radha Bai Prabhu, 40/78, IInd Cross street, Collectorate Colony, Aminjikarai, Chennai 600 029, Tamil Nadu, India.
Email: radhaprabhu54@ymail.com

Accepted 1 May 2019. Published Online 31 May 2019.

Objectives To explore whether non-tuberculous mycobacteria (NTM) are associated with tubal disease leading to infertility.

Design Prospective observational study.

Setting Teaching hospital.

Population Women with tubal factor infertility.

Methods In all, 173 infertile women with tubal disease were investigated for genital tuberculosis, *Chlamydia trachomatis* and *Neisseria gonorrhoeae* using polymerase chain reaction, culture and histopathological examination. On culture, NTM were grown in 23.7% of endometrial samples. The mycolic characteristics of these organisms were analysed.

Main outcome measure Whether NTM are associated with tubal disease leading to infertility.

Results The organisms identified in association with tubal disease were *Mycobacterium tuberculosis* in 30%, gonococci in 1.7%, *Chlamydia* in 7.5% and NTM in 23.7% of cases. *Mycobacterium chelonae* was the predominant organism identified by high-performance liquid chromatography. In ten women, for whom there was laparoscopic evidence of tubal disease, the only organism that was grown was NTM, and the tests for other organisms were negative. Tests for possible environment (theatre, instruments) contamination was reported negative.

Conclusion While evaluating infertile women for tubal disease, culture studies revealed a high prevalence of NTM in the endometrium. In the absence of *M. tuberculosis*, gonococci and *Chlamydia* infection, the presence of NTM suggests the possibility that these organisms may be responsible for tubal damage leading to infertility.

Keywords Gonococci, *Mycobacterium tuberculosis*, non-tuberculous mycobacteria, tubal disease.

Tweetable abstract On evaluating the causes of tubal disease, NTM were associated with tubal disease.

Please cite this paper as: Radha Bai Prabhu T, Pandiyan N, Sujatha N, Jawahar MS. Significance of isolating non-tuberculous mycobacterial organisms in infertile women with tubal disease: an observational study. BJOG 2019; 126 (S4): 66–71.

Introduction

Non-tuberculous mycobacteria (NTM) are generally considered to be non-pathogenic environmental organisms. However, in the last few decades, increased interest has been shown in these organisms. According to the American Thoracic Society, this high level of interest in NTM disease is the result of two major recent trends: the association of NTM infection with AIDS, and, the recognition that NTM lung disease is encountered with increasing frequency in the non-AIDS population. Furthermore, NTM infections are emerging in previously unrecognised settings, with new clinical manifestations.[1] In recent years there are reports on isolating NTM in laparoscopic port site infections, lymph nodes and, pulmonary infections.[2,3] There is a great deal of controversy as to the pathogenicity of NTM, but the American Thoracic Society has recognised NTM as human pathogens and has given clear guidelines regarding the diagnosis and treatment of pulmonary disease caused by NTM.[1] Studies have shown a prevalence in infertility of 12.6% in the southern states and 12.0% in the northern states of India.[4,5] Among women with tubal

factor infertility, a high prevalence of *Mycobacterium tuberculosis* (MTB) infection has been reported by Indian researchers.[6–8] Interestingly, studies on female genital tuberculosis have also shown positive cultures for NTM.[9,10] Recent reports suggest that NTM are important pathogens causing genital infections and infertility.[11] However, the clinical importance and significance of isolating these organisms in endometrial samples of women presenting with tubal infertility had not been ascertained. While evaluating the causes for tubal disease in infertility, it was found that there was a high prevalence of NTM reported on culture. Therefore, a study was undertaken to see whether NTM reported on culture are, in fact, contaminants or could be considered possible pathogens associated with tubal damage.

Objectives

The objective of this study was to identify the possible microbiological causes for tubal disease in female infertility and to see whether NTM are associated with tubal damage leading to infertility.

Methods

This prospective observational study was conducted at Meenakshi Medical College, Kancheepuram, and the Institute of Obstetrics and Gynaecology, Chennai, from January 2008 to December 2016. Sampling used the convenience sampling technique. A total of 173 women with suspected or proven tubal factor infertility were included in the study and women with other causes of infertility were excluded from the study.

