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Original Article

Female Genital Tuberculosis in Pakistan – A Retrospective Review of 10-Year Laboratory Data and Analysis of 32 Cases

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Abstract

Background: Female genital tuberculosis (FGTB) is an underobserved clinical entity owing to diagnostic challenges stemming from difficulty of obtaining diagnostic specimens and paucibacillary nature of the disease. Yet, FGTB is a cause of infertility, pelvic pain, or menstrual irregularities in high-burden countries. To assess laboratory and microbiology diagnostic utilization for FGTB in Pakistan, we have collected data from 2007 to 2016 to inform the need for improved laboratory diagnostics. The objectives of this study were to determine the proportion of FGTB as culture-confirmed extrapulmonary tuberculosis (EPTB) and to describe the characteristics of women with culture-confirmed FGTB in a nationwide laboratory network in Pakistan. **Method:** A retrospective database was established by accessing laboratory archives and analyzed by sex and source to determine extrapulmonary cases among women. Data were checked for quality, and after removing patient identifiers and duplicate samples, frequencies were calculated in MS Excel. Clinical characteristics of patients were derived from a linked hospital database for those patients who were diagnosed and managed at the affiliated university hospital in Karachi, Pakistan. **Results:** Over 10 years, 410,748 mycobacterial cultures were received from multiple geographic sites throughout Pakistan and processed at the study laboratory. The overall mean culture positivity rate was 5.9% ± 3.5%, while the mean culture positivity rate among females was 2.8% ± 0.8%. Among female culture-confirmed tuberculosis cases, the pulmonary-to-EPTB ratio of infection was 5. Over 10 years, a total of 32 FGTB cases were reported on the basis of positive cultures for *Mycobacterium tuberculosis*; 3 (9.4%) were rifampin resistant. **Conclusions:** FGTB currently constitutes a small but significant proportion of culture-confirmed EPTB. A fewer number of laboratory requisitions suggest the need to increase awareness and testing. The advent of high-sensitivity molecular testing on extrapulmonary specimens has the potential to improve diagnostic accuracy and improved detection of FGTB cases in high-burden regions.

Keywords: Diagnosis, endometrial, female genital tuberculosis, histopathology, mycobacterial culture, tubo-ovarian

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Introduction

Female genital tuberculosis (FGTB) classically presents with infertility, pelvic pain, or menstrual irregularities, with or without other systemic symptoms of tuberculosis.[1] Such symptoms and resulting debilitation as well as economic costs can have serious detrimental effects on women's quality of life, sexual and reproductive health, mental health, and productivity. Yet, the burden of FGTB is unclear – remaining an underdiagnosed form of extrapulmonary tuberculosis (EPTB), even among women in high-burden settings.[2]

The widely held belief that FGTB is a rare site for EPTB[3,4] is due to the challenges of limited health-care utilization by women, covert symptoms, and paucibacillary nature of FGTB. The focus of national tuberculosis control programs on pulmonary tuberculosis (PTB) exacerbates this underestimation of prevalence. However, among women presenting with pelvic inflammatory disease (PID) and/or infertility, published data from high tuberculosis burden settings indicate a high prevalence of FGTB.[5]

Data from the 2016 national survey of tuberculosis control program health-care facilities in Pakistan indicate that rates of

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EPTB are higher among females as compared to males,[6] as has also been observed in studies from South Asia.[7] However, tuberculosis control program data are not disaggregated by the site of EPTB and sex-disaggregated data on incidence cannot be used to estimate FGTB burden.

In an effort to better define the FGTB epidemiology in the high-burden country of Pakistan, we examined data from a referral laboratory to report culture-confirmed FGTB rates among females evaluated for tuberculosis. These data are important to inform the need for guideline development, facilitated referral for improved health-care delivery and quality of life in women, physicians training, and further research in diagnostics.

Method

Setting

We report on data from Pakistan, a high-burden country with an incidence of tuberculosis of 263 per 100,000 population,[8] 41% of total cases occurring in women. This study was performed at the Aga Khan University Clinical Laboratories, with collection outlets in 289 different locations across Pakistan. Samples were collected in accordance with laboratory policies and transported within 48 h to the central mycobacteriology laboratory in Karachi, a biosafety level 3 facility. The laboratory has been accredited by the College of American Pathologists (CAP) since 2016 and is compliant with their standards. Laboratory records are archived for 2 years. However, electronic patient investigation records of essential variables are archived on a separate server at the Aga Khan University Information Technology Unit.

Study design and data sources

This was a retrospective descriptive analysis of FGTB rates among culture-confirmed EPTB cases among females, with an analysis of FGTB cases based on information obtained through linked electronic records. Data were retrieved for the years of 2007–2016.

