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Technique with Rapid Turnaround Time for Mycobacterial Tuberculosis in Granulomatous Lesions in Tissue Section

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KEYWORDS

Immuhistochemistry, Granulomatous inflammation, Histopathology

ABSTRACT:

Introduction: Tuberculosis (TB) is devastating infectious disease. Patient usually has enlarged lymph nodes (LN). Mycobacterial culture is considered gold standard. Ziehl-Neelsen stain has low sensitivity. New test like immunohistochemistry (IHC) can confirm involvement by MTB and demonstrate antigen immunolocalization. This study attempts to study details of IHC using Anti MTB polyclonal antibody in suspected cases of TB.

Objectives: To compare immunohistochemistry study with AFB culture, ZN stain and Gene Xpert.

Methods: A cross sectional, Observational study was done where H & E slides of 76 tissue sections of granulomatous inflammation were gathered. IHC was performed using anti-MTB polyclonal antibody (Dako Envision FLEX/HRP) and interpretated based on proportion and intensity of staining in epithelioid cells, giant cells and caseous necrosis. Grading score for proportion of staining: 0-(0-4%), 1 (5-25%), 2-(25-50%), 3-(50-100%), intensity: 0-weak, 1-mild, 2-moderate, 3-strong. Total combined score was 18. IHC score ≥5 considered cut off.

Results: Out of 76 cases majority were LN. Out of 76 cases, 63 (82.9%) showed IHC positivity for MTB with 94.6% sensitivity and 30.6% specificity.

Conclusions: IHC has potential to reveal any mycobacterial antigen. Intact cell wall is not prerequisite. It can aid to diagnose granulomatous inflammation of mycobacterial etiology as it requires less time than culture.

1. Introduction

Tuberculosis (TB) is a devastating infectious disease in developing nations like India. Incidence rate (new cases per 100 000 population per year) increased by 3.6% between 2020 and 2021[1]. It poses social and economical threat. It is paramount to diagnose and treat the TB infection early. Patient frequently only has enlarged lymph nodes (LN), which can also be seen in lymphoproliferative disorders, metastasis of various carcinomas or in variety of infections as well[2].

Mycobacterial culture is considered as gold standard test having longer turn around time (4-8 weeks) which delays the diagnosis[3-4]. Ziehl-Neelsen (ZN) stain is quicker method but has relatively low sensitivity (0 to 44%) and requires 10^4 bacilli per slide for diagnosi[3,5-6]. On biopsy from tissues, histological changes of chronic granulomatous inflammation are used to diagnose TB but it can be found in other diseases also making it more difficult to diagnose[2]. ZN stain, Gene Xpert and CBNAAT are also used for diagnosis of tuberculosis[7]. Polymerase chain reaction (PCR) is also used for TB diagnosis in which amplification of IS6110 is done which is specific for M. tuberculosis complex organisms from formalin fixed tissue biopsies[8-11]. PCR is fast and highly sensitive but it does not allow localization within tissues[5,12].

Immunohistochemistry (IHC) has potential to reveal any mycobacterial antigen and it demonstrate antigen immunolocalization[13]. Compared to AFB culture, IHC has superior sensitivity, while ZN stain showed higher

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specificity suggesting that IHC may serve as a valuable adjunct to ZN stain for detecting mycobacteria[13-14].

2. Objectives

To check the utility of immunohistochemistry for detecting Mycobacterium tuberculosis in granulomatous lesions in tissue section.

3. Methods

A cross-sectional observational study over a period of one and half year was conducted at Bharati Vidyapeeth Deemed To Be University Medical College, Hospital and Research Centre, Pune with a sample size of 76 whose tissue sections of granulomatous lesion were taken. Data were retrieved from test request forms, histopathology reports and HIS. Inclusion criteria is all tissue sections of granulomatous lesion of suspected cases of tuberculosis and exclusion criteria are Inadequate and non-representative sample.

The study was approved by the Institutional Review Board of Bharati Vidyapeeth Medical College, Pune. (IRB No. DHR Reg No. EC/NEW/INST/2020/656) and performed in accordance with the principles of the Declaration of Helsinki. Written informed consents were obtained.

