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Case Report

First report of cervicofacial lymphadenitis due to *Mycobacterium haemophilum* in an immunocompromised adult patient

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Abstract

We report the first case of an immunocompromised adult patient presenting with cervicofacial lymphadenitis due to *Mycobacterium haemophilum*, confirmed using *hsp65* gene sequencing and line-probe assays. In resource-limited settings, especially in developing countries, appropriate culture methods and rapid molecular diagnostic tools such as *hsp65* gene sequencing for identification of this organism may not be readily available. This may cause *M. haemophilum* infections to go unrecognised or lead to delays in diagnosis. Lack of heightened awareness about the potential for this mycobacterial species to cause infections may also contribute to possible underestimation of *M. haemophilum* cases in the developing world.

Key words: *Mycobacterium haemophilum*; cervicofacial lymphadenitis; immunocompromised patient; *hsp65* gene sequencing.

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Introduction

M. haemophilum is a slow-growing, fastidious nontuberculous mycobacterium (NTM), that preferentially grows at 30°C to 32°C [1,2]. In recent years, *M. haemophilum* has emerged as an important human pathogen, causing mainly opportunistic infections in severely immunocompromised patients [3]. *M. haemophilum* infections have been previously reported in other patients receiving immunosuppressives after renal transplantation, with skin lesions being the most common clinical presentations [4,5]. Multiple skin lesions tend to occur and can present as erythematous papules, plaques, nodules, necrotic abscesses, or chronic ulcers, which are found most commonly on the extremities [6]. Although extracutaneous infections such as septicaemia, pneumonitis, septic arthritis, osteomyelitis, epididymal abscess, and pyomyositis have been described previously in immunocompromised patients [6,7], this patient presented with cervicofacial lymphadenitis, which has only been reported so far in immunocompetent adults and children [6]. Therefore, this case, to the best of our knowledge, is the first report of cervicofacial lymphadenitis caused by this mycobacterial species in an immunocompromised adult.

Case Report

The patient was a 43-year-old woman who has been receiving immunosuppressive treatment with mycophenolate mofetil, prednisolone, and tacrolimus since undergoing renal transplantation 13 years earlier, due to end-stage renal disease caused by poorly controlled hypertension. She presented to us with a painful purulent skin lesion over the left maxillary area and bilateral swelling in the submandibular region. The swelling on the left side was markedly bigger than the right and showed skin discolouration (Figure 1). She experienced no fever, cough, night sweats, malaise, loss of appetite or loss of weight. There was no history of trauma, insect bite, acupuncture treatment, dental manipulation or exposure to aquatic environments.

Incision and drainage of the maxillary skin lesion was performed, and a pus specimen obtained was sent for bacterial culture, which yielded negative growth. Histopathological examination of a skin lesion biopsy revealed inflamed granulation tissue with granulomatous inflammation. Subsequent staining

with Ziehl-Neelsen and Wade-Fite staining showed acid-fast bacilli (AFB).

A contrast-enhanced computed tomography (CT) scan of the neck revealed enlarged bilateral cervical lymph nodes and an abscess measuring 2.5 by 2.3 by 1.9 cm in size in the left submandibular region (Figure 2). Cytopathological examination of smears prepared from pus specimens obtained by fine-needle aspiration biopsy of the affected lymph nodes revealed multiple histiocytes admixed with neutrophils, consistent with lymphadenitis. Although no well-formed granulomas were seen, numerous AFB were observed on Ziehl-Neelsen stain. The pus specimens from the submandibular abscess and the involved lymph nodes were sent for mycobacterial culture, smears of which were heavily positive for AFB on Auramine O and Ziehl-Neelsen stain. However, no growth was detected in Lowenstein-Jensen (LJ) medium and liquid Mycobacteria Growth Indicator Tube medium (Bactec MGIT 960; BD Diagnostic Systems, Sparks, USA) at 37°C. In view of these findings, and due to a high clinical suspicion at this stage of a possible infection with a fastidious mycobacterial species, the specimens were incubated in LJ medium and liquid MGIT medium at 30°C, without the addition of iron supplementation. Conventional polymerase chain reaction (PCR) and subsequent sequencing of a 422-bp segment of the hsp5 gene encoding the 65-kDa heat shock protein (*hsp65*) was performed directly on one of the pus specimens, using the primers 5 - ATCGCCAAGGAGATCGAGCT-3 (forward) and 5 - AAGGTGCCGCGGATCTTGTT-3 (reverse), as previously described [8]. The partial *hsp65* DNA sequences generated were aligned and compared with those of mycobacterial reference strains. The sequence was found to be 99% identical to that of *Mycobacterium haemophilum.* In addition, a positive signal was detected in the liquid MGIT medium after 1 week of incubation, and the mycobacterium was also identified as *M. haemophilum* by the line-probe assays Genotype Mycobacterium CM (Hain Lifescience GmbH, Nehren, Germany) and Genotype Mycobacterium AS (Hain Lifescience GmbH, Nehren, Germany). No growth was detected in LJ medium.