A detailed history was taken regarding demographic details, gynaecological symptoms, menstrual disturbances, exposure to sexually transmitted infections, and past history of tuberculosis and pelvic inflammatory disease. Details of previous investigations were also noted to rule out other causes of infertility. Besides haemoglobin %, total count, differential count, erythrocyte sedimentation rate, tuberculin test, chest X-ray, and HIV types I and II tests, all women underwent ultrasonography and laparoscopy. A hysterosalpingogram was carried out in 131 women. At the time of laparoscopy, any fluid present in the pouch of Douglas (POD) was aspirated for investigations. To ascertain the microbiological causes of tubal disease, material for culture was collected from four sources: endocervical swabs, endometrium, POD aspirate and urine.

Specific diagnostic tests

Detection of *Chlamydia trachomatis* and *Neisseria gonorrheae* was done by Amplicor CT/NG PCR (Roche Diagnostic Systems, Inc.,) test on endocervical samples.

Detection of MTB was done by acid fast bacilli (AFB) smear examination, culture, histopathological examination (HPE) and polymerase chain reaction (PCR) studies on the endometrial, POD fluid and urine samples. All cultures and PCR tests were carried out at the Tuberculosis Research Centre of the Indian Council of Medical Research, Chennai. Culture of MTB was carried out as per the guidelines of the Indian Council of Medical Research, inoculating into multiple media, namely Lowenstein–Jensen medium, Lowenstein–Jensen enriched with sodium pyruvate and selective Kirchner's medium. After 8 weeks, the culture was studied for the presence of MTB or any other organisms. Biochemical tests were also carried out for conventional assignment of the species of MTB. Twenty-five endometrial samples that were positive for NTM were analysed by high-performance liquid chromatography to identify the mycolic characteristics of various NTM. The PCR testing for MTB was carried out using IS6110 and TRC4 probes as per the laboratory protocol of the Tuberculosis Research Centre.

Because of the high prevalence of NTM in culture, to rule out the possibility of contamination, 16 sets of samples were taken for culture on 10 different days at an interval of 15–20 days from the theatre tables, and, from the instruments which were used for curettage and laparoscopy.

Statistical analysis was performed in OPEN EPI VERSION 2.2.1 software. Descriptive statistics such as frequencies, percentages, ranges and means were used to describe demographic variables and specific tests.

Ethical committee approval was obtained and informed consent was taken from each woman. The participants and public were not involved in the development of this research.

Results

Clinical profile

Women were aged between 20 and 37 years. The mean age of presentation was 27.4 years; 93.1% of women had primary infertility and 12 (6.9%) had secondary infertility. Infertility was the only complaint in 56.6% of the women. Menstrual disturbances were recorded in 48 (27.7%) women, chronic vaginal discharge in five and chronic pelvic pain in nine. Ten women had been treated for sexually transmitted infections in the past and past history of tuberculosis was present in 11 (6.4%) women. Erythrocyte sedimentation rate was elevated in 27 (15.6%) women, chest X-ray showed old healed lesions of tuberculosis in four women (2.3%) and the tuberculin test (Mantoux) was positive in 37 (21.4%) women. Among the 37 women who showed a positive reaction to the Mantoux test, 24 had evidence of MTB by HPE, culture and PCR, and in five women the NTM were grown in culture. All participants were negative for HIV infection.

Laparoscopy findings

During laparoscopy, in 93 women the tubes and pelvis appeared normal. In 80 women, there was evidence of tubal damage with findings such as granulomas, caseation, calcification, hydrosalpinx, tubal blocks and adhesions.

Tests for gonococcal and chlamydial infection

The PCR tests on endocervical samples were positive for gonococcal infection in three (1.7%) women and for chlamydial infection in 13 (7.5%) women.

Tests for MTB

The AFB smear was positive in 8/173 (4.6%)of the endometrial samples and 5/81 (6.2%) of the samples of POD fluid. Among eight cases positive, on culture of the endometrial sample, none grew MTB, but NTM was grown in three cases. In two of these cases, NTM was positive both in the endometrium and in the POD fluid.

