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Original Article

Non-tuberculous mycobacterium in chronic breast lesions in a tertiary care hospital in Dhaka, Bangladesh

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ABSTRACT: Mycobacterial association in breast lesions with or without abscess is often overlooked and misdiagnosed as carcinoma or pyogenic abscess because specimens are paucibacillary and investigations such as microscopy and culture are frequently negative. Over the past few decades incidence of infection caused by non-tuberculous mycobacterium (NTM) has increased worldwide. Sometimes NTM infection is misdiagnosed as tuberculosis because NTM can also produce granulomatous lesion and take acid fast bacilli (AFB) stain like mycobacterium tuberculosis (MTB). We previously reported presence of NTM in variety of clinical specimens from patients attended in our hospital. This time we evaluated mycobacterium tuberculosis (TB) complex and NTM by real time PCR in chronic primary breast lesions in 45 patients attended in our hospital. The target DNA sequences were amplified with IS6110-specific primers for MTB complex and ITS-specific primers for NTM. NTM was detected in 4 breast biopsy tissues, 3 nipple discharge and 2 breast ulcer specimens from 9 patients (20%) and MTB was detected in pus and fluid aspirate from 2 (4.44%) patients out of total 45 patients. Histopathology record of 7 NTM PCR positive specimens showed 5 granulomatous mastitis, 2 fibrocystic diseases. These data show high association of NTM in chronic breast lesions for the first time in the country which were responded well by combination of clarithromycin and ciprofloxacin and warrants large scale study.

Keywords: NTM, Breast abscess, Bangladesh

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INTRODUCTION

Most breast abscesses develop as a complication of mastitis secondary to bacterial infection. Mastitis usually affects lactating women, but it can also occur in non-lactating women. Among bacteria most common is staphylococcus, streptococcus and anaerobes. More uncommon pathogens may include mycobacteria, *Actinomyces*, *Brucella*, fungi (*Candida* and *Cryptococcus*), parasites, and maggot infestation [1]. Mycobacteria are subdivided into three groups: the *Mycobacterium tuberculosis* complex, the non-tuberculous mycobacteria called NTM or MOTT (Mycobacteria Other Than Tuberculosis) and *Mycobacterium leprae*. Despite high prevalence of tuberculosis breast tuberculosis is rare with an incidence of less than 0.1% of all breast lesions in Western countries and 4% of all breast lesions in TB endemic countries [2-3].

NTM are widely distributed in the environment with high isolation rates worldwide; they can be found in soil, water, animals, and dairy products [4-9]. NTM can cause a broad spectrum of diseases: (a) pulmonary infections resembling tuberculosis; (b) extra pulmonary infections affecting lymph nodes, skin and soft tissue; (c) multi-focal disseminated infections; (d) infections in immunocompromised individuals, such as AIDS and transplant patients, [10] and (e) nosocomial infections with outbreaks related to inadequate disinfection/ sterilization of medical devices [11]. Thus, NTM has

drawn increasing attention worldwide and over the past few decades, the incidence of infections caused by NTM has also increased [12-15].

The Center for Disease Control (CDC) guidelines call for strict isolation of patients suspected of having tuberculosis in order to prevent spread to health care workers and other patients. Isolation precautions are not deemed necessary for NTM infections. Effective adherence to these guidelines requires a microbiologic diagnosis of the species of mycobacterium causing the clinical illness in a given patient. Furthermore, treatment of tuberculosis is different from that of NTM infections. Early identification of the species of mycobacterium causing illness in a patient would have significant clinical impact. Unfortunately, early species identification is not possible neither by AFB stain nor by histopathology as both the method cannot discriminate NTM from MTB. On the other hand, species identification of mycobacteria by culture is time consuming and laborious and not undergoing in the country. We previously showed real time PCR can be utilized for rapid identification and discrimination of NTM from MTB in variety of clinical specimens [16].

Though few studies in Bangladesh were done on breast tuberculosis there is no study about NTM in breast lesion [17-18]. Here, for the first time we evaluated both mycobacterial tuberculosis complex and non-tuberculous mycobacteria by real time PCR in primary chronic benign breast lesions attended in our hospital.

