Nontuberculous mycobacteria in fistula-in-ano: A new finding and its implications

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ABSTRACT

Objective/background: Nontuberculous mycobacteria (NTM) are not known to be associated with fistula-in-ano. NTM was detected in three fistula-in-ano patients in our series. In this study, related data was reviewed to find the mycobacterial disease in patients in our database. Methods: In this study, 311 consecutive fistula-in-ano patients operated over 2 years were analyzed. The histopathology of anal fistula tract epithelial lining of every operated patient was analyzed and other tests (real-time-polymerase chain reaction [RT-PCR], GenXpert, and mycobacterial culture) were conducted in patients with high index of suspicion of having mycobacterial disease. Results: Two patients had histopathological features suggestive of mycobacterial disease. Of these, one patient had NTM and the other had Mycobacterium tuberculosis (MTB) on RT-PCR. Four patients had normal histopathology features but tested positive on RT-PCR (2 each for NTM and MTB). Therefore, a total of six patients were tested for mycobacterial disease (3 each for NTM and MTB). Mycobacterium culture was performed in two patients (both NTM) but the result was negative. Five of six patients (NTM = 2, MTB = 3) presented with delayed recurrences after operation (6–18 months after complete healing). Conclusion: NTM can cause fistula-in-ano. It could be an undiagnosed contributory factor in fistula recurrence. Mycobacterial disease (both tuberculous and nontuberculous) may be associated with delayed recurrence of fistula. RT-PCR is highly sensitive and can differentiate between NTM and MTB. It should perhaps be performed in all recurrent and refractory cases.

Introduction

Although Mycobacterium tuberculosis (MTB) infection is considered to be a diminishing clinical problem in industrialized countries, it continues to be a dominant public health concern in many developing ones [1]. However, the incidence of nontuberculous mycobacteria (NTM) infections has increased in developed countries with the global human
immunodeficiency virus/acquired immunodeficiency syndrome epidemic and the use of immunosuppressive agents [1]. Unlike MTB, which is transmitted from one person to another, NTM are abundant in nature, soil, and water, and are believed to be acquired by environmental exposure. Different species of NTM prefer different environmental conditions, and thus, they are also known as environmental bacteria.

Extrapulmonary TB is responsible for 3–46% of all types of TB cases worldwide [2]. Of these, perianal TB is a rare type accounting for only 0.7% of these cases [3]. However, it is very likely that the prevalence of perianal TB is underestimated as it remains undiagnosed or gets misdiagnosed as Crohn’s disease or other granulomatous diseases [4]. NTM is not known to be associated with perianal or fistula-in-ano disease.

NTM was detected in three fistula-in-ano patients in our series. The data were reviewed to find the mycobacterial disease in patients in our database.

Materials and methods

A retrospective analysis of 311 consecutive fistula-in-ano patients operated in a referral fistula center between August 2013 and October 2015 was performed.

The protocol of our center was to send the histopathology of anal fistula tract epithelial lining of every operated patient for further analysis. Because NTM was not known to be associated with fistula-in-ano, patients with histopathological features suggestive of mycobacterial disease (granuloma formation, caseation necrosis, epithelioid cells, or Langhans giant cells; Figs. 1 and 2) were assumed to be suffering from MTB and standard antitubercular therapy was started in these patients. This practice was continued until NTM was detected on real-time-polymerase chain reaction (RT-PCR). Since then, RT-PCR was performed in cases in which mycobacterial disease was suspected clinically. However, it was not performed in all operated fistula cases due to cost constraints. RT-PCR is highly sensitive and can differentiate between TB and NTM but has low specificity (due to false positives and contamination) [5]. By contrast, both histopathological analysis and mycobacterial culture have low yield [6,7]. Therefore, RT-PCR positivity and the overall clinical picture were correlated to arrive at a diagnosis. Other related tests (chest X-ray, Mantoux test, GeneXpert, Mycobacterium culture) were performed as deemed necessary.

Delayed recurrence of fistula-in-ano was defined when a patient with fistula-in-ano was completely cured after the fistula surgery but had a recurrence 6–18 months after the operation.

Results

Histopathology of anal fistula tract epithelium was analyzed in 311 fistula-in-ano patients and only two patients had features suggestive of mycobacterial disease. Of these, one patient had NTM and the other had MTB on RT-PCR. A total of six patients were tested for mycobacterial disease (3 each for NTM and MTB) on RT-PCR (Table 1). Four (2 each for NTM and MTB) of these six patients had normal histopathology features. Mycobacterium culture was done in two patients (NTM positive on RT-PCR) but the result was negative. Five of the six patients (NTM = 2, MTB = 3) presented with delayed recurrences after the operation (6–18 months after complete healing; Table 1). All patients diagnosed with NTM received an oral antibiotic combination (clarithromycin + sulfonamide/faropenem/doxycycline), which empirically covered all common skin and soft-tissue infections causing NTM (Mycobacterium fortuitum group, Mycobacterium abscessus, Mycobacterium chelonae, and Mycobacterium marinum) [8]. The two-drug combination was given for 3 months and all the patients responded well with complete healing of their fistula.

