Clinical Case Report

Polymerase chain reaction to identify *Mycobacterium tuberculosis* in patients with tuberculous lymphadenopathy

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ABSTRACT

Tuberculous lymphadenopathy is often diagnosed and treated on clinical and cytopathological grounds as *Mycobacterium tuberculosis* remains undetected in tissue specimens from such patients. At times, lymph nodes are known to respond sluggishly to and reappear during antitubercular therapy. We report a polymerase chain reaction-based approach to confirm the presence of *M. tuberculosis* in 4 such patients.

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INTRODUCTION

Tuberculous lymphadenopathy commonly involves the lymph nodes of the head and neck region.[1–3] During antitubercular therapy, up to one-fourth of such patients may experience changes such as enlargement, fluctuation with or without drainage of pus in the existing nodes, and appearance of new nodes.[4] Despite efforts at identifying the causative organism, *Mycobacterium tuberculosis* most often remains elusive in tissue specimens from such patients necessitating commencement of antitubercular therapy on the basis of clinical suspicion. We used a polymerase chain reaction (PCR)-based method to substantiate the aetiology in 4 such patients who responded sluggishly to antitubercular therapy.

METHODS

Four patients presented with a history of fever, malaise and a lymph node mass in the neck. Their ages ranged from 12 to 42 years. All were city dwellers having no household contact with patients or animals suffering from tuberculosis. None gave a history of having suffered from a similar illness in the past. The duration of the presenting symptoms varied from 1 to 4 months. There was no clinical or laboratory evidence of immune compromise in any of the patients. Lymph nodes in the anterior cervical triangle were involved in 3 patients and in the posterior triangle in 1. The lymph nodes were solitary in 2 patients and matted in the others. They were firm, non-tender and mobile with no evidence of fluctuation. No evidence of pulmonary or any other systemic involvement was apparent in any of the patients. All were tuberculin positive and non-smokers. Their blood levels of glucose, urea, creatinine, bilirubin and transaminases, and urinalysis and chest X-ray were within normal limits. On the basis of clinical examination, a positive tuberculin test and fine-needle aspiration cytology suggestive of a tuberculous aetiology, the patients were given 2 months of standard antitubercular therapy comprising isoniazid, rifampicin, pyrazinamide and ethambutol.

However, acid-fast bacilli could not be demonstrated in any of the specimens. All the patients showed an initial response with defervescence and a subjective sense of well-being. The lymph nodes regressed in size after 6–10 weeks in 2 patients. One of them developed enlargement and fluctuation, and required antigravity drainage of pus in the seventh week of therapy. The second patient developed 2 small lymph nodes adjacent to the original lesion 8 weeks after the commencement of therapy. The third patient did not show any response to therapy till 10 weeks, when he developed two new lymph nodes in the posterior triangle; the original lesion being in the anterior triangle. The fourth patient also showed no response to therapy in terms of regression in size at the end of 2 months; on the contrary, her lymph node enlarged, became fluctuant and tender, and required repeated antigravity aspiration and ultimately resection at the end of 3 months of antitubercular therapy.

Specimen collection, cytological, histopathological, bacteriological and PCR examination

A lymph node biopsy was performed through an antigravity approach in all these patients under local anaesthesia, using an automatic, 18 gauge, core biopsy system echogenic needle of 15 cm length and 17 mm channel cut-specimen notch (Microvasive®, Boston Scientific Corporation). The specimen was subjected to analysis by histopathology, smear microscopy by Ziehl–Neelsen staining, PCR for detection of *M. tuberculosis* and culture for the same bacillus (on Lowenstein–Jensen medium). Sample preparation for smear microscopy, culture and PCR was carried out using the Universal Sample Processing (USP) method described by us.[5] PCR assays were performed to amplify target gene sequences—*devR*[6] and *IS 6110*[7] of *M. tuberculosis*.

RESULTS

All the four specimens demonstrated granulomatous inflammation with caseation necrosis, epithelioid cells and Langhan giant cells suggestive of tuberculosis of the lymph node. Acid-fast bacilli could not be demonstrated in any of the nodes and all were sterile on culture for *M. tuberculosis*. All the four specimens tested positive for both the target sequences by PCR.

The initial intensive phase of antitubercular therapy using 4 drugs was, therefore, extended by 1 month in all the patients, on clinical grounds of sluggish response and based on the PCR results. The continuation phase comprising 3 drugs was extended to 6 months in 3 patients and 9 months in the fourth patient in view of their sluggish response to treatment. All the 4 patients completed antitubercular therapy (9 months in 3 patients and 12 months in 1 patient) with complete response. None showed any adverse reaction to the drugs or relapsed while on follow up.

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DISCUSSION

Tuberculous cervical lymphadenitis is a common manifestation of extrapulmonary tuberculosis. It has been known to affect various groups of lymph nodes in the neck and response to therapy is the rule. However, the nodes may remain palpable due to scarring and fibrosis long after there is evidence that the disease has subsided. Uneventful resolution of the lesions is seen in approximately 70% of patients.[8] Appearance of fresh lymph nodes and their enlargement can occur during treatment, usually followed by resolution. At the end of therapy, about 10% may be left with residual nodes.[9] Such transient enlargement or appearance of fresh lymph nodes does not imply relapse, nor does the persistence of nodes presage relapse. In one series, 2% of the patients demonstrated enlargement of nodes during the latter half of their treatment.[9] In another report of 113 patients, 2 developed fresh lymph nodes and 10 showed an increase in the size of the nodes while on treatment. Although tubercle bacilli have not been demonstrated in such lymph nodes, they show clinical features of tuberculosis suggesting that the enlargement may be due to a reaction to a constituent of *M. tuberculosis*.[4]

All the 4 patients had clinical and histopathological evidence of a tuberculous aetiology. Since *M. tuberculosis* could not be demonstrated in any of the tissue specimens, biopsy specimens from all the 4 patients were subjected to PCR analysis for the detection of *M. tuberculosis* and were found positive. None of the patients was immunocompromised (HIV–AIDS, neoplasms, drugs, etc.). All were compliant with appropriate antitubercular therapy.[10] No evidence of drug resistance could be identified in any patient as the culture was negative. However, they demonstrated features of deterioration (enlargement, abscess formation, fresh nodes) while on therapy. The probable reasons for these include allergic manifestations to *M. tuberculosis* and inability of the drugs to reach the target areas in the lesions. The presence of *M. tuberculosis* DNA in tissue specimens was demonstrated by PCR, which established the specific microbial aetiology. The patients received an extended duration of therapy and showed satisfactory response.

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Potential conflicts of interest—none.

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