Case Report

Post Cesarean Surgical Site Infection With *Mycobacterium Abscessus Sp. Massiliense*

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Abstract

Objective: Surgical site infections (SSIs) owing to nontuberculous Mycobacteria (NTM) have emerged as an important cause of hospital-acquired SSI leading to great morbidity and mortality. Among NTM, *Mycobacterium abscessus* is reported in these sites. Epidemiology and transmission of *M. abscessus* in humans is noticing crux changes.

Case report: We hereby describe a case of SSI after lower segment cesarean section (LSCS), presenting as a skin and soft tissue infection (SSTI) owing to a NTM.

Conclusion: Clinicians should be aware of the possibility of infections caused by M. abscessus in patients who develop SSIs, particularly if they do not respond to conventional first-line antimicrobial therapy.

Keywords: Surgical Wound Infection; Nontuberculous Mycobacteria; Mycobacterium Abscessus; Heat-Shock Protein 65

Introduction

Surgical site infections (SSIs) are the infections presented on the surgical sites within 30-90 days based on the type of surgery and implants used. Centre for Disease Control and prevention- National Healthcare Safety Network (CDC NHSN) 2019 report describes SSI resulting in substantial increase in morbidity and mortality, prolonged hospitalization, incurring heavy expenses and sometimes ending with poor prognosis (1). In India, post-discharge follow-up has been a challenging area of surveillance. It is further complicated by the infections with *Mycobacterium species* (2). Global incidence of infections owing to nontuberculous Mycobacteria (NTM) is on rise and is usually associated with pulmonary infections, skin and soft tissue infections (SSTI) and disseminated disease (3). A few of the possible sources responsible for rise in incidence are fomites, iatrogenic, or a direct wound contamination following trauma, aerosolized airway secretions, contaminated hospital and public water supply. A

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role in climate change and relative genetic susceptibility in Asian population is also evolving (4). The Indian data on incidence rate and risk factors associated with post cesarean SSI is not adequate. The incidence rate varies from 3-15% depending on methods and intensity of surveillance used for pathogen identification, patient population and antibiotic prophylaxis (5).

The recommended therapy for such infections is the combination of surgical and antimicrobial interventions. The choice of antimicrobial treatment is variable and depends upon the NTM isolated (6). We hereby describe a case of SSI after lower segment caesarean section (LSCS), presenting as a SSTI owing to a NTM.

Case report

22-year-old female, G2P1L1, with previous history of LSCS and without any known complaints of hypertension, diabetes, asthma or tuberculosis, was admitted for safe institutional delivery. Bowel and bladder habits were normal throughout the pregnancy. Routine antenatal investigations were within normal limits. Indication for previous emergency LSCS was premature rupture of membranes and failure to progress. LSCS was planned and healthy female baby was delivered. Post op period was uneventful. Suture removal was done post-op day (POD)-7. Wound was healthy and the patient was discharged. After one month patient came to the out-patient department (OPD) with complaints of non-healing LSCS wound and abscess formation at and above the incision line of the LSCS scar. A gaping LSCS scar with two sinus formation was noted, one at the centre of the wound and the other at the right corner of the wound (Figure 1a). There was no local rise of temperature, tenderness or regional lymphadenopathy. Serosanguinous exudate was collected in two universal containers with the blunt end of the sterile scalpel after cleaning the wound margins and superficial area thoroughly with normal saline. One specimen was sent for regional National Tuberculosis Elimination Program (NTEP) which is the regional reference lab for Xpert MTB/RIF (Rifampicin) Assay for detection of *M. tuberculosis* (MTb) and drug resistance. The second specimen was subjected to a Grams stain, Ziehl– Neelsen (ZN) stain, and a potassium hydroxide (KOH) mount. Furthermore, sample was inoculated on Blood agar (BA) and Mac Conkey agar (MA) for aerobic bacterial culture, Lowenstein Jensen (LJ) media and Sabouraud's dextrose agar (SDA) for mycobacterial and fungal cultures, respectively.

Liver and renal function tests were normal. X-ray chest was clear. The enzyme linked immunosorbent assay for human immunodeficiency virus (HIV) was nonreactive. Patient was posted for exploration of the sinuses followed by debridement of the wound under spinal anaesthesia in view of chronic non healing discharging sinuses due to any infectious agent or foreign body reaction by prolene material. Debridement of the entire wound including two sinus tracts excision was done up to rectus sheath and the prolene suture material was completely removed followed by the wound closure with the use of delayed absorbable sutures. Patient was prescribed oral ampicillin/clavulanic acid 625mg TDS and metronidazole 400mg TDS for 5 days and was discharged. The surgical specimen was sent for histopathological examination. Gross specimen received showed two sinus tracts (Figure 1b). Hematoxylin and eosin-stained sections examined showed multiple epithelioid cell granulomas with foreign body and Langhans giant cells, foci of caseous necrosis, collection of lymphocytes and congested capillaries (Figure 1c-d). ZN stain for acid fast bacilli (AFB) done on the section showing necrosis and multiple granulomas. It revealed singly scattered pink, slightly curved, beaded rod-shaped bacilli.