The study clearly indicated that histopathology had lower sensitivity as compared with RT-PCR. It would have been interesting to compare the sensitivity of histopathology and RT-PCR. As we know, the sensitivity of a test is the probability that a test will indicate disease among those with the disease. Because there is no gold standard test to diagnose
Mycobacterium, it was not possible to know how many patients with fistula-in-ano in this study were actually suffering from the mycobacterial disease. In the absence of knowledge of total number of patients suffering from mycobacterial disease, it was not possible to calculate the sensitivity of either of the tests (histopathology and RT-PCR). Second, RT-PCR was not performed in all patients, but only in patients with high index of suspicion. This would have led to a significant sampling bias if these data were used to calculate sensitivity. The only parameter that can be calculated is prevalence of mycobacterial disease on histopathology ($2/317 = 0.63\%$). This cannot be calculated for RT-PCR due to sampling bias.

**Discussion**

This is a first time that NTM have been shown to be associated with fistula-in-ano. This finding has a number of important implications.

The diagnosis of mycobacterial disease is not easy and straightforward. Most of the mycobacterial bacilli are slow growing and ubiquitous. This leads to difficulty in culturing these microorganisms and problem of contamination. Although a number of tests are available, the interpretation of their results is not simple. Ziehl-Neelsen carbol-fuchsin staining for acid-fast bacillus and histopathological features can neither differentiate between MTB and NTM [6] nor between different species of NTM [6,7]. Some recent studies have outlined characteristics to differentiate MTB from NTM (neutrophil infiltration, interstitial granuloma, small vessel proliferation, and increased numbers of bacilli were found to be associated more with NTM, whereas giant cells, plasma cells, tuberculoid granulomas, and necrosis were associated more with TB) [6]; however, these need to be confirmed in more studies before they can be routinely used.

Mycobacterial culture is more specific and sensitive than histopathology but requires weeks of incubation and cannot differentiate between infection and contamination. PCR is now routinely available, but requires careful control to prevent contamination and false-positive results [9]. RT-PCR can differentiate between TB and NTM [5] but it cannot differentiate between different species of NTM. Gene sequence analysis (PCR-amplified hypervariable regions of the 16S ribosomal RNA gene) can differentiate between species of NTM [10–12] but it is quite costly and will not help to know about antibiotic sensitivity. GeneXpert (Xpert MTB/RIF) is an automated, heminested RT-PCR that not only detects TB but also tests for rifampicin sensitivity using molecular beacons [13]. It is also rapid as the result can be obtained within 2 h. However, it cannot differentiate between TB and NTM.

NTM is not known to be a causative agent in fistula-in-ano. The findings of this study bring to light a new factor that could be responsible for fistula causation or recurrence. At the same time, it raises several questions and adds to the dilemma of treating fistula-in-ano.

1. NTM could be responsible for repeated recurrences (refractoriness to treatment) in some proportion of fistula-in-ano cases.
2. Up to now, histopathology was the test routinely done to detect MTB in fistula-in-ano. The findings of this study reaffirm that histopathology has low sensitivity and perhaps misses a significant proportion of mycobacterial cases.
3. Mycobacterial disease on histopathology of anal fistula (granuloma, caseation necrosis, epithelioid cells, and Langhans giant cells) was routinely diagnosed as MTB and treated by standard antitubercular therapy. NTM gives a similar picture on histopathology as TB (Figs. 1 and 2). Therefore, the histopathological features of mycobacterial disease cannot be diagnosed as TB and should be evaluated further to ascertain whether they are because of TB or NTM.
4. Histopathology cannot differentiate between TB and NTM and the yield of histopathology is lower than RT-PCR. This raises the following question: “Should RT-PCR be performed in all biopsy samples of fistula-in-ano patients or in only those with histopathology suggestive of mycobacterial disease?”
5. RT-PCR cannot give details about different species of NTM. Only mycobacterial culture can differentiate between species of NTM and can help to determine their antibiotic sensitivity. However, the culture takes a long time (4–6 weeks) and has low yield. Different species of NTM have different