The patient was then commenced on a triple chemotherapeutic regimen consisting of azithromycin, ciprofloxacin, and rifampicin. The lesions significantly improved after 2 months of antibiotic treatment. A repeat CT scan of the neck performed 3 months after therapy commencement showed resolution of the previously noted left-sided submandibular abscess and bilateral cervical lymphadenopathy. The patient showed clinical resolution of her lesions at follow-up visit at the infectious diseases outpatient clinic, 4-months post-treatment. Therefore, the decision was made to continue the antibiotic regimen to complete a total of at least 12 months of therapy.

Figure 1. Clinical photo showing a left maxillary skin lesion that was incised and drained and a swelling in the ipsilateral submandibular region with skin discolouration.

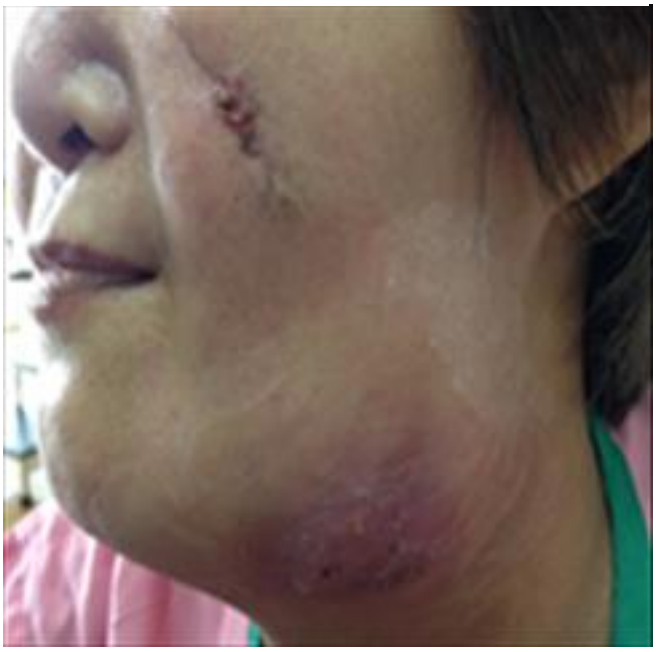
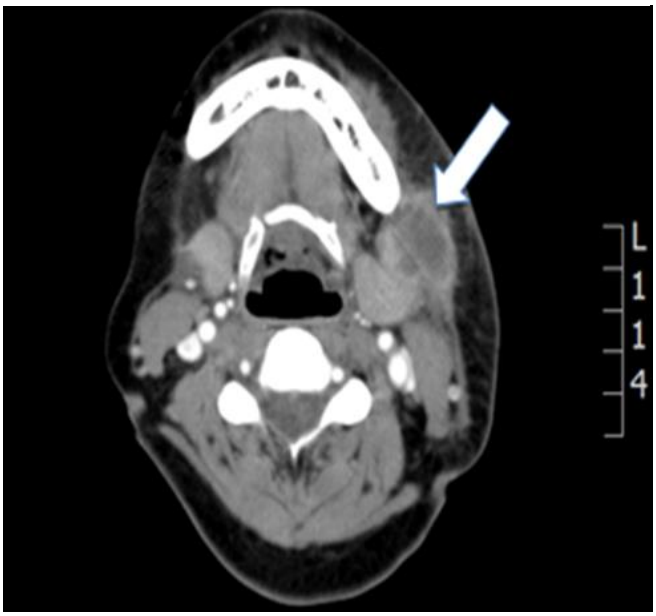


Figure 2. CT scan of the neck with contrast enhancement showing an abscess in the left submandibular region (arrow).