Supplementary Figure 1 describes data retrieval, abstraction, and secondary database creation stages and sequence. Laboratory records disaggregated by sex from 2007 to 2016 were retrieved, and duplicates were removed to generate denominators for the rates calculated for male versus female cultures requested and the proportion of positive cultures. A second database with culture results was retrieved from 2007 to 2016, and other variables in the database included sex, age, specimen type, cultured isolate identification (*Mycobacterium tuberculosis* or nontuberculous mycobacteria), susceptibility testing results, dates of specimen collection, and reporting. Linked laboratory identifiers allowed retrieval of histopathology requisitions and results, and linked hospital identifiers allowed examination of patient management details, where available.

We used the ISPOR[9] and STROBE[10] checklists to evaluate the standard of reporting for this descriptive analysis. An intersection of both checklists for this analysis is available in Supplementary Table 1.

Laboratory methods

From 2007 to 2016, the laboratory used both liquid and solid media on specimens processed with 2% sodium hydroxide and N-acetyl-L-cysteine method and high-speed centrifugation. Extrapulmonary specimens (except cerebrospinal fluid) were decontaminated and liquid cultures were continuously monitored for evidence of growth for 6 weeks. The laboratory subscribed to proficiency testing program by CAP during the study period and obtained satisfactory results. Culture contamination rates during the 10-year study period remained within the recommended benchmark of 5%–8%.[11]

Data abstraction and analysis

Data downloaded from laboratory servers were extracted from records using a preexisting coding manual for laboratory variables and by date (year). After initial extraction, data were abstracted into a separate Excel database by creating drop-down categories for the following variables: year, sex, specimen types, cultured isolated identification, and drug susceptibility (sensitive or resistant) for rifampin, isoniazid, and ofloxacin. Duplicate entries for specimen type and patient identification numbers were removed in batches. Data quality checks and verification were performed by comparison to source data. Any conflicts were resolved by consultation with the author group. Data were refined by type of FGTB specimen source, including endometrial tissue, endometrial curettage sample, tubo-ovarian samples, ovarian fluid/pus/tissue, and endocervical sample. For culture-confirmed FGTB samples, linked histopathology records were retrieved from the laboratory database and linked radiology and clinical records were retrieved from the hospital databases and a final relational database was created in MS Excel for the culture-confirmed FGTB cases. Frequencies and means or median values were calculated in MS Excel and Chi-square test for proportions was performed where applicable using Chi-square test calculator from www.socstatistics.com (https://www.socscistatistics.com/tests/chisquare2/default2.aspx).

Ethical approval

The study was reviewed and exempted from individual informed consent by the Ethical Review Committee of the Aga Khan University, Pakistan.

Results

Study population and laboratory utilization

From 2007 to 2016, the study laboratory received 410,748 specimens for mycobacterial culture. The male: female ratio of all requests was 1.3. During the 10 study years, the mean culture positivity rate was 5.9% ± 3.5%, with 2.8% ± 0.8% of these positive cultures from females of all age groups. Data are presented in Supplementary Figure 2 to show the variance across years.

Culture positivity and extrapulmonary tuberculosis rates among females

Among a total of 177,463 cultures from females, 11,544 (6.5%) were positive for *M. tuberculosis* complex (MTB), and

1889 (16.4%) of these positive cultures were from extrapulmonary specimen sources. Rifampin resistance (RR) was observed more frequently in PTB in females at 47.5% (n = 4583), while it remained low in EPTB cases in females at 15.8% (n = 298) (P < 0.0001; Chi-square). FGTB was responsible for 1.8% of all culture-positive EPTB specimens in females. Figure 1 shows cultures received, positivity rates, and RR among PTB and EPTB in females of all age groups.

Culture-confirmed female genital tuberculosis

A total of 36 FGTB specimens were culture positive for *Mycobacterium* species. Two of these samples were excluded from this analysis due to growth of nontuberculous mycobacteria. Of the 34 MTB culture-positive specimens in 32 patients (two tubo-ovarian and one endometrial specimens were received from one patient), 31 were females 15–49 years, with a median age of 26.5 (32–25) years. Clinical indications, age, and linked specimen positivity details are presented in Table 1.

Among culture-confirmed FGTB, infertility was the most common indication for evaluation. Only one patient had a positive smear for acid-fast bacilli. Molecular tests (i.e., Xpert MTB/RIF or a nucleic acid amplification test) were not requested on any of the patients. Histopathological details were available in 28 patients, with 22 (78.6%) showing agreement with culture confirmation, i.e., histopathological examination showed chronic granulomatous inflammation with epithelioid cells and giant cells with or without caseous necrosis. RR was observed in 3 patients in endometrial specimens. Of the two RR patients, histopathological examination was available in two and did not show chronic granulomatous inflammation. One patient had a benign late secretory endometrium while the second had abnormal secretory endometrium, with no signs of inflammation.