Microbiological examination of the exudate revealed occasional Gram-positive beaded bacilli with plenty of pus cells. Few acid-fast bacilli were seen on ZN stain (Figure 2a). A KOH mount for fungal elements was negative. Smooth colonies were visible on BA and LJ after 2 days of incubation at 37°C (Fig. 2b-c). Non-lactose colonies were seen on MA (Fig. 2d). SDA showed tiny colonies after 3-days of incubation (Fig. 2e). ZN stains of the smears from LJ and other culture positive media demonstrated AFB (Fig. 2f), and a gram-positive slender bacillus on gram's staining. (Fig. 2f-g). Due to the rapid growth of the AFB on the medium (within one-week), rapidly growing mycobacterium (RGM) of *M. fortuitum* group (*M. chelonae*, or *M. abscessus*) was considered. An Xpert MTB/RIF assay was negative, which ruled out Mtb.

Diagnosis of SSI owing to RGM was made. Patient was put on cefixime 200mg BD for 15 days on follow up visit. The isolate was later submitted for molecular confirmation.

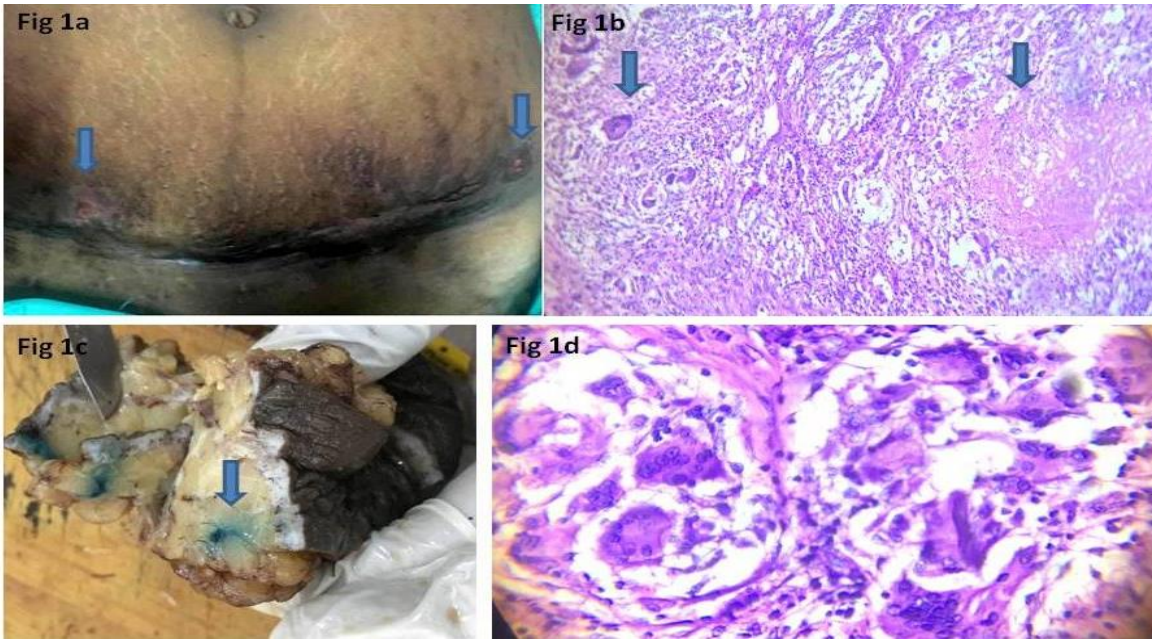

Figure 1: (a) LSCS scar with two sinus formation (arrows), one at the centre of the wound and the other at the right corner of the wound. (b) Gross specimen showing sinus tract. (c) H&E stained section, 10X, showing multiple epithelioid cell granulomas, foci of caseous necrosis and collection of lymphocytes. (d) H&E stained section, 40X, showing granulomas with both Langhan's and foreign body giant cells.

First a Polymerase chain reaction (PCR) amplification of 16S-23S ribosomal DNA (rDNA) of intergenic spacer primers (IGS) was performed. The two IGS primers used were Sp1 (59-ACC TCC TTT CTA AGG AGC ACC-39) and Sp2 (59-GAT GCT CGC AAC CAC TAT CCA-39).