Discussion

M. haemophilum is widely regarded as a "blood-loving" mycobacterium due to its requirement for media supplemented with a source of iron such as hemin or ferric ammonium citrate for cultivation [9-11]. However, the strain isolated from this patient grew in liquid MGIT medium without the addition of either of these compounds. This indicates that iron supplementation is not an absolute requirement for growth of *M. haemophilum* in liquid MGIT medium. However, negative growth in LJ medium suggests that iron supplementation may be a strict requirement for recovery of this mycobacterial species in this medium, although we did not attempt to repeat culture with the addition of an iron source.

The application of molecular assays for direct detection of *M. haemophilum* in clinical materials using the 16S rRNA and *hsp65* genetic markers has been described in a number of case reports [7,12,13]. However, the target gene most suitable for identification of this mycobacterial species, like for other NTMs, is still unclear [6]. The *hsp65* gene has been reported to be more variable than the 16S rRNA gene sequence [12]. Therefore, the former may potentially be a better genetic marker for use in molecular assays compared to the latter, as it allows better discrimination between *M. haemophilum* and genetically-related species like *Mycobacterium leprae* [14], thus allowing more accurate identification of *M. haemophilum*. The successful application of *hsp65* gene sequencing for direct detection of *M. haemophilum* in this patient's pus specimen supports its utility as a rapid tool in the identification of this organism in immunocompromised patients who present with lymphadenitis. The use of a rapid molecular diagnostic tool like *hsp65* gene sequencing [15] in the identification of *M. haemophilum* is particularly important, as culture is difficult and time-consuming due to the fastidious and slow-growing nature of this organism. The excellent utility of this molecular assay for direct detection in pus specimens has also been shown in another reported *M. haemophilum* case [7].

Almost all published reports of *M. haemophilum* so far have been from developed countries [6]. The paucity of reported cases from developing countries could be attributed to the lack of optimal diagnostic facilities such as appropriate culture and molecular methods for identification of this organism. Lack of heightened awareness about the potential for this mycobacterial species to cause infections may also contribute to possible underestimation of *M. haemophilum* cases.

Antibiotic susceptibility testing for *M. haemophilum* is not recommended as there is currently no standardised method for testing [16]. In addition, correlation between in vitro susceptibility test results and treatment response has not been clearly defined [16-18]. However, most of the published literature agree that patients should be given a multidrug regimen that include some combination of clarithromycin, ciprofloxacin, and one of the rifamycins for a minimum duration of 12 months [18].

This case report illustrates the importance of including *M. haemophilum* in the differential diagnosis of cervicofacial lymphadenitis in immunocompromised patients, especially when AFB are visualised in smears but cultures at routine temperatures (35°C to 37°C) are negative. This may be especially important if cervicofacial lymphadenitis is the sole clinical presentation in the absence of cutaneous manifestations typically associated with *M. haemophilum* infections in immunocompromised adults. In such situations, specimens from the facial skin lesion and affected lymph node(s) should be cultured in iron-supplemented media and incubated at lower growth temperatures (30°C to 32°C). *hsp65* gene sequencing can be used for rapid identification of this organism in clinical specimens. However, in resource-limited settings, where appropriate culture and molecular methods for detection of *M. haemophilum* are not readily available, specimens from suspected cases should be sent to a mycobacteriology reference laboratory that has the capability to grow as well as accurately and rapidly identify this mycobacterial species, to avoid infections going undetected or delays in diagnosis.

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