



AN OUTBREAK OF NON TUBERCULOUS MYCOBACTERIA IN POST SURGICAL WOUND INFECTION IN A TERTIARY CARE CENTRE IN WESTERN UTTAR PRADESH

Dr Ruchi Kotpal* MD, Assistant Professor, Department of Microbiology *Corresponding Author

Dr. Neetika Agarwal MD, Consultant Pathologist, Department of Pathology

Dr. Savita Yadav MD, Consultant Pathologist, Department of Pathology

ABSTRACT **Purpose** – Non tuberculous mycobacteria (NTM) are environmental organisms that can cause skin and soft tissue infections at incision site in patients undergoing surgical procedures. Most of the NTM causing these infections are not treated by first line standard anti tubercular drugs.

Methods – it is a case series of 30 patients who presented to our hospital with nodular swelling followed by discharging sinus at the site of incision almost one month after they underwent surgery all over the period of 6 month from May to October 2018,. Pus was collected and examined for isolation and identification of the causative agents. Debrided sinus tract was also sent for histopathological examination.

Result - Of the 30 patients examined, all presented with discharging sinus, with no systemic symptoms, none of them responded to pyogenic antibiotics. None of the pus sample was positive for gram staining and all were sterile for pyogenic culture, 60% positivity for ZN staining, 80% grew Mycobacteria on LJ media and 100% showed granulomatous inflammation on histopathology. All responded to oral clarithromycin for 4-6 weeks

Conclusion - Present study indicate that non-tuberculous mycobacterial infections should be considered in wounds that show delayed healing, do not respond to antibiotics used for pyogenic infections and have sterile aerobic cultures. Empirical therapy should be initiated immediately. Also smear positivity and culture positivity are not the sole criteria for considering mycobacterial infections.

KEYWORDS : post surgical, wound infections, Non tuberculous mycobacteria (NTM), discharging sinus

INTRODUCTION

Non tuberculous mycobacteria (NTM) are widely distributed in nature having been isolated from natural water, tap water, soil and water used in showers and surgical solutions^[1]. The highest rates of NTM colonization in potable water systems are found in hospitals and hemodialysis and dental offices, with rates ranging from 60% to 100%^[2,3]. Most frequently isolated NTM from hospital water sources and causing nosocomial infections are *M. avium*, *M. xenopi*, and rapid growing mycobacteria (RGM) i.e *Mycobacterium fortuitum*, *Mycobacterium chelonae* and *Mycobacterium abscessus*^[4].

NTM are associated with wide variety of nosocomially acquired infections one of these are skin and soft tissue infections in post surgical patients. These infections are a source of significant morbidity for patients recovering from laparoscopic surgeries. Erroneous sterilization of instruments is almost always responsible for such outbreaks and makes it a problem mainly affecting developing countries. Thus proper sterilization of such instruments is essential to prevent the occurrence of post laparoscopic wound infections with atypical mycobacteria^[5]. Various studies have reported Rapid growing mycobacteria as one of the important cause of post surgical wound infections^[6,7].

Rapidly growing mycobacteria are of significant importance as they are not treated by standard first line antitubercular drugs used for the treatment of infections caused by them^[8]. Thus, the standard treatment consist of combinations of second line anti-tubercular drugs including macrolides such as clarithromycin, quinolones such as ciprofloxacin, tetracyclines such as doxycycline, and aminoglycosides such as amikacin are used for the treatment of disease caused by NTM. Moreover, the course of therapy is also significantly differ for the disease caused by Rapid growers, non rapid growers mycobacteria & other mycobacterial species^[9]. Once there is manifestation of clinical symptoms, the standard treatment consists of a 28 day regimen of oral clarithromycin and ciprofloxacin or amikacin^[10].

We report a case series of 30 patients who underwent surgery both laparoscopic and open procedures at our hospital over the period of 6 month from May to October 2018. All presented with typical symptoms of post surgical wound infection not responding to antibiotics used for pyogenic infections.

MATERIALS AND METHODS

thirty patients (14 male and 16 female) mean age 49.25 ± 14.29 underwent surgical procedures at our Hospital (15 underwent laparoscopic cholecystectomy, 8 had undergone inguinal mesh

hernioplasty, 4 undergone percutaneous nephrolithotomy and 3 underwent male breast reduction surgery) all over the period of 6 month from May to October 2018. All presented with similar symptoms of nodular swelling at the site of incision with mild to moderate pain along with tenderness one month after the surgery (Fig 1). This swelling progressed to sinus formation with serosanguinous discharge over the period of 2 weeks, typical of symptoms incision site infections. Non of them responded to antibiotics for pyogenic infections. At the time of discharge none of the patients showed any signs of infections and were discharged with healthy wounds. None of the patients complained of febrile illness and there were no other signs of systemic infection. White blood cell and differential count were normal thus confirming absence of systemic infection.