By culture, only 6 (3.5%) endometrial samples were positive for MTB and none of the POD aspirate and urine samples grew MTB. However, NTM were grown in 41/173 (23.6%) endometrial samples, 16/81(19.8%)POD fluid and 6/52 (11.5%) urine samples (Table 1). High-performance liquid chromatography analysis of the NTM was performed on 25 endometrial samples and the most common NTM species characterised in our study were *Mycobacterium chelonae* and *Mycobacterium abscessus* (sixteen samples), followed by *Mycobacterium fortuitum* (four samples), *Mycobacterium simiae* (two samples), and one sample each of *Mycobacterium kansasii*, *Mycobacterium intracellulare* and *Mycobacterium marinum* (Table 2). On HPE, seven (4%) of the endometrial samples were reported positive for tuberculosis. PCR was positive in 45/160 (28.1%) of the endometrial samples, 16/81 (19.8%) of the POD aspirates and 4/52(7.7%) of the urine samples. Using a combination of culture, HPE and PCR testing, a diagnosis of MTB was made on 52 samples (30%). On analysing the above results, the organisms identified in association with tubal disease were; MTB in 30%, gonococci in 1.7%, chlamydia in 7.5% and NTM in 23.7%. No organisms were isolated in 36.9% of cases (Table 3).

Table 2. Characterisation of non-tuberculous mycobacterium organisms by high-performance liquid chromatography

Organism	Number positive	Percentage
Mycobacterium chelonae	16	64
Mycobacterium fortuitum	4	16
Mycobacterium kansasii	1	4
Mycobacterium simiae	2	8
Mycobacterium intracellulare	1	4
Mycobacterium marinum	1	4

Correlating NTM positivity with other results

To see whether NTM could be a possible pathogen, NTM positivity was corroborated with other results and it was seen that, among the 41 women in whom NTM was positive in the endometrium, in 24 there was laparoscopic evidence of tubal disease. In all these 24 women, MTB culture, and tests for chlamydia and gonococci were negative. However, MTB PCR was positive in 14 women. Therefore, in ten women, where there was laparoscopic evidence of tubal disease, the only organism that was grown was NTM. Tests for environmental contamination showed that samples taken from the instruments and theatre environment were all negative for NTM. However, during the same period, 6/16 (37.5%) endometrial samples were positive for NTM (Table 4).

Discussion

Main findings

Important organisms causing tubal disease leading to infertility include *N. gonorrhoeae*, *C. trachomatis* and MTB.[12] Our study showed that organisms responsible for tubal disease in our participants were: gonococci in 1.7%,

Table 1. Culture results of *Mycobacterium tuberculosis* for various sources

Result	Endometrium (n = 173)	POD fluid (n = 81)	Urine (n = 52)
Positive for MTB	6 (3.5%)	0	0
Negative for MTB	124 (71.7%)	63 (77.8%)	46 (88.5%)
NTM-positive	41 (23.7%)	16 (19.8%)	6 (11.5%)
Contamination	2 (1.2%)	2 (2.5%)	0

MTB, *Mycobacterium tuberculosis*; NTM, non-tuberculous *Mycobacterium*; POD fluid, pouch of Douglas fluid.

Table 3. Organisms identified in association with tubal disease from 173 tested endometrial samples

Organism	n	%
MTB	52	30.0
Gonococcus	3	1.7
Chlamydia	13	7.5
NTM	41	23.7
No organisms grown	64	36.9

MTB, *Mycobacterium tuberculosis*; NTM, non-tuberculous *Mycobacterium*.