Immunohistochemistry (IHC) was performed using anti mycobacterium tuberculosis (Anti-MTB) polyclonal antibody made by Medaysis company using 100% dilution. Positive control taken as known case of leprosy and negative control taken as known case of foreign body granuloma.

IHC interpretation was done based on proportion score (PS) and intensity score (IS). Both of these scores were evaluated separately in epithelioid cells (E), giant cells (G) & caseous necrosis (C). For proportion Score (PS), Grading Score for proportion of staining in each of 3 parameters were evaluated as follows: 0- 0 to 4%, 1- 5 to 25%, 2- 25 to 50%, 3- 50 to 100%. PSE(0 to 3) + PSG(0 to 3) +PSC(0 to 3) = PS(0 to 9)[15]. Similarly, for Intensity Score (IS) Grading Score was evaluated in each of 3 parameters as follows: 0- Weak, 1- Mild, 2- Moderate, 3- Strong. ISE(0 to 3) + ISG(0 to 3)+ISC(0 to 3)=IS (0 to 9)[15]. Total score was ranging from 0 to 18.

For IHC cut off score, evaluation at various IHC score level were done and compared with gold standard test AFB culture. Final cut off was taken as Cut off score: ≥ 5 with sensitivity- 94.6%, specificity- 30.6%, positive predictive value- 58.3%, negative predictive value- 84.6%.

Table 1: IHC cut off score evaluation

	CUT OFF SCORE		
	≥4	**≥5**	**≥6**
Sensitivity (Sn)	97.3%	94.6%	73.0%
Specificity (Sp)	22.2%	30.6%	36.1%
Positive predictive value	56.3%	58.3%	54.0%
Negative predictive value	88.9%	84.6%	56.5%

Statistical analysis was done using SPSS software version 28.0. For qualitative data, frequency and percentage tables were created and Chi-square test applied. For quantitative data, T test was applied. ROC analysis, specificity and sensitivity analysis was used to compare AFB culture and IHC. Throughout result 5% level of significance was used. All results were shown with 95% of confidence. Predictive value (P value) <0.05 is considered significant.

4. Results

Out of 76 cases, 30 were lymph nodes followed by 24 bone and soft tissue, 10 Git tissue, 7 respiratory tissue, 3 involving central nervous system and 2 were involving reproductive system. Most common system involved by granulomatous inflammation is lymph node 30/76 (39.4%) followed by bone and soft tissue (31.5%). On immunohistochemistry 63/76 (82.9%) showed positivity of which Lymphnode is 25/76 (32.9%) followed by bone and soft tissue 21/76 (27.6%).

IHC and ZN stain on tissue were performed on 76 samples whereas data for AFB culture, ZN stain on sputum, Gene-Xpert and CBNAAT were available for 73, 75, 73 and 11 samples respectively.

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Table 1: Comparison of immunohistochemistry with other methods

Test	Positive	Negative	Total	P value
IHC	63 (82.9%)	13 (17.1%)	76 (100%)	
AFB culture	37 (50.7%)	36 (49.3%)	73 (100%)	<0.001
ZN stain on tissue	11 (14.5%)	65 (85.5%)	76 (100%)	<0.001
ZN stain on sputum	13 (17.3%)	62 (82.7%)	75 (100%)	<0.001
Gene Xpert	52 (71.2%)	21 (28.8%)	73 (100%)	<0.118
CBNAAT	05 (45.5%)	06 (54.5%)	11 (100%)	<0.204

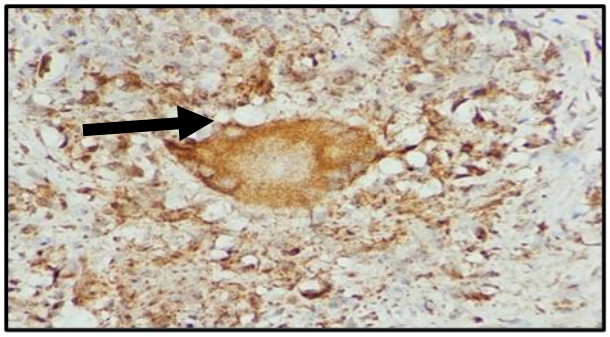

Figure 1: H&E section showing well-formed granuloma comprising of epithelioid cells, giant cell and lymphocytes.