MATERIALS AND METHODS

Specimen processing and decontamination

All patients with chronic primary breast lesions visited Apollo Hospitals Dhaka over a period of four years (2015 to 2019). 49 breast specimens (15 breast abscess, 13 nipple discharge, 12 breast biopsy, 7 breast lump and 2 ulcer) were received at the Molecular Diagnostic Laboratory of Apollo Hospitals Dhaka for the DNA detection of *Mycobacterium tuberculosis* complex and NTM by PCR. For breast tissue biopsy, a tiny suspected part of tissue was collected in a sterile container with normal saline by the clinician. Tissue was taken in a petridish and chopped with BP blade, 1ml distilled water was added and the tissue mixture was transferred into 1.5ml microcentrifuge tube. After vigorous vortexing, the tube was centrifuged at 13000 rpm for 3 minutes. Supernatant was discarded leaving pellet with 100 µl solution and DNA extraction was done thereafter.

For nipple discharge and swab from ulcer, 1ml PBS was added into the swab tube and vortexing was done to mix the specimen properly with PBS. The specimen was transferred into 1.5ml microcentrifuge tube and centrifugation was done at 13000 rpm for 3 minutes. Supernatant was discarded and cell pellet was resuspended with 100 ul of 1x pretreatment solution 1 (LyteStar, ADT Biotech) provided in the kit (10x stock solution was diluted to 1x with water) and then DNA extraction was done according to manufacturer's instruction.

DNA extraction

1ml of 1x pretreatment solution 2 (LyteStar, ADT Biotech, diluted to 1x with water) was added to the pellet and vigorous vortexing was done and then centrifugation was done at 13000 rpm for 3 minutes. Supernatant was discarded and the same procedure was followed again. 50 µl well mixed extraction buffer (provided in the kit) was dispensed on pellet (100 µl for large pellet) and the mixture was heated at 100°C for 20 minutes. The tubes were then spinned, vortexed and centrifuged at 13000 rpm for 4 minutes and the supernatant (30 ul) containing DNA was collected into a fresh 1.5 ml microcentrifuge tube and 5 ul used in PCR reaction.

LyteStar TB/NTM PCR Principle and procedure

The LyteStar TB/NTM PCR kit 2.0 is based on real-time PCR technology, utilizing polymerase chain reaction (PCR) for the amplification of specific target sequences and target specific probes for the detection of the amplified DNA. Target DNA sequences are amplified with IS6110-specific primers for MTBC, and ITS-specific primers for NTM. The probes are labeled with fluorescent reporter and quencher dyes. Probes specific for MTBC and NTM DNA are labeled with the fluorophore FAM and HEX, respectively. The probe specific for the internal control (IC) is labeled with the fluorophore Cy5. Using probes linked to distinguishable dyes enables the parallel and differential detection of MTBC and NTM-specific DNA and the Internal Control in the corresponding detector channels of the real-time PCR instrument. The test consists of two processes in a single tube assay; 1. PCR amplification of target DNA and internal control 2. Simultaneous detection of PCR amplicons by fluorescent dye labeled probes. 5 ul Primer-Probe Mix and 10 ul 2X PCR Mix were used per sample. 15 ul master mix and finally 5 ul extracted DNA was used into each PCR tube. 5 ul Positive Control and 5 ul Negative Control was also used into one PCR tube each. Real Time Cycler Rotor Gene Q (Qiagen, Germany) was programmed according to the kit manufacturer's instruction. Specific guidelines were followed for data analysis and result interpretation.

RESULTS

We analyzed the record of patients with primary breast lesions from 2015 to July 2019 attended in our hospital. Patients are mostly (>75%) adults of 21- 40 years age and multiparous (table 1).

Table 1. PCR tested patient's age distribution

Age range (years)	No of patients	%
≤20	3	6.67
21 - 30	23	51.11
31 - 40	11	24.44
41 - 50	3	6.67
51 - 60	4	8.89
>60	1	2.22
Total	45	100.00

Nipple discharge from only one male patient was also tested. Molecular lab tested 49 specimens (table 2) from 45 patients by real time PCR.