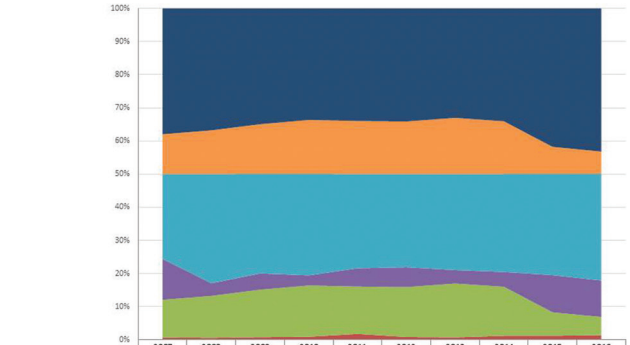
Discussion

FGTB accounts for a small proportion (1.8%) of culture-positive EPTB in this countrywide laboratory network. This proportion, however, is not indicative of the incidence or prevalence of FGTB. Data from evaluation cohorts among women of reproductive age with infertility and PID in Pakistan have reported higher rates of FGTB (3%–3.5%).[12,13] Furthermore, when performed in subsets of women with infertility or associated symptoms, the yield of laparoscopic diagnosis is 44%, and of mycobacterial culture and/or nucleic acid, detection by polymerase chain reaction-based tests is 10%,[14] and the two approaches are complementary. Laparoscopic diagnosis of tuberculosis among women with chronic pelvic pain from Pakistan has also demonstrated higher rates (20%), but the laparoscopic diagnosis was not confirmed on culture or histopathological examination of endometrial tissue.[15] An integrated approach to diagnosis of FGTB is therefore recommended, with availability of all these services as part of national tuberculosis control programs in liaison with health services for women. Particularly, Xpert Ultra may be a useful tool in improving the yield of molecular diagnosis.

The low proportion of culture-confirmed tuberculosis observed in our data, despite the expected high prevalence of FGTB, may be a function of any of the following factors: paucibacillary nature and low culture yield of genital specimens (endometrium), lack of availability or reduced utilization of molecular methods with presumed higher sensitivity,[14] use of private laboratory services with data that are not captured by national programs or in this study, or lack of access to health-care services required for the appropriate diagnosis and management of FGTB. Each of these factors likely contributes to the traditional understanding of FGTB as a "rare" form of tuberculosis in women even in high-burden settings. A recent evaluation of EPTB in Pakistan for the year 2016 in public health-care facilities notifying tuberculosis to national authorities, however, reveals that data are either not segregated by source or the proportion of genitourinary specimens submitted for women was very low,[6] again pointing to low service utilization. Moreover, tuberculosis notification rates were based on smear positivity and/or Xpert MTB RIF (which is available in 5.6% of reporting facilities) and not culture or histopathological diagnosis.[6]

The data presented demonstrate good agreement between histopathological diagnosis of FGTB and culture positivity. Two cases where RR MTB were cultured were not confirmed on histopathology and may well be representative of sample contamination during collection and transport or laboratory cross-contamination. Lower rates of RR among EPTB cases in Pakistan also support the hypothesis that culture results are a result of contamination rather than RR FGTB.[16]

The data highlight the need for improving FGTB algorithms to facilitate improved diagnosis and management in Pakistan. Development of new and improved diagnostic tools and



	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016
MTB culture positive in females	1156	869	1428	1482	1522	1426	1601	1061	574	425
RR	365	311	612	717	717	663	823	495	112	66
PTB positive	781	779	1228	1349	1276	1176	1406	923	420	317
EPTB positive	375	90	200	133	246	250	195	138	154	108
PTB RR	346	299	585	682	641	629	789	461	97	54
EPTB RR	19	12	27	35	76	34	34	34	15	12
FGTB	3	2	6	6	4	2	2	4	2	2

Figure 1: Culture-confirmed tuberculosis among females in Pakistan based on data from a large laboratory network 2007–2016. Figures in the table represent total numbers of culture-positive specimens. MTB: *Mycobacterium tuberculosis* complex, RR: Rifampin-resistant tuberculosis, PTB: Pulmonary tuberculosis, EPTB: Extrapulmonary tuberculosis, FGTB: Female genital tuberculosis

Table 1: Demographics, clinical indications, and microbiological and histopathological details of 32 women with culture-confirmed female genital tuberculosis diagnosed at a countrywide laboratory network, 2007-2016