<table>
<thead>
<tr>
<th>Patient</th>
<th>Histopathology (anal fistula tract lining)</th>
<th>Real-time-polymerase chain reaction</th>
<th>Culture</th>
<th>Delayed recurrence after operation</th>
<th>Response to standard antitubercular therapy</th>
<th>Response to empirical NTM therapy (clarithromycin + sulfonamides)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Positive</td>
<td>NTM</td>
<td>Negative</td>
<td>Yes</td>
<td>Not given</td>
<td>Successful</td>
</tr>
<tr>
<td>2</td>
<td>Negative</td>
<td>NTM</td>
<td>Negative</td>
<td>Yes</td>
<td>Given but failed</td>
<td>Successful</td>
</tr>
<tr>
<td>3</td>
<td>Negative</td>
<td>NTM</td>
<td>Not done</td>
<td>No</td>
<td>Not given</td>
<td>Successful</td>
</tr>
<tr>
<td>4</td>
<td>Positive</td>
<td>MTB</td>
<td>Not done</td>
<td>Yes</td>
<td>Successful</td>
<td>Not applicable</td>
</tr>
<tr>
<td>5</td>
<td>Negative</td>
<td>MTB</td>
<td>Not done</td>
<td>Yes</td>
<td>Successful</td>
<td>Not applicable</td>
</tr>
<tr>
<td>6</td>
<td>Negative</td>
<td>MTB</td>
<td>Not done</td>
<td>Yes</td>
<td>Successful</td>
<td>Not applicable</td>
</tr>
</tbody>
</table>

Note: The empirical therapy of NTM given was clarithromycin + sulfonamides/faropenem/doxycycline. This was done because the common NTM species that cause extrapulmonary abscesses and soft-tissue infections (Mycobacterium fortuitum group, Mycobacterium abscessus, Mycobacterium chelonae, and Mycobacterium marinum) are responsive to these antibiotics. MTB = Mycobacterium tuberculosis; NTM = nontuberculous mycobacteria.
Table 2 – Common NTM species that cause extrapulmonary abscesses and soft-tissue infections and their antibiotic sensitivity.

<table>
<thead>
<tr>
<th>Species of NTM</th>
<th>First line</th>
<th>Second line</th>
<th>Second line</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mycobacterium fortuitum</td>
<td>Clarithromycin</td>
<td>Sulfonamides (trimethoprim–sulfamethoxazole)</td>
<td>Amikacin</td>
</tr>
<tr>
<td>Mycobacterium abscessus</td>
<td>Clarithromycin</td>
<td>Amikacin</td>
<td>Imipenem, cefoxitin, ciprofloxacin, &amp; doxycycline</td>
</tr>
<tr>
<td>Mycobacterium chelonae</td>
<td>Clarithromycin</td>
<td>Amikacin</td>
<td>Imipenem &amp; cefoxitin</td>
</tr>
<tr>
<td>Mycobacterium marinum</td>
<td>Clarithromycin</td>
<td>Sulfonamides (trimethoprim–sulfamethoxazole)</td>
<td>Doxycycline, rifampin, ethambutol, &amp; minocycline</td>
</tr>
</tbody>
</table>

Note: NTM = nontuberculous mycobacteria.

antibiotic sensitivity (some NTM respond to conventional antituberular therapy, whereas others respond to different conventional antibiotics). In such a scenario and in the paucity of any data or guidelines in the literature as to which species of NTM cause fistula-in-ano, any treatment (even empirical) is difficult to start.

In this study, empirical treatment was started in patients diagnosed with NTM infection (diagnosis made on RT-PCR along with clinical picture). In the absence of any guidelines in the literature, simple scientific strategy was followed. The literature was analyzed to tabulate the most common NTM-causing skin and soft-tissue infections and their known antibiotic sensitivity (Table 2) [8]. The oral antibiotic combination (clarithromycin + sulfonamides/faropenem/doxycycline), which covered all these NTM (M. fortuitum group, M. abscessus, M. chelonae, and M. marinum), was selected [8]. The two-drug combination was recommended for 3 months. Probiotics were also recommended along with these.

Delayed recurrence (recurrence of fistula 6–18 months after complete healing of fistula) was observed in 83.3% (5/6) of patients who tested positive for mycobacterial diseases on RT-PCR. A possible explanation for this could be that most of the Mycobacteria are slow growers and take a few weeks to months to multiply. Usually, a fistula heals after operation within 6–10 weeks. This duration is too short for Mycobacteria to multiply to substantial numbers. Therefore, presence of Mycobacteria perhaps does not stop fistula from healing but causes delayed abscess formation and recurrence after few months once they get sufficient time to multiply and grow.

To conclude, both NTM and TB can cause fistula-in-ano. They should be especially suspected in patients having a delayed recurrence (recurrence after 6–18 months). Histopathology alone cannot be relied upon to detect mycobacterial disease. RT-PCR should be done in patients with high index of suspicion for mycobacterial disease. More data need to be accumulated across the world and guidelines about how to approach (diagnose and treat) mycobacterial disease (TB and NTM) in fistula-in-ano cases should be formulated.

Conflicts of interest

The author declares no conflicts of interest.

REFERENCES