

ABSTRACT

Background: The diagnosis of extrapulmonary tuberculosis in tissue sections is challenging and this causes delayed diagnosis, missed diagnosis and misdiagnosis of tuberculosis. Immunohistochemistry is believed to improve the accuracy of the diagnosis of tuberculosis in formalin-fixed, paraffin-embedded tissue sections. However, the diagnostic utility of immunohistochemistry for the diagnosis of extrapulmonary tuberculosis has not been assessed in Uganda.

Objective: Our aim was to assess the diagnostic utility of anti-*Mycobacterium tuberculosis* antibody staining for the detection of mycobacterial antigens in tissue specimens at Mbarara Regional Referral Hospital.

Study Methods: This was a cross-sectional study conducted between September 2023 and November 2023; the research involved 87 archived tissue specimens of patients with clinical suspicion of EPTB. Tissue blocks were selected based on their preservation status, availability of medical records and their suitability for sectioning for further analysis.

Demographic data were collected from the register review process, Hematoxylin and Eosin (H&E) and Ziehl Neelsen (ZN) staining were repeated to confirm EPTB diagnosis. Immunohistochemistry (IHC) was performed on FFPE tissue sections using concentrated anti-*Mycobacterium tuberculosis* antibody (catalogue no. ab905, Abcam, Cambridge, UK) diluted (1:100). Indirect manual IHC technique with one-step envision method (HRP-Streptavidin-biotin method, Dako, Germany) with diaminobenzidine (DAB) as chromogen was used. The staining outcomes and diagnostic utility of anti-*Mycobacterium tuberculosis* antibody staining was determined against ZN and/or Histopathology as a composite reference standard for diagnosis. Data analysis employed Stata version 17; sensitivity, specificity, positive and negative predictive values of anti-*Mycobacterium tuberculosis* antibody staining were reported as proportions with 95% confidence intervals. Receiver Operating Characteristic curve was used to determine the predictive performance of IHC for EPTB.

Results: A total of 87 specimens retrieved from the bio-repository were analyzed. Lymph nodes constituted majority (n=50; 57%) of the specimens. On histopathology, 52.87% (n=46) specimens had granulomatous inflammation suggestive of EPTB while 47.12% (n=41) were not suggestive. Of the 87 specimens analyzed, 17 (19.5%) stained positive by the ZN technique while 70 (80.5%) were negative. The sensitivity, specificity, PPV and NPV of anti-*Mycobacterium tuberculosis* antibody staining were 74.47%, 80.00%, 81.40% and 72.73% respectively.

Conclusions: This study concluded that the sensitivity and specificity of anti-*Mycobacterium tuberculosis* antibody staining was 74.47% and 80.00% respectively. Positive and negative predictive values respectively were 81.40% and 72.73%. This demonstrated a relatively high and balanced sensitivity, specificity, positive and negative predictive values. This makes it a potentially valuable diagnostic tool for extrapulmonary tuberculosis.

Though the study found a relatively high and balanced sensitivity, specificity, PPV and NPV of anti-*Mycobacterium tuberculosis* antibody (ab905) staining, further validation of its application in diagnostics is recommended through large prospective studies employing monoclonal anti-*Mycobacterium tuberculosis* antibodies.

What this study adds: This research provides additional insights into the potential role of immunohistochemistry in the diagnosis of extrapulmonary tuberculosis in tissues.

Key words

Anti-*Mycobacterium tuberculosis* antibody (ab905); Extrapulmonary tuberculosis; Formalin-fixed paraffin-embedded; Hematoxylin & Eosin; Immunohistochemistry; Ziehl-Neelsen.