Result of IHC was compared with various other methods like AFB culture, ZN stain on tissue and on sputum, Gene Xpert and CBNAAT. IHC showed positivity in 63/76 cases (82.9%) whereas other methods like AFB culture showed positivity in 37/73 cases (50.7%), ZN stain on tissue showed positivity in 11/76 cases (14.5%), ZN stain on sputum showed positivity in 13/75 cases (17.3%), Gene Xpert showed positivity in 52/73 cases (71.2%) and CBNAAT showed positivity in 5/11 cases (45.5%) all of which are less compare to IHC.

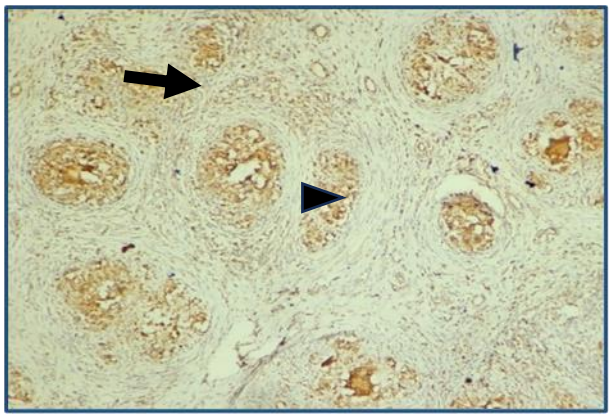

Figure 2: In well-formed granuloma, immunohistochemistry showing positivity in giant cell (Black arrow) and in TB bacilli (arrow head)

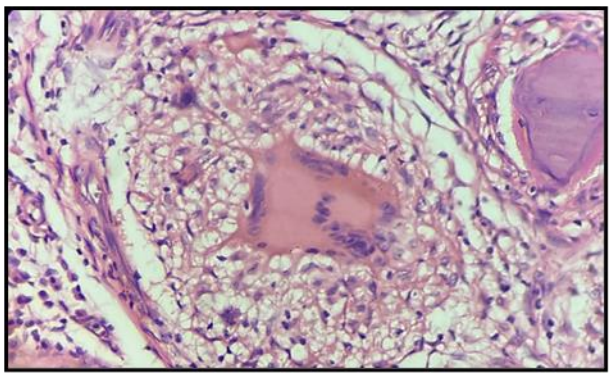

Figure 3: Immunohistochemistry showing positivity in granulomas

Statistically, when IHC result was compared with AFB culture, P value derived was <0.001. Similarly, when IHC results were compared with ZN stain on tissue section and ZN stain on sputum, P value derived was <0.001. P Value <0.001 is statistically significant indicating that IHC is showing more positivity for detecting mycobacterial infection and IHC is more sensitive compare to AFB culture and ZN stain. Thus, IHC can be useful for detecting mycobacterial infections. When IHC was compared with Gene Xpert, P value derived was 0.118 which is statistically not significant indicating that IHC is less sensitive compare to Gene Xpert. When IHC was compared with CBNAAT, P value derived was 0.204 which is statistically not significant. This insignificance between IHC and CBNAAT can be explained by considering that CBNAAT is performed in a smaller number of cases.

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5. Discussion

TB still ranks as the top cause of death among infectious diseases worldwide[3]. In current study most common organ involved is lymph node followed by connective tissue. Diagnosis of TB is challenging. Definitive diagnosis of TB is made by using AFB culture which has longer turnaround time. ZN stain has lower sensitivity and requires intact cell wall[14,16]. Presence of mycobacterial antigen can be evaluated by IHC[16]. Within granuloma antigen was mainly detected in epithelioid cells and giant cells. Necrotic centers were negative in majority of cases[13]. IHC is positive even when there are 10 bacilli per slide[17]. Thus, IHC can be applied when only small number of bacilli is present like in primary stage of TB infection[16].

