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CASE REPORT

APPLICATION OF PCR FOR THE DETECTION OF TUBERCLE BACILLI FROM RETROPHARYNGEAL AND THYROID ABSCESS: CASE REPORTS

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ABSTRACT

The diagnosis of extra-pulmonary tuberculosis by *Mycobacterium tuberculosis* infection in retropharyngeal and thyroid abscess is challenging due to a lack of rapid, sensitive and specific diagnostic assay. Hence, we evaluated the performance of highly sensitive polymerase chain reaction (PCR) to detect the *M. tuberculosis* in the retropharyngeal and thyroid abscess. The microscopy and culture were negative test result however; PCR result had demonstrated the presence of *M. tuberculosis* in the same specimens with retropharyngeal and thyroid abscess. Therefore, it is recommended to perform PCR to detect undiagnosed cases, which were not detected by conventional approaches for the diagnosis, of extra-pulmonary tuberculosis.

INTRODUCTION

Tuberculosis remains a major public health problem worldwide. Retropharyngeal abscess occurs mainly due to pyogenic infection, and tuberculous retropharyngeal abscess is very rare.[1] In case of tuberculous retropharyngeal abscess, it is usually due to spinal tuberculosis and is seen mostly in children.[2] A definitive and accurate diagnosis is important because satisfactory results can be achieved with chemotherapy alone, obviating surgery.

In most of the cases, fine needle aspiration cytology (FNAC) has provided an alternative and easy procedure for collection of material for cytomorphologic and bacteriologic examination. But the detection rate for *M. tuberculosis* from the aspirate material is still low with Ziehl-Neelson stain and even with culture. Therefore, Polymerase chain reaction (PCR) were performed in department of microbiology at B. P. Koirala Institute of Health Sciences (BPKIHS), Dharan, Nepal in order to confirm the presence/absence of *M. tuberculosis* bacteria in retropharyngeal and thyroid abscess.[3] These two different samples were received from ENT department of BPKIHS from two different undiagnosed patients. These samples were processed in the laboratory to obtain high quality DNA by using DNeasy Blood and Tissue kit (Qiagen, Germany). Briefly, the tuberculosis specific PCR primers (T4: 5'-CCT GCG AGC GTA GGC GTC GG-3' and T5: 5'-CTC GTC CAG CGC CGC TTC GG-3') were used to amplify the insertion sequence IS*6110* in *M. tuberculosis* genome and the positive PCR result had band size of 123 bp.[4,5] PCR Mastermix were prepared in 25 µl final volume using the HotStarTaq Master mix kit (Qiagen, Germany) and amplification was carried out in a Mastercycler Pro S (Eppendorf, Germany). After PCR amplification, the PCR products were electrophoretically separated on 2% agarose gels and visualized on a UV-light transilluminator (Syngene).

CASE REPORT 1

A 17 years old male was admitted at ENT ward of BPKIHS, Dharan with the complaints of a rapidly progressive swelling of the neck below the lower jaw in right side, with difficulty in breathing and dysphasia for solid food from one week, and low grade irregular fever for 10 days. There was no history of pain in neck, impaction of foreign bodies, infection in ear, dental extraction, endoscopy or any other invasive procedure, blood transfusion, sexual exposure. He had a past history of tuberculosis three years back which was diagnosed by FNAC for which he had tak-

en Anti Tubercular Drugs (ATT) for 6 months.

On examination, the patient was of average built and nutrition. There was mild anemia. There was no pedal edema, icterus, cyanosis, nor clubbing of fingers. Pulse rate was 80/min, regular, blood pressure 130/70 mm Hg. There was a palpable mass, 7 cm x 2 cm in size projecting beneath the anterior border of sternomastoid, deep to the muscle, on the right side. It was irregular in outline, cystic in consistency and non-tender. There was no bony tenderness in the cervical spine, no lymphadenopathy nor splenomegaly nor hepatomegaly. Examination of cardiovascular, respiratory and nervous systems did not show any abnormality.

Investigations revealed – haemoglobin (Hb) 9.5 g%, total leucocytes 10,600/mm^3, differential leucocyte counts (DLC)- neutrophils (N) 45, leucocytes (L) 55, eosinophils (E) 1 and monocytes (M) 1. Platelet count 258000/mm^3, erythrocyte sedimentation rate (ESR) 19/1st hour, blood sugar (random)-70 mg%, sodium-129mmol/L, potassium-3.6mmol/L, prothrombin time-18 sec, blood group-AB positive, total calcium-7.8mg/dL, urea 19 mg%, creatinine 0.7 mg%. FNAC report was negative for tuberculous. Chest X-ray showed no evidence of pulmonary tuberculosis. CT Scan of neck showed a huge retropharyngeal abscess pushing the trachea and oesophagus to the left.

Findings by indirect laryngoscopy were: i) fullness of right pyriform fossa, ii) larynx normal and, iii) right pharyngeal wall swollen.

Aspiration of pus was done from the bulging site of the lesion. Pus from the retropharyngeal abscess was: i) negative for bacteriological culture, ii) negative for AFB and iii) positive by PCR for *M. tuberculosis*. The patient was treated with 4 antituberculosis drugs (HRZE: Isoniazid, Rifampicin, Pyrazinamide and Ethambutol) and was discharged to continue the drugs from DOTs centre.