Table 4. Test result of samples taken for environmental contamination

Samples	No. of endometrial samples sent	NTM positivity in endometrium	NTM positivity in laparoscopy instruments	NTM positivity in uterine curette	NTM positivity in theatre table
Batch I	Sample 1	Negative	Not done	Negative	Not done
	Sample 2	Positive	Not done	Negative	Not done
Batch II	Sample 1	Positive	Negative	Negative	Negative
Batch III	Sample 1	Negative	Not done	Negative	Not done
	Sample 2	Negative	Not done	Negative	Not done
	Sample 3	Negative	Negative	Negative	Not done
Batch IV	Sample 1	Negative	Negative	Negative	Not done
	Sample 2	Positive	Not done	Negative	Not done
Batch V	Sample 1	Positive	Negative	Negative	Not done
Batch VI	No samples taken	Not done	Negative	Negative	Negative
Batch VII	Sample 1	Negative	Negative	Negative	Not done
	Sample 2	Negative	Negative	Negative	Not done
	Sample 3	Positive	Negative	Negative	Not done
Batch VIII	Sample 1	Negative	Negative	Negative	Negative
Batch IX	Sample 1	Negative	Negative	Negative	Not done
	Sample 2	Negative	Negative	Negative	Not done
Batch X	Sample 1	Positive	Negative	Negative	Not done

NTM, non-tuberculous *Mycobacterium*.

chlamydia in 7.5% and MTB in 30%. Interestingly NTM were grown in 23.7% of women. Further work was done to see whether NTM was associated with tubal damage leading to female infertility.

Non-tuberculous mycobacterial organisms are widely distributed in the environment. They live in the soil and water sources throughout the world and human disease is suspected to be acquired from environmental exposure.[13] These organisms gain entry into the body through inhalation, via open wounds exposed to contaminated water, and via surgical procedures that use contaminated instruments. There is little evidence of person-to-person spread of NTM. Most NTM infections have been linked to cutaneous diseases[14] and cultured in extra-pulmonary sites such as lymph nodes.[15] Singh et al.[16] isolated NTM in 12.5% of cases with lymphadenitis, the highest rate so far reported from India. Few studies have reported NTM in culture while investigating cases of infertility for genital tuberculosis.[9,10] Gangania et al.[9] evaluated infertile women with suspected genital tuberculosis using AFB smear staining, BACTEC culture and multiplex real-time PCR to isolate MTB and NTM. For NTM and MTB differentiation the SD MPT 64 card test was carried out and specific primers were used for the detection of NTM by PCR. On analysing 217 samples from various sources, NTM was positive by culture in 5.5%, and by multiplex real-time PCR in 11.0% of samples. The authors suggested that NTM may lead to female infertility. The mechanism by which NTM could lead to tubal disease is not known. Being an environmental pathogen, it is possible that NTM could gain entry into the upper genital tract through the vagina. Chatterjee and Basak[17] evaluated 120 women with unexplained infertility for MTB and NTM infections using a real-time PCR technique in menstrual blood samples. In their study, none of the samples exhibited any association with NTM infection. Our study, similar to that of Gangania et al., was conducted on women with proven or suspected tubal pathology, which may explain the high prevalence of NTM in our study.

While evaluating cases of infertility, a positive Mantoux test should alert the clinician to the possibility of MTB or NTM infections, as in 29 of the 37 women with positive Mantoux test, either MTB or NTM were positive. Similarly, a positive AFB smear could be due to MTB or NTM and must be followed by culture to determine the species of *Mycobacterium*. Studies have shown that PCR can be used to distinguish between MTB and NTM in cases of smear-positive disease.[18] Various investigations including molecular methods are available for species specification and the identification of NTM. Our diagnosis of NTM was made primarily by culture and biochemical analysis. With real-time PCR, Premraj et al.[19] have shown that, in extra-pulmonary samples, NTM positivity was high in comparison to MTB, with menstrual blood samples showing the highest positivity (22.9%) for NTM. In our analysis, although NTM were positive in 63 samples, the initial positive reports were presumed to be due to contamination and further investigations were not performed. However, as NTM were reported more frequently, the mycolic

characteristics of NTM were studied on 25 endometrial samples and the commonest organism identified was *Mycobacterium chelonae*. To rule out the possibility of contamination we also conducted culture studies on the theatre environment which proved to be negative.

