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Histopathological and microbiological findings and diagnostic performance of GeneXpert in clinically suspected tuberculous lymphadenitis

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ABSTRACT

Objectives: The primary objective was to determine the association between histopathological and microbiological findings in patients clinically suspected to have tuberculous lymphadenitis. A secondary objective was to assess the diagnostic utility of GeneXpert in lymph node specimens.
Method: This was a single-centre prospective cohort study, performed in the Infectious Disease Clinic at The Indus Hospital. Three hundred and forty-one adult patients with chronically enlarged, accessible lymph nodes were enrolled after obtaining verbal consent, between February 2013 and April 2016. Tissue specimens were processed for histopathology, acid-fast bacillus (AFB) microscopy, AFB culture, and GeneXpert. Based on these results, anti-tuberculosis therapy (ATT) was prescribed. Clinical characteristics and treatment outcomes were recorded.
Results: There were 297 evaluable patients; 74.4% were diagnosed with tuberculous lymphadenitis (TBLA), 6.7% with a malignancy, and 12.8% with reactive nodes. TBLA was diagnosed on suggestive histopathology in 89.6% of cases, followed by GeneXpert (32.6%), mycobacterial culture (26.6%), and AFB smear positivity (12.5%). The sensitivity of GeneXpert was 65.7% when assessed against AFB culture. Drug resistance was displayed by 8.2% of GeneXpert-positive cases and 11.7% of culture-positive cases. The majority of TBLA patients (88.7%) responded favorably to ATT.
Conclusions: In light of laboratory evidence, a quarter of patients suspected of TBLA had an alternative diagnosis, highlighting its importance in avoiding over-treatment and diagnostic delays in malignancy. Although sensitivity is poor, the demonstration of drug resistance by both GeneXpert and AFB culture represents a useful tool to guide treatment.

Introduction

Pakistan is included in both the 20 high tuberculosis (TB) burden countries and the 20 high multidrug-resistant TB (MDR TB) burden countries in lists compiled by the World Health Organization (WHO), with an incidence of 510 000 new TB cases in 2015, 4.2% of which were MDR TB (World Health Organization, 2015). Tuberculous lymphadenitis (TBLA) is the most common form of extrapulmonary TB (EPTB) (Polesky et al., 2005). However, TBLA closely mimics other pathological conditions and obtaining tissue specimens for microbiological diagnosis is difficult, thus it remains a challenging entity to diagnose and treat (Iqbal et al., 2010; Singh et al., 2000). For this reason, clinicians are compelled to treat the disease empirically, resulting in a strain on TB services, unnecessary side effects of anti-tuberculosis therapy (ATT), and missed cases of malignancy.

Fine needle aspiration cytology (FNAC) and trucut biopsy are simple and minimally invasive procedures for obtaining lymph node samples for histopathology and other relevant tests, i.e., mycobacterial culture, smear microscopy, and molecular tests (Ligthelm et al., 2011). Mycobacterial culture remains the gold

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standard for definitive diagnosis, but its major limitations are a turnaround time of 2–4 weeks (Chihota et al., 2010) and poor sensitivity. Acid-fast bacillus (AFB) smear positivity likewise is insensitive (Vadwai et al., 2011). Chronic granulomatous inflammation on tissue histology supports a diagnosis of probable TB in AFB-negative, culture-negative cases, but it may be seen in other infectious and non-infectious conditions (Fontanilla et al., 2011; Asano, 2012). GeneXpert, a novel automated point-of-care real-time nucleic acid amplification test (NAAT) that simultaneously detects the DNA of *Mycobacterium tuberculosis* complex and the *rpoB* mutation associated with rifampicin resistance from clinical specimens in less than 2 h, has a sensitivity comparable to culture (Boehme et al., 2011; Churchyard et al., 2015). It was endorsed by the WHO for the rapid diagnosis of pulmonary tuberculosis (PTB) in 2010, and in 2014 was conditionally recommended over conventional tests (microscopy, culture, and histopathology) for improving case detection in EPTB, albeit with very low-quality evidence (World Health Organization, 2014). It shows favorable results on gastric aspirate, urine, and stool (Hillemann et al., 2011). Diagnostic performance-related data for lymph node specimens from high TB burden regions are limited.

The aim of this study was to determine the association between histopathological and microbiological findings in patients clinically suspected of having TBLA. In addition, it was aimed to assess the diagnostic performance of the GeneXpert assay for direct detection of *Mycobacterium tuberculosis* (MTB) and rifampicin resistance from lymph node specimens.