Table 2. PCR tested breast specimen type and distribution

Type of specimen	Number	%
Pus from abscess	15	30.61
Nipple discharge	13	26.53
Tissue biopsy	12	24.49
Fluid/tissue from lump	7	14.29
Ulcer	2	4.08
Total	49	100

Two different breast specimens were tested from each 4 patients. NTM was detected in 9 (20%) patients and MTB was detected in 2 (4.44%) patients. Both the MTB positive patients were previously diagnosed case of TB and one was of disseminated TB and were receiving ATT but not responding. Clinical presentation, investigation details, therapy given and response of NTM positive cases were shown in table 3.

Table 3. NTM positive patient's brief clinical presentation, investigations, therapy given, duration and response

S/L	Clinical presentation	Specimen type tested for PCR	Histopathology	Routine culture (not mycobacteria)	Therapy given	Duration	Response
1.	Breast swelling, pain, h/o multiple surgery on lt. breast	Breast tissue biopsy	Granulomatous mastitis	No growth from pus	ATT with Clarithromycin	12 months	Cured
2.	Non healing wound with satellite nodule, h/o abscess	Breast ulcer with nipple discharge	Granulomatous mastitis	Staphylococcus epidermidis	Clarithromycin Ciprofloxacin ATT*	6 month	Cured
3.	Pain & swelling rt. Breast, weakness & anorexia	Breast tissue biopsy	Fibrocystic disease	N/A	Clarithromycin Ciprofloxacin	6 months	Cured
4.	Post breast surgery wound infection, nipple discharge	Nipple discharge	Fibrocystic disease	N/A	Clarithromycin Ciprofloxacin	6 months	Cured
5.	Rt. Breast abscess, non healing wound, lumpiness	Nipple discharge	Not done	No growth	Clarithromycin Ciprofloxacin	From 19/03/19	N/A
6.	Lt. breast lump with multifocal abscess, axillary lump lt. side	Brest tissue Nipple discharge	Granulomatous mastitis	Staphylococcus hominis	Clarithromycin Ciprofloxacin	From 17/01/19	N/A
7.	Left breast swelling with pain	Nipple discharge	Not done	Staphylococcus epidermidis	Clarithromycin Ciprofloxacin	From 10/07/19	improved
8.	Swelling left breast	Breast tissue biopsy	Granulomatous mastitis	Not done	ATT	From 15/07/19	Improved
9.	Non-healing ulcer after rt. breast I/D twice for mastitis	Swab from ulcer	Granulomatous mastitis	Staphylococcus hominis	Clarithromycin Ciprofloxacin	From 03/09/19	improved