In ten women, for whom there was laparoscopic evidence of tubal disease, the only organism that had grown was NTM. Moreover, in nine of these women, NTM were positive both in the endometrium and in the POD aspirate. These findings raise an unresolved question as to whether NTM are responsible for tubal damage leading to infertility. There have been reports on concomitant infection with MTB and NTM in pulmonary disease.[20,21] Our study also showed co-infection with MTB and NTM in 14 women. In the study by Jain et al.[22] one of the risk factors found to be associated with NTM infection was a past or present history of tuberculosis.

Strengths and limitations

The strength of this study is that the culture studies were carried out as per protocols and mycolic characterisation was performed on NTM-positive samples. There is also clinical evidence to suggest that NTM could be possible pathogens.

The limitations of this study are that the tests for possible contamination were made only on a small number of samples and there was no control group to explore the prevalence of NTM among women without tubal disease.

Interpretation

Based on the evidence presented in this study, we suggest that NTM could be possible pathogens causing tubal damage leading to infertility. Reports from the literature as well as our findings suggest the need for increased clinical awareness as to the possibility of co-existing MTB and NTM infections. These are two different pathogens that have many similar clinical and epidemiological features but that have different treatment regimens.[23]

Conclusions

Though, there is profound controversy surrounding the pathogenicity of NTM, they have been recognised as human pathogens and international guidelines are available for the diagnosis and management of NTM-related diseases. The evidence presented in this study is preliminary and further research is required to see whether there is a definite association between NTM, genital infection and infertility.

Disclosure of interests

The authors declare no conflict of interest. Completed disclosure of interest forms are available to view online as supporting information

Contribution to authorship

TRBP was responsible for conceiving the idea, conducting the research, analysing the data and preparing the draft. TSN and MSJ were responsible for the technical support and guidance for conducting this research at Tuberculosis Research Centre, Chennai. NP was responsible for giving valuable guidance throughout the research.

Details of ethics approval

Ethical clearance was obtained from the Human Ethical Committee of the Institute of Obstetrics and Gynaecology and the Government Hospital for Women and Children, Chennai, 600 008, on 11 October 2003.

Informed consent

The authors declare that informed consent was obtained from each participant in this study.

Funding

No funding was received for this study.

Acknowledgements

We sincerely thank and acknowledge the Director and Staff of the Institute of Obstetrics and Gynaecology and Dean Meenakshi Medical College and Research Institute, Kancheepuram, for help in conducting this research. We also acknowledge the technical staff of the Tuberculosis Research Centre, Indian Council of Medical Research, Chennai. We also thank Dr Narayanan, Director of Tuberculosis Research Centre for allowing us to conduct the major part of the study at Tuberculosis Research Centre. We also acknowledge the support and guidance rendered by Dr Latha at IOG, Chennai. ■

References

1 Griffith DE, Aksamit T, Barbara A, Elliott B, Catanzaro A, Daley C, et al. American Thoracic Society documents. An official ATS/IDSA statement: diagnosis, treatment, and prevention of nontuberculous mycobacterial diseases. *Am J Respir Crit Care Med* 2007;175:367–416.
2 Sasmal PK, Mishra TS, Rath S, Meher S, Mohapatra D. Port site infection in laparoscopic surgery: a review of its management. *World J Clin Cases* 2015;3:864–71.
3 Grubek-Jaworska H, Walkiewicz R, Safianowska A, Nowacka-Mazurek M, Krenke R, Przybyłowski T. Nontuberculous mycobacterial infections among patients suspected of pulmonary tuberculosis. *Eur J Clin Microbiol Infect Dis* 2009;28:739–44.
4 Adamson PC, Krupp K, Alexandra HF, Jeffery DK, Arthur LR, Purnima M. Prevalence and correlates of primary infertility among young women in Mysore, India. *Indian J Med Res* 2011;134:440–6.
5 Mittal A, Yadav S, Yadav SS, Bhardwaj A, Kaur R, Singh P, et al. An epidemiological study of infertility among urban population of Ambala, Haryana. *IJIMS*, 2015;2:124–30.
6 Tripathy SN, Tripathy SN. Infertility and pregnancy outcome in female genital tuberculosis. *Int J Gynaecol Obstet* 2002;76:159–63.
7 Singh N, Sumana G, Mittal S. Genital tuberculosis: a leading cause for infertility in women seeking assisted conception in North India. *Arch Gynecol Obstet* 2008;278:325–7.