Materials and methods

Study setting and participants

A prospective cohort study was conducted from February 2013 to April 2016 in the Infectious Disease Clinic of The Indus Hospital, Karachi, Pakistan, in which patients clinically suspected of having TBLA were enrolled. Patients were >14 years of age with one or more superficial lymph nodes (i.e., cervical, axillary, and inguinal nodes) measuring >2 cm in largest diameter and persisting for more than 1 month, with or without constitutional symptoms of fever, anorexia, and weight loss. Patients with superficial glands with diagnostic work-up done at any other healthcare setting before presenting to The Indus Hospital or those on ATT before enrolment were excluded.

Study procedures

Demographic and clinical data were collected on a structured questionnaire after obtaining verbal consent from the participants. A baseline complete blood count, chest radiograph, and erythrocyte sedimentation rate (ESR) was performed. Using standard asepsis, a tissue sample was obtained by ultrasound-guided trucut biopsy, fine needle aspiration, or both, based on the size and suppuration of involved nodes; this was performed by an interventional radiologist. Samples for microbiological tests were sent in normal saline to the laboratory immediately, while those for histopathology and cytology were outsourced. Mycobacterial growth on culture or the detection of MTB on GeneXpert or AFB on microscopy was labeled as confirmed TBLA, whereas the diagnosis was labeled as presumed TBLA if the histopathological picture was suggestive but there was an absence of microbiological evidence. If reactive or malignant changes were found on histology, the patients were likewise diagnosed with reactive nodes or malignancy, respectively. Patients with reactive nodes were kept under observation without any therapy, whereas those with malignancy were referred to oncology units.

ATT was commenced in patients diagnosed with TBLA. New cases were prescribed a four-drug regimen comprising isoniazid (H), rifampicin (R), ethambutol (E), and pyrazinamide (Z) for 2 months in the intensive phase, followed by 4 months of H and R with a weight-based dosage in the continuation phase. Retreatment cases were treated for 8 months with the five drugs H, R, E, Z, and streptomycin (S) in the intensive phase and three in the continuation phase (H, R, E). For drug-resistant cases identified on culture or GeneXpert, directed second-line ATT was prescribed. Monthly clinical follow-up was done to monitor side effects and the treatment response. The treatment outcome was reported as treatment completed (in cases where there was complete resolution) or significant regression of the affected glands (at least 50% reduction in size from baseline), treatment failure, default, or death due to any cause.

Laboratory methods

Specimens were processed for microscopy, culture, and GeneXpert testing. A fraction of each specimen was decontaminated by conventional *N*-acetyl-l-cysteine and sodium hydroxide (NALC–NaOH) method, neutralized with phosphate buffered saline (PBS), and centrifuged to obtain a sediment, which was resuspended in 1–2 ml PBS. It was then subjected to microscopy by Ziehl–Neelsen staining and cultured on BACTEC MGIT 960 automated liquid medium and Lowenstein–Jensen slopes at 37 °C for growth detection. Confirmed cultures underwent phenotypic drug susceptibility testing on liquid medium (MGIT 960). The GeneXpert assay was performed according to the manufacturer's instruction. Homogenized tissue was mixed with GeneXpert reagent, and after brief incubation at room temperature it was transferred to a cartridge and inserted into the GeneXpert device; the automatically generated results were read after 2 h.

Formalin-fixed specimens were embedded in paraffin blocks, cut, and stained with hematoxylin and eosin (H&E). To prevent reading bias, all histology and cytology slides were interpreted by the same histopathologist. The histological patterns considered suggestive of TBLA were the presence of chronic granulomatous inflammation, acute necrotizing or suppurative inflammation, or only extensive caseous necrosis. Suggestive histopathology was taken as the composite reference standard (CRS) for comparison purposes.

Ethical considerations

The study was approved by the Institutional Review Board of The Indus Hospital (IRB#: 2013_01_002).

Statistical analysis

Data were entered and analyzed using IBM SPSS Statistics version 21.0 (IBM Corp., Armonk, NY, USA). The mean ± standard deviation or median and interquartile range was computed for quantitative variables, while the Chi-square test/Fisher's exact test was applied to assess significant associations between various qualitative variables, as appropriate. Performance calculations, including test sensitivity, specificity, predictive values, and area under the curve were computed to compare the diagnostic performance of the GeneXpert and smear microscopy to the reference standard (culture positive for MTB). Likewise, the diagnostic performance of AFB smear, GeneXpert, and AFB culture were compared with histopathology using the latter as the CRS. A p-value of <0.05 was considered statistically significant.