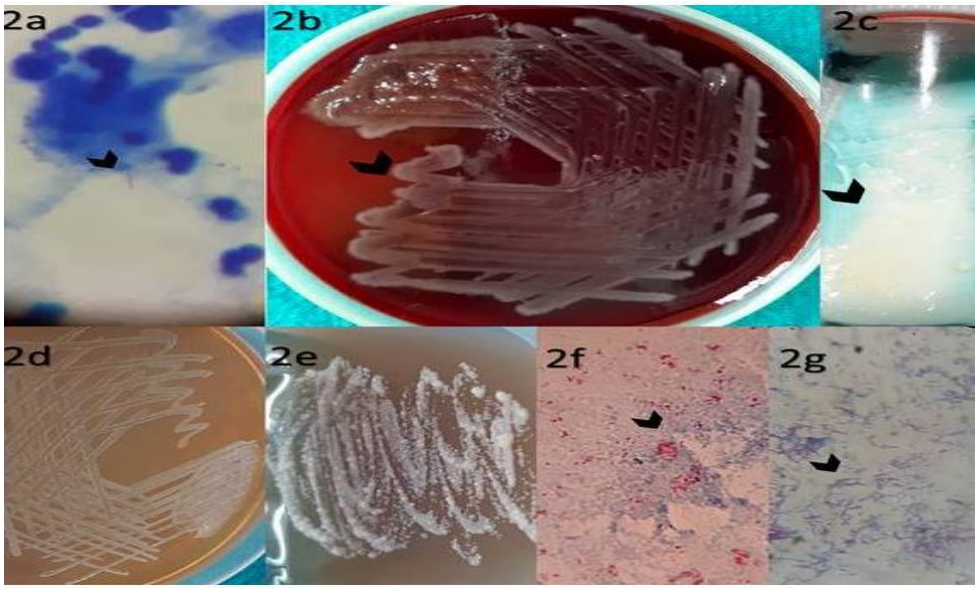

Figure 2: (a) Acid-fast bacilli seen on the direct smear preparation of exudate from LSCS. (b) Smooth, mucoid colonies on BA after 2 days of incubation at 37◦C (c) Rapid growth on LJ medium (with 2 days) at 37◦C. (d) Non lactose fermenting colonies on MacConkey agar. (e) Growth on SDA with Chloramphenical-3days of incubation at 37◦C. (f) ZN stain showing AFB from LJ medium culture. (g) Gram stain showing slender Gram positive bacilli.

The amplified products were digested separately with restriction enzyme HaeIII, CfoI, TaqI, MspI (Sigma, Germany). Electrophoresis on 4% agarose gel in the presence of ethidium bromide at 65 V for 2-3 hours was performed for Restriction Fragment Length Polymorphism (RFLP) analysis. It depicted a positive fragment band with a sequence of 201 nucleotides along with a control and a 100-bp ladder. Furthermore, identification and taxonomic separation of RGM PCR sequencing of heat shock protein 65 (hsp65) was performed. PCR for RFLP was performed on the extracted DNA by using the primers Tb12 [59-CTTGTCGAACCGCATACCCT]) which represents the 65-kDa hsp65 gene products. The amplified PCR positive mixture was processed for sequencing using the primers Tb11 and Tb12 and sequencing and analysis by ABI Prism 3130XL automated Genetic Analyzer (Applied Biosystems) using ABI PRISM BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA, USA).

The IGS sequence was 100% similar to *M. abscessus* IGS sequence (Accession no. AY498740.1) and was submitted with the accession number and MN891919, as *M. abscessus subsp. massiliense*. Comparison of the hsp65 gene and protein sequence from *M. abscessus subsp. massiliense* [Accession number EF486339.1 (gene) & AIK24802.1 (Protein)] showed 98.64%, and 100% similarity on both nucleotide basic local alignment search tool (nBLAST) and protein (pBLAST) tools of NCBI (National Centre for Biotechnology Information), respectively. The hsp65 sequence of the present case was submitted in the GenBank Banlt with submisison ID 2341238 as *M. abscessus subsp. massiliense*. Clarithromycin was started after the final diagnosis. Outcome was satisfactory with complete remission of tissue erythema, tenderness and swelling. No discharge was noted and there was good healing of cesarean section wound.

Discussion

SSTIs are the second most common infectious complications reported following urinary tract infections after LSCS (7). The most common causative microbes for LSCS wound infection are Streptococci, Staphylococcus aureus, Escherichia coli and Bacteroides species (8). NTM infection following LSCS is not commonly reported. Among the NTM, *M. abscessus* is known to be the major cause of skin infections and also the most frequent NTM recovered from clinical samples (9,10). The diagnosis is potentially challenging as routine blood cultures are usually performed and it often fails to yield *M.abscessus* due to insufficient growth and time given in general practice. Risk factors considered for developing SSIs following LSCS include age and race of the patient, anemia, previous LSCS as indication for present LSCS procedure, associated comorbid conditions like hypertension and diabetes, intraoperative blood transfusion, operative time and prophylactic antibiotics (5,11). Several studies documented that excessive intraoperative blood loss during LSCS induces immunosuppression in patients by reducing the cytotoxic T-cell and natural killer cells. Blood transfusion, if done after prolonged storage, calls forth a non-specific inflammatory response, which may divert the immune system from a more appropriate focus. Hypoalbuminemia and edema may also contribute to the development of SSI (5). The probable sources of M. abscessus are contaminated solutions such as gentian violet, instrumentation, intravenous medications, implantable devices, tap water, and improper sterilization techniques (6).