8 Thangappah RBP, Paramasivan CN, Narayanan S. Evaluating PCR, culture & histopathology in the diagnosis of female genital tuberculosis. *Indian J Med Res* 2011;134:40–6.
9 Gangania PS, Bisht D, Singh VA. Detection of mycobacterial infections (MTB & NTM) by different molecular, staining and culture techniques among infertile females. *Global J Med Res* 2016;16:25–31.
10 Kumar P, Shah NP, Singhal A, Chauhan DS, Katoch VM, Mittal S, et al. Association of tubercular endometritis with infertility and other gynaecological complaints of women in India. *J Clin Microbiol* 2008;46:4068–70.
11 Gangania PS, Bisht D, Singh VA. Current concepts of diagnosis for mycobacterial infections in female genital tract. *Indian J Microbiol Res* 2017;4:7–13.
12 Novy M, Eschenbach D, Witkin S. Infections as a Cause of Infertility. Global Library of Women's Medicine, (ISSN: 1756 – 2228) 2008; https://doi.org/10.3843/glown.10328
13 Johnson MM, Odell JA. Nontuberculous mycobacterial pulmonary infections. *J Thorac Dis* 2014;6:210–9.
14 Wentworth AB, Drage LA, Wengenack NL, Wilson JW, Lohse CM. Increased incidence of cutaneous nontuberculous mycobacterial infection, 1980 to 2009: a population-based study. *Mayo Clin Proc* 2013;88:38–45.
15 Drobniewski FA, Amin AK, Balabanova Y. Non-pulmonary tuberculosis and mycobacterial infection. *Medicine* 2009;37:649–53.
16 Singh S, Gopinath K, ShahdadS KM, Singh B, Sharma P. Nontuberculous mycobacterial infections in Indian AIDS patients detected by a novel set of ESAT-6 polymerase chain reaction primers. *Jpn J Infect Dis* 2007;60:14–8.
17 Chatterjee T, Basak AK. *Mycobacterium tuberculosis* and non-tubercular mycobacterium infection in women with unexplained infertility from eastern India. *Int J Reprod Biomed (Yazd)* 2018;16:557–62.
18 Kim Y, Min KK, Na CH, Hyung LJ, Sung PH, Yun JK, et al. Clinical usefulness of PCR for differential diagnosis of tuberculosis and nontuberculous mycobacterial infection in paraffin-embedded lung tissues. *J Mol Diag* 2015;17:597–604.
19 Premraj S, Vinay B, Rachna V, Sarjana D. Prevalence of MTB/NTM infection in pulmonary and extrapulmonary samples among tuberculosis suspects in India. *IJSR* 2014;3:695–7.
20 Huang H-C, Yu W-L, Shieh C-C, Cheng K-C, Cheng H-H. Unusual mixed infection of thoracic empyema caused by *Mycobacteria tuberculosis*, nontuberculosis mycobacteria and *Nocardia asteroides* in a woman with systemic lupus erythematosus. *J Infect* 2007;54:25–8.
21 Ishiekwene C, Subran M, Ghitan M, Kuhn-Basti M, Chapnick E, Shia Lin Y. Case report on pulmonary disease due to co-infection of *Mycobacterium tuberculosis* and *Mycobacterium abscessus*: difficulty in diagnosis. *Respir Med Case Rep* 2017;20:123–4.
22 Jain S, Sankar MM, Sharma N, Singh S, Chugh TD. High prevalence of non-tuberculous mycobacterial disease among non-HIV infected individuals in a TB endemic country – experience from a tertiary center in Delhi, India. *Pathog Glob Health* 2014;108:118–22.
23 Sarro YD, Kone B, Diarra B, Kumar A, Kodio O, Fofana DB, et al. Simultaneous diagnosis of tuberculous and nontuberculous mycobacterial diseases: time for a better patient management. *Clin Microbiol Infect Dis Clin Microbiol Infect Dis* 2018;3:1–4.