IHC showed coarse granular cytoplasmic positivity[16]. Positivity is observed in fragments of bacilli and antigenic dust[18]. Intensity and extent of staining varied[13]. In current study, Epithelioid cells showed more positive staining compare to giant cells and caseous necrosis. Epithelioid cells outside granuloma showed more positivity compared to necrotic zone, in contrast to ZN stain that mostly reveals intact bacilli at center of necrotic zone[16,19]. IHC positivity also noted in fibroblasts, plasma cells, lymphocytes. Presence of fragments of TB bacilli in macrophages, fibroblasts, plasma cells, lymphocytes and in endothelial cells outside the granuloma indicates that these cells play an active role in histopathogenesis of TB and hence showed positivity[16].

Considering AFB culture as the gold standard evaluation was done for the utility of IHC for detecting mycobacteria[13]. Total IHC score was ranging from 0 to 18. Final IHC cut score was evaluated as various level as shown in table 2. Sensitivity, specificity, PPV and NPV were derived and compared with Kohli et al study. In Kohli et al sensitivity, specificity, PPV and NPV were 95.56%, 35.06%, 30.56%, 96.43%. When cut off taken as ≥4 sensitivity, specificity, PPV and NPV were 97.3%, 22.2%, 56.3%, 88.9% respectively which when compared with Kohli et al showed that sensitivity will be higher but specificity dropped down. When cut off taken as ≥5 sensitivity, specificity, PPV and NPV were 94.6%, 30.6%, 58.3%, 84.6% respectively. These values were nearly similar with Kohli et al. When cut off taken as ≥6 sensitivity, specificity, PPV and NPV were 73.0%, 36.1%, 54.0%, 56.5% respectively, which when compared with Kohli et al showed that sensitivity dropped down and specificity is nearly similar to Kohli et al[3].

After comparing all these different cut off level, final cut off score was taken as ≥5 as at this cut off level sensitivity and specificity both has near similar value to Kohli et al[3]. IHC evaluation was done at three level by pathologist for achieving better results.

Result in current study is comparable different other studies reporting 64 to 100% sensitivity for IHC indicating that IHC has higher sensitivity[3,14,20]. Sensitivity of IHC depends on various factors including distribution of antigen in granuloma, clinical stage of disease, treatment duration and specificity of antibody[3].

This study showed lower specificity for differentiating different mycobacterial antigen[21]. Eliminating background with proper technique could provide greater specificity[16]. In order to achieve this, attention must be given to dilution and IHC technique with polyclonal antibody or else fine granular staining in background can cause false positive reaction which is the main limitation of working with this type of antibody[16]. Background staining caused by non-specific binding of antibody can obscure true positive signals, leading to misinterpretation of results[22].

In this study, known case of leprosy was taken as positive control showed positivity for anti MTB antibody which indicate that this antibody has cross reactivity for mycobacterial antigen also other than MTB. Other than this, one case of granulomatous inflammation with differential diagnosis of Crohn's disease and TB was showing negativity on IHC indicating that immunostaining positivity with this antibody rules out other differential diagnosis of nontuberculous granulomas.

Advantages of IHC is its ability to provide spatial information about the distribution of Mycobacterium tuberculosis (MTB) within tissue samples[16].

IHC has lower specificity as it is positive in other cells like endothelial cells, colonic mucosa, lymphocytes, fibroblasts, plasma cells etc[16,21]. It can be positive in other mycobacterial infection like mycobacterium leprae, mycobacterium avium etc. More studies need to

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be carried out specially using monoclonal antibody to increase the specificity but availability which is still an issue[16]. IHC results were not compared with PCR.

To conclude, this was cross sectional, observational study in which the objective was to compare immunohistochemistry results with mycobacterial culture which is the gold standard test and other tests like ZN stain, Gene Expert and CBNAAAT to detect TB was carried out. Being highly sensitive, IHC has a potential to reveal any mycobacterial antigen where intact cell wall is not a prerequisite[3]. It has rapid turnaround time, reagents are stable at 4°C and can be used for longer period of time[3]. Pathologist must be familiar with staining pattern, elimination of background staining and type of antibody used[16]. To conclude, IHC can be useful to provide a diagnosis in high endemic areas and aid to diagnose granulomatous inflammation of mycobacterial etiology as it requires less time than culture[3,16]. It may be a useful adjunct to conventional methods to reach an equivocal diagnosis of mycobacterial infection[3].

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