Pus/discharge from the post-operative wounds were collected either with the help of sterile cotton swabs or ultrasound guided aspiration and were sent to the Microbiology Department immediately. Pus from all the patients were collected from the nodules and were smeared for Gram staining, Ziehl Neelsen staining and cultured for aerobic pyogenic organism on Blood agar, Mac Conkey agar and brain heart infusion broth and incubated at 37°C for 48 hours. The pus sample was also cultured directly (without decontamination) on Lowenstein Jensen culture media for Mycobacterial growth and incubated at 37°C for 8 weeks^[11]. Twelve out of 30 patients underwent sinus tract debridement under general anaesthesia with primary suturing and the sinus tract was sent for histopathology to the Department of Pathology. Remaining 18 patients responded to oral clarithromycin and did not require sinus tract debridement. One of the patient underwent ultrasound guided aspiration from stitch line which was also sent for cytology to the department of Pathology.

All isolates grew on LJ medium was further processed and tested for the following additional characteristics: growth within 3-8 days on LJ medium, acid fastness of the isolates. Antibiotic susceptibility was performed using disc diffusion method and showed resistance to cefoxitin, ceftriaxone, ciprofloxacin and polymyxin B and susceptible to azithromycin, amikacin and vancomycin

RESULT

A total of 30 patients with delayed post-operative wound infections were included in this study. All the patients underwent surgery either open or laparoscopic at our hospital in the month of April 2014. All presented with similar symptoms of appearance of nodular swelling and induration (1 month to 2month after the surgery) followed by breakdown of wound and suppuration and discharging sinuses, with absence of systemic symptoms and lack of response to antibiotics used

for pyogenic infections.

Pus from all 30 patients were examined for Gram staining and all of them showed abundant polymorphonuclear cells with no organism. On Ziehl Neelsen staining, 18 sample was positive for acid fast bacilli (Fig 2) and 24 sample grew on LJ media within 1 week of incubation and was identified as NTM by staining with ZN Staining. Aerobic culture of all the 30 samples were sterile. All 24 pus samples grew white non-pigmented, smooth colonies on Lowenstein-Jensen (LJ) medium within 1 week of incubation at 37°C (Fig 3). AFB staining from the culture revealed acid fast bacilli.

Twelve out of 30 patients underwent sinus debridement and tissue were sent for histopathology. Gross examination of submitted tissue mostly consisted of single piece of tissue measuring 2.5 cm x 1.5cm approximately. Microscopy revealed diffuse infiltration of tissue by lymphocytes, plasma cells and polymorphs. Few ill defined granulomas comprising of lymphocytes, plasma cells and giant cells both foreign body and langhan's type. Features were suggestive of granulomatous inflammatory lesion (Fig 4a,b). Ultrasound guided fluid aspiration from nodule of umbilical stitch line of one of the patient also revealed ill defined granulomas on cytology (Fig 5).

All the 30 patients were started on oral Clarithromycin for 4-6 weeks and responded to it (Fig 1b). (Table 1)

DISCUSSION

Infections at the incision site of surgery can be classified as early type due to pyogenic organisms or delayed type. Delayed type is caused by atypical mycobacteria which includes those mycobacterial species that are not part of the *M. tuberculosis* complex, have an incubation period of 3 to 4 weeks, do not respond to common antibiotics^[12]. We focused on delayed type of portal site infections due to Non tuberculous mycobacteria.

From contaminated water NTM contaminate the surgical instruments including laposcopes which are not properly sterilized. These unsterilized instruments leave the mycobacterial bacilli at the portal site and cause infections limited to skin and soft tissue. These bacteria have an affinity for the dermis and the subcutaneous area and protective factors within the peritoneum destroy the mycobacteria and prevent infection within the peritoneal cavity hence donot disseminate^[13].

Typically it presents with nodules which appear 4 weeks after the surgery. The nodules get bigger in size, become more tender and inflamed and eventually form a sinus discharging white pus. Lastly the area develops into a chronic sinus discharging white fluid followed by darkness of the area with necrosed skin. If left untreated, the infection can continue for months and multiple nodules appear in different areas^[13]. Patients in our study also presented with these typical symptoms.

Many studies in India like one in Bangalore^[14] a series of 145 laparoscopy port site infections due to *M. chelonae* were found in 35 patients following laparoscopy at a single hospital over a 6-week period. The contaminating source was ultimately identified as the rinsing water used for washing chemically disinfected instruments. Apart from these many sporadic cases^[5,6,15,17] of soft tissue infections due to NTM have being reported from various parts of India post surgery. Our study also report 30 cases of post surgical wound infections, some due to Mycobacterium abscesses or other NTM all presenting with same clinical symptoms. In our study the medical instruments such used were washed with tap water and autoclaved whereas the laposcopes used were washed with tap water, disinfected with 2% gluteraldehyde and then rinsed with normal saline.