CASE REPORT 2

Another 21 years old male was admitted at the same ward of BPKIHS, Dharan with the complaints of swelling of the anterior neck which was insidious in onset, gradually progressive for about 3 weeks, pain over the neck which was throbbing in nature on and off and iii) low grade irregular fever for about 10 days. There was no history of pulmonary tuberculosis, trauma, and diabetes mellitus. No history of sexual contact. No any family history of tuberculosis, diabetes mellitus.

On physical examination, 3x3cm, single, globular, swelling seen in anterior neck, which was soft in consistency, smooth in surface, mobile, non-adherent to skin.

On laboratory investigation, Mantoux test: > 10 mm indurations, sputum microscopy for AFB (all three samples): Negative, Pus for bacteriological culture: Negative, FNAC: Negative for AFB, USG: Thyroid Abscess, PCR: Positive for *M. tuberculosis*. The diagnosis was thyroid abscess, four antitubercular drugs (HRZE) was started to the patient and discharged to continue the drugs from DOTs centre.

DISCUSSION

Definitive and rapid diagnosis of extra-pulmonary tuberculosis is challenging since conventional techniques have suffered from several limitations. Among the several factors for diagnostic challenges, the most common reasons are: receiving of the inadequate sample amounts or volumes for analysis, the apportioning of the sample for various diagnostic tests (histology/cytology, biochemical analysis, and microbiology), resulting in no uniform distribution of microorganisms; the paucibacillary nature of the specimens; and the lack of an efficient sample processing technique universally applicable on all types of extra-pulmonary samples.

The poor performance of conventional techniques in extra-pulmonary specimens has stimulated the increased use of PCR tests in the laboratory diagnosis of tuberculosis.[4,6] Moreover, increased sensitivity of conventional IS*6110* PCR (66.7%) in extra-pulmonary tuberculosis diagnosis compared to other assays such as microscopy (7.3%), culture (11.3%) has been reported from India.[7] Whereas the real time PCR assay amplifying IS*6110* target has sensitivity of 90.3% for diagnosing the extra-pulmonary tuberculosis.[8] Therefore, the PCR assay used in this case study to diagnose the extra-pulmonary tuberculosis is more accurate and reproducible. The validity of diagnostic results were in this study was also confirmed by using *M. tuberculosis* bacterial culture DNA in each batch of experiment as a positive control (PC) and PCR grade water as a no template control (NTC). In addition, to further improve the sensitivity of PCR assay several approach such as multiplex PCR, DNA biosensor.[7,8,9] However, precise diagnostic role of PCR assay for *M. tuberculosis* in high-prevalence areas for tuberculosis has to be assessed in appropriate control groups, particularly in the case of extra-pulmonary tuberculosis.[5,10]

In conclusion, despite of expensive laboratory set up, PCR could be the better marker to diagnose the extra pulmonary samples and the cost of the test could be minimized by testing the large number of samples in each run and shared the laboratory set up to diagnose other infectious diseases. Indeed, these would be the exploratory recommendation to implement the test in future.

REFERENCES:

1. Carroll N, Bain RJ, Tseung MH, Edwards RH. Tuberculous retropharyngeal abscess producing respiratory obstruction. Thorax. 1989;44(7):599-600. **[DOI]**
2. Mathur NN, Bais AS. Tubercular retropharyngeal abscess in early childhood. Indian J Pediatr. 1997;64(6):898-901. **[DOI]**
3. Chakravorty S, Dudeja M, Hanif M, Tyagi JS. Utility of universal sample processing methodology, combining smear microscopy, culture, and PCR, for diagnosis of pulmonary tuberculosis. J Clin.Microbiol. 2005;43(6):2703-8. **[DOI]**
4. Harirzadeh S, Kazemi MJ, Babakhani S. Identification of Mycobacterium tuberculosis isolated from culture-negative pulmonary and extra-pulmonary samples in cases of suspected tuberculosis. GMS.Hyg.Infect.Control. 2019;14:Doc09. **[DOI]**
5. Deshpande PS, Kashyap RS, Ramteke SS, Nagdev KJ et al. Evaluation of the IS6110 PCR assay for the rapid diagnosis of tuberculous meningitis. Cerebrospinal.Fluid Res. 2007;4:10. **[DOI]**
6. Chakravorty S, Tyagi JS. Novel multipurpose methodology for detection of mycobacteria in pulmonary and extrapulmonary specimens by smear

microscopy, culture, and PCR. J Clin.Microbiol. 2005;43(6):2697-702. [DOI]

7. Sharma K, Appannanavar SB, Modi M, Singh M et al. Role of multiplex polymerase chain reaction using IS6110 and Protein b for the diagnosis of extra-pulmonary tuberculosis: North India. Indian J.Pathol.Microbiol. 2015;58(1):27-30. [DOI]

8. Raveendran R, Wattal C. Utility of multiplex real-time PCR in the diagnosis of extrapulmonary tuberculosis. Braz.J.Infect.Dis. 2016;20(3):235-41. [DOI]

9. Bai L, Chen Y, Liu X, Zhou J et al. Ultrasensitive electrochemical detection of Mycobacterium tuberculosis IS6110 fragment using gold nanoparticles decorated fullerene nanoparticles/nitrogen-doped graphene nanosheet as signal tags. Anal.Chim.Acta. 2019;1080:75-83. [DOI]

10. Clarridge JE, III, Shawar RM, Shinnick TM, Plikaytis BB. Large-scale use of polymerase chain reaction for detection of Mycobacterium tuberculosis in a routine mycobacteriology laboratory. J Clin.Microbiol. 1993;31(8):2049-56. [DOI]