Brief Clinical history of patients

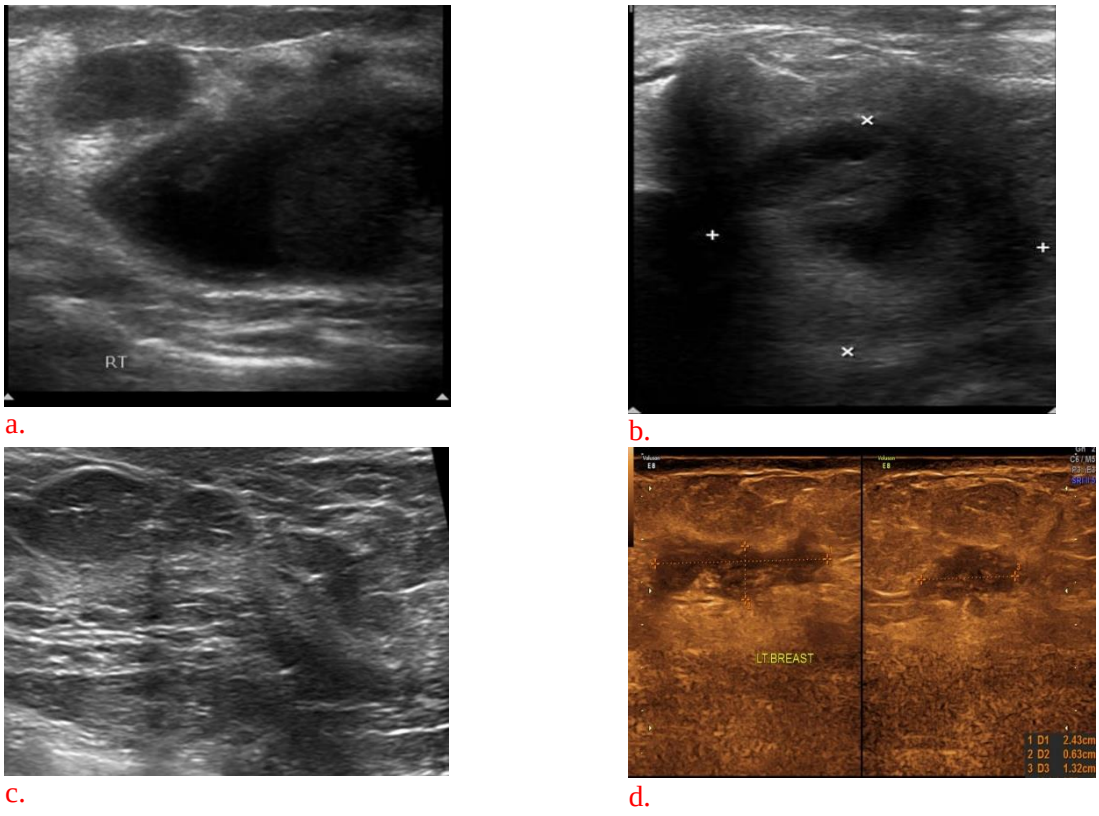
Patient usually presented with history of firm to hard swelling in the affected breast with multiple sequential micro and macro abscess with skin discoloration usually overlying the mass. Most gave history of this lump growing in size and refractory to prolonged treatment taken outside. Many have undergone incision and drainage to drain the abscesses leading to a non-healing ulcer. Some gave history of low-grade fever also.

Clinical examination

A disfigured breast with a large indurated, tender mass with areas of micro and macro abscesses and or sinuses with puckering of the skin in the affected areas. There was serous / seropurulent nipple discharge or from the raw wound of an incision and drainage done to evacuate the abscess. The mass was not fixed to the underlying structures but was tethered to the skin at the point of the abscesses and or sinuses. There was associated axillary lymphadenopathy on the affected side.

Investigation

Ultrasonography of the breasts (figure 1) revealed a large irregular lump with inspissated debris and pus, but no localized pool of pus as normally seen in an abscess.



a. b. c. d.

Figure 1. Ultrasound image of breast abscess of four (a-d) patients at time of diagnosis.

There was evidence of multiple sinuses in some cases with serous or seropurulent discharge showing no growth on culture and sensitivity. MTB/NTM PCR of nipple discharge/swab from the drained raw ulcer/ultrasonography guided tissue core biopsy led to positive identification of the NTM bacilli. The histopathology of core biopsy was reported as chronic granulomatous mastitis (with a suggestion of tuberculosis or to rule out tuberculosis) in 5 NTM positive cases and fibrocystic breast in 2 NTM positive cases. In two cases histopathology data was not available as it was not requested by the concerned clinician. Intermittent nipple discharge/ulcer swab culture was done to rule out superadded bacterial infection which is common in these patients. Monthly follow up with clinical examination and/or ultrasonography was carried out. Ultrasonography was repeated after 3 months and compared to the one that was done before instituting therapy.

Treatment

Treatment was instituted with ciprofloxacin and clarithromycin in doses of 500mg twice daily each with local antibiotic ointment dressing over the ulcers and micro abscesses till they heal. Patient was counseled that the treatment will be prolonged, and she must be in regular follow-up. Patient was also informed that the infection will subside slowly and there may be further small satellite abscesses that may appear (smoldering infection) during the therapy and that should not worry her. In case of superadded bacterial infection, it was treated with appropriate antibiotics. End point of treatment was decided through clinical and ultrasonography evaluation of the disease to assess whether the infection and the lump has resolved or not. Treatment was carried out till there was complete resolution of the lump and or healing of the non-healing breast ulcer/wound.