Most of the clinical diagnosis is made based on these signs and symptoms since AFB staining rate and mycobacterial culture positivity rate of the pus collected from surgery site is very poor. One of the study in India showed 60% direct AFB staining rate and 80% culture positivity rate^[15]. Chaudhary et al^[13] reported all 19 cases with typical symptoms of port site infections to be negative for acid fast bacilli. Though many sporadic cases^[5,6,15,17] of infection caused by NTM have being reported with some being positive for AFB, some being positive for culture and some being positive for granulomatous inflammation on histopathology. All our patients showed typical symptoms of post surgical site infection, with average incubation period of 1 month to 2 months, with absence of systemic symptoms

with no response to antibiotics used for pyogenic infections. Out of 30 cases 18 were AFB positive from direct pus also showing culture positivity for Mycobacteria. 100% showed granulomatous inflammation on histopathology and 100% response to oral clarithromycin. Though we could not isolate Mycobacterium spp from hospital water source. Hence standard treatment consists of a 28 day regimen of oral clarithromycin and ciprofloxacin or amikacin should be started once there is manifestation of clinical symptoms^[12] without waiting for AFB staining or culture report as it may take few more weeks resulting in increased suffering to the patients.

To prevent these nosocomial infections the instruments should be thoroughly cleansed after each use, with complete dismantling of parts to ensure removal of all organic soil^[18]. Secondly, limit the use of gluteraldehyde disinfectants and replace it with ethylene oxide gas sterilization, as it is highly effective in reducing atypical mycobacterial infections following laparoscopy^[14]. When gluteraldehyde is to be used, then use it in higher concentrations (3.4%) with increased exposure time to 8-12 hours to activate sporicidal activity. Furthermore, the water used to rinse the instruments should be autoclaved to prevent recontamination with spores^[14].

CONCLUSION

These small measures if taken can reduce the incidence of post surgical wound infections in patients and decrease the incidence of morbidity and suffering in the patients coming to the hospital for surgical procedures.

LEGENDS OF IMAGES



Fig 1a : Discharging sinus in the infraumbilical area before treatment



Fig 1b : Healed sinus after treatment with oral clarithromycin

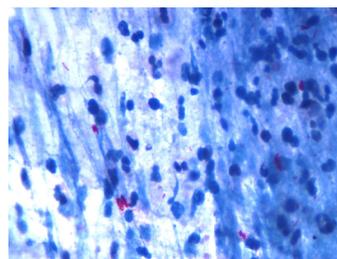


Fig 2 : Direct smear showing Acid Fast bacilli on Ziehl Neelsen stain from pus sample



Fig 3 : white non-pigmented smooth colonies on Lowenstein-Jensen medium on 5th day of incubation at 37 degree C

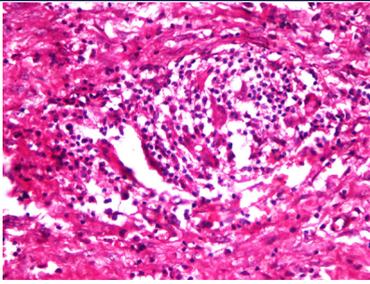


Fig 4a : Ill defined granulomas seen in debrided sinus tract biopsy on 10X

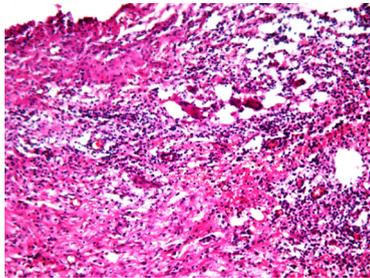


Fig 4b : Ill defined granulomas seen in debrided sinus tract biopsy on 40X

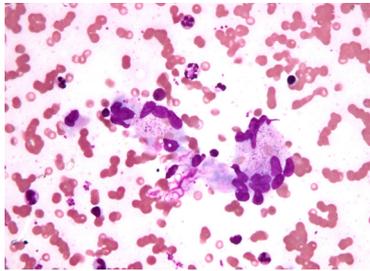


Fig 5 : Langhan's type of giant cells seen on FNAC from stitch line on 100X

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Table I. Number and percentage of patients showing the following parameters :

Parameter	No of Patients (30)	Percentage
Symptoms of Nodular Swelling with/without discharging sinus	30	100
Average duration of appearance of symptoms 1-2 months post surgery	30	100
Absence of Systemic symptoms	30	100
No Response to pyogenic antibiotic	30	100
Gram staining showing no organism only polymorphs	30	100
No growth on Pyogenic culture	30	100
Presence of AFB on Ziehl Nelson staining	18	60
Growth on LJ medium	24	80
Granulomatous inflammatory reaction on Histopathology/cytology	12	40
Response to oral Clarithromycin for 4 weeks	30	100

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