Tsao et al, defined the “certain” diagnosis of SSIs due to NTM if the patient had subcutaneous inflammatory lesions, purulence, or other findings consistent with an infection at the LSCS site in association with positive culture for NTM and/or consistent histopathological examination with NTM infection from a postoperative tissue specimen. However, diagnosis was labeled “probable” if the patient had subcutaneous lesion consistent with an infection, but the smear, culture, or histopathological examination were negative for NTM (6). There is wide diversification in cutaneous manifestations by *M. abscessus* which can cause delayed healing: ulcerations, abscesses, draining sinuses or nodules. Our patient presented with two abscesses with discharging sinuses and consistent histopathological findings of the postoperative sample. Hence it can be labeled as “certain” diagnosis of SSI due to NTM.

M. abscessus produces two types of colonies, rough and smooth variants. The rough variant is produced by *M.abscessus subsp. abscessus* and *bolletii*, which are predominately acquired through respiratory route and lead to an invasive infection. The smooth variant is produced only by *M. abscessus subsp. massiliense*. It has an ability to form biofilms and can be acquired through the environment source or a wound contamination (12,13). Smooth colonies were observed in our isolate. Identification of RGM

by conventional phenotypic and biochemical methods is cumbersome as these methods frequently fail to identify closely related species such as *M. abscessus* and *M. chelonae*. Genotypic methods like 16S-23S ribosomal DNA (rDNA) gene intergenic partial spacer, 16s rRNA, and hsp65 gene sequencing are successfully utilized in identification of these closely related NTMs. Among the above mentioned methods, hsp65 gene is genetically highly variable and more sensitive method in comparison with 16s RNA, and has been widely used in algorithms of identification of a diverse Mycobacterial species (14). The present isolate showed a positive band on ITS (16S-23S ribosomal) PCR, and hsp65 gene sequencing, thereby confirming its species similarity with *M. abscessus subsp. massiliense*.

The importance of differentiating the *M. abscessus* complex into three subspecies has been emphasized recently by utilizing the multigene sequencing and analyses, presence of erythromycin ribosome methyltransferase 41(erm) gene, in vitro antibiotic susceptibility results, and response to antibiotic therapy (9,15). The American Thoracic Society/Infectious Disease Society of America recommends combination therapy for the treatment of most postsurgical infections involving the *M. abscessus* complex. Concomitant use of macrolides with amikacin, fluoroquinolones, imipenem/cilastatin, or cefoxitin is advised (8).

In cases of *M. abscessus subsp abscesssus* infection, the induction of erm 41 gene can lead to macrolide resistance, which gives the explanation for the lack of efficacy of clarithromycin-based treatments. However, this inducible macrolide resistance has not been found in *M. massiliense* infection. Hence clarithromycin is found very effective in these cases. Most published regimens included clarithromycin alone or in combination therapy (8). Our patient underwent surgical debridement and was lastly prescribed clarithromycin after the final diagnosis. Patient had satisfactory outcome without any complication till date.

Specimens from the operation theatre such as tap water, cleaning liquid soap, povidone iodine solution, swabs from inner wall of washbasins and sterilized saline solution were collected for bacterial and mycobacterial cultures. *M. massiliense* was not detected in any of these cultures. Identification of an infection focus was difficult and strict compliance with infection control measures was done to prevent SSIs in other patients.

Conclusion

Knowledge of risk factors associated with SSIs is essential to implement specific preventive measures. Clinicians should be aware of the possibility of infections caused by *M. abscessus* in patients who develop SSIs, particularly if they do not respond to conventional first-line antimicrobial therapy as the presenting symptoms and signs of *M. abscessus* infections are nonspecific and indolent. Combination therapy of macrolides with imipenem, amikacin, or cefoxitin has been reported to be the optimal therapy. The advancement of subspecies differentiation has allowed for more effective management. Unlike *M. abscessus* subsp. *abscessus*, subsp. *massiliense* does not have inducible resistance to clarithromycin. Therefore, knowing that a patient's infection is due to *M. abscessus* subsp. *massiliense* enables the physician to confidently administer clarithromycin.

Conflict of Interests

Authors have no conflict of interests.

Acknowledgments

The authors declare no conflict of interests.

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