Clinical indication	Age	Source	City	Year	Microbiological			Histopathological
					Smear	Culture	Rifampin	
Infertility	23	E	Karachi	2008	-	MTB	S	Chronic granulomatous inflammation
	23	E	Karachi	2009	-	MTB	S	Chronic granulomatous inflammation
	25	E	Karachi	2009	-	MTB	S	Chronic granulomatous inflammation
	25	E	Peshawar	2010	-	MTB	R	Benign late secretory endometrium
	30	E	Karachi	2010	-	MTB	S	Chronic granulomatous inflammation
	27	E	Karachi	2012	-	MTB	S	NA
	28	E	Karachi	2013	-	MTB	S	Normal endometrial tissue
	34	E	Karachi	2007	-	MTB	S	Normal endometrial tissue
	27	E	Karachi	2009	-	MTB	S	Chronic granulomatous inflammation and necrosis
	26	E	Karachi	2010	-	MTB	S	Normal endometrial tissue
	41	E	Karachi	2010	-	MTB	S	Benign endometrial epithelium
	26	E	Karachi	2011	-	MTB	S	Chronic granulomatous inflammation
	30	E	Karachi	2014	-	MTB	S	Chronic granulomatous inflammation and necrosis
	35	E	Karachi	2014	-	MTB	S	Chronic granulomatous inflammation and necrosis
	31	TO	Karachi	2007	-	MTB	S	Chronic granulomatous inflammation
	25	TO	Quetta	2016	-	MTB	S	Chronic granulomatous inflammation and necrosis
Menstrual irregularities/ amenorrhea	26	E	Karachi	2007	-	MTB	S	Benign endometrial epithelium
	22	PA	Karachi	2009	-	MTB	S	Chronic granulomatous inflammation
Postcoital bleeding	34	C	Karachi	2008	-	MTB	S	Cervical polyp, chronic nonspecific inflammation
Abdominal pain	30	E	Karachi	2015	-	MTB	S	Chronic granulomatous inflammation
	30	TO	Karachi	2010	-	MTB	S	Chronic granulomatous inflammation
	17	TO	Karachi	2014	-	MTB	S	Chronic granulomatous inflammation
	52	E	Mianwali	2015	-	MTB	S	Chronic granulomatous inflammation
Ectopic pregnancy	25	TO + E	Karachi	2009	-	MTB	S	Endometrium: Chronic granulomatous inflammation
Unknown	32	E	Karachi	2009	+	MTB	R	NA
	15	E	Karachi	2011	-	MTB	S	Chronic granulomatous inflammation
	24	E	Karachi	2011	-	MTB	S	NA
	25	E	Karachi	2011	-	MTB	S	Chronic granulomatous inflammation with necrosis
	26	E	Karachi	2013	-	MTB	S	NA
	35	E	Karachi	2010	-	MTB	R	Abnormal secretory endometrium with glandular and stromal breakdown
	26	E	Karachi	2014	-	MTB	S	Chronic granulomatous inflammation
	45	E	Abbottabad	2012	-	MTB	S	Chronic granulomatous inflammation

E: Endometrium, TO: Tubo-ovarian, C: Cervical, PA: Pelvic adhesions, MTB: *Mycobacterium tuberculosis* complex, NA: Not available, R: Resistant, S: Susceptible

their application to diagnosis of FGTB should be advocated to improve case detection and confidence in diagnosis.[17] Implementation of these diagnostic modalities would require strategies to improve access and service delivery to impact the health of women through reducing FGTB in Pakistan. Lack of published data on etiology of PID, especially granulomatous endometritis in Pakistan, also highlights the need for research to evaluate appropriate and innovative methods for specimen collection and integrated diagnosis.

HIV status of women was not known and linked laboratory and hospital databases for the 32 women with FGTB did not reveal if any of them had been evaluated for diabetes or prediabetes. Some limitations of this study which stem from the study design are important to note. The retrospective nature of the study did not allow us to address the proportion of FGTB among all women of reproductive age group who were evaluated for gynecological complaints. Specimen source-wise negative cultures could not be extracted from the laboratory's electronic database. We were also unable to access an independent database of histopathological examinations to evaluate the culture-negative proportion of granulomatous endometritis and salpingitis. Limitations of this study noted above highlight the need for program linkages and electronic databases.

Improved surveillance for FGTB paired with uptake of complementary and enhanced diagnostic methods in women is needed in Pakistan. Neither Xpert nor histopathology services are widely available to allow improved diagnosis which highlights the need for facilitated referral. The low positivity rates of culture in FGTB should lead to advocacy for research into and implementation of improved diagnostic methods and strategies.

Ethical clearance

The study was reviewed and exempted from individual informed consent by the Ethical Review Committee of the Aga Khan University, Pakistan.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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