DISCUSSION

Breast swelling, pain, lumpiness was most common presentation and nipple discharge, delayed wound healing, nodules, sinuses and ulcer were more common in NTM positive cases. These kinds of features were previously reported in post-surgical would infection caused by NTM [18]. Granulomatous mastitis was found in 5 NTM positive cases. Variety of pathology by NTM was already reported and tuberculosis-like granuloma is not found in all patients with NTM infection [19].

We could not document the presence of NTM by mycobacterial culture as the service is neither available in the hospital nor in the country, unfortunately. Right now, PCR is the only method to show the existence of NTM in the country and we previously showed the existence of NTM by PCR in variety of clinical specimen [20]. Granuloma negative specimens were found NTM positive by PCR and the method is more sensitive than slide smear staining by *acid-fast bacilli* (AFB). Moreover, our PCR method can simultaneously detect and differentiate MTB and NTM whereas AFB

stain can detect both but cannot discriminate NTM and MTB [21].

Common antibiotics for NTM infections were reported previously where clarithromycin is said the drug of choice [22]. However, to avoid resistance against clarithromycin combination with another antibiotic was recommended. Most of our cases were offered to treat by clarithromycin 500mg and ciprofloxacillin 500 mg twice daily for two months initially and then extended up to 6 months. Though histopathology finding was not the same among these patients (granuloma in case no. 2 and case no. 6; fibrocystic disease in case no. 3 and case no. 4) combination of clarithromycin and ciprofloxacillin worked well. Our medicine consultant preferred conventional anti-TB therapy (Rifampicin, INH and Ethambutol) with clarithromycin (case number 1) or without clarithromycin (case number 8) for 12 months. Case number 1 is already cured by this treatment. Treatment is continuing for case number 5, 8 and 9 and the case number 6 is unable to reach for follow up.

Paucibacillary nature of NTM and other mycobacteria in tissues is well known and this cause disease diagnosis difficult either by PCR or by culture [23]. Among the NTM PCR negative patients 8 cases were diagnosed "Clinical NTM infection" based on clinical features such as recurrent mastitis with or without abscess, non-healing wound after surgical drainage, sinus tract, satellite nodule. On histopathology these were benign lesion and on routine microbiology culture there was no growth. These cases were treated with clarithromycin 500mg and ciprofloxacillin 500 mg twice daily for few months depended on individual response. Mastitis were resolved in 4 of these patients. Two patients are still getting these combination drugs and already improved a lot. One patient discontinued due to drug intolerance and one did not accept the diagnosis and medication.

Breast abscess by NTM was described in association with breast reduction surgery, augmentation mammaplasty [24-25]. Infection usually involves the implant, often resulting in removal of the device and significant morbidity. In addition, spontaneous chronic breast abscess secondary to NTM infection was also reported [26-27].

Most of our NTM positive cases had recurrent infections despite antibiotic treatments and few were suffering prolonged wound infections with or without nodule and sinus formation after surgical drainage. Although we could not demonstrate the bacteria by culture, the biopsy tissue or pus or nipple discharge requested by the regular physician was positive for NTM. These patients were not immunocompromised and had no history of breast surgery, including implant insertion. Therefore, the exact cause of the NTM infection could not be determined.

CONCLUSION

NTM infection of the breast is not a very uncommon disease and is often misdiagnosed as a chronic/acute abscess/inflammatory carcinoma/tuberculosis. These need not be drained or operated upon and can be treated conservatively with appropriate antibiotics after proper clinical/laboratory and radiological investigations. It is necessary for the surgeon/clinician to be aware of this disease while treating breast lumps which appear to present like acute/chronic abscesses. Many of these are treated with conventional antibiotics or go under the knife or put on empirical ATT with no relief or resolution. Any chronic granulomatous mastitis should be investigated for NTM infection before instituting a therapy. High percentage of NTM infections in breast lesions observed among immunocompetent individuals in a TB endemic country in this report, emphasizes the need for increased awareness of these emerging human pathogens and importance of molecular methods to reduce morbidity resulting from these diseases.

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