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Original Article

Relative Value of Immunohistochemistry in Detection of Mycobacterial Antigen in Suspected Cases of Tuberculosis in Tissue Section

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Abstract

OBJECTIVE: Due to its infectious nature, complex immunological response, chronic progression, and the necessity for long-term treatment, tuberculosis has always been a major health burden. Immunohistochemistry (IHC) has the capacity to highlight the occurrence of mycobacterial antigens for tissue diagnosis. This study was conducted to understand the advantage of immunostaining over culture of *Mycobacterium tuberculosis*.

MATERIAL AND METHODS: A cross-sectional study was conducted on 30 samples of suspected cases of tuberculosis. Specimens received were fixed in 10% formalin and processed; 3-5 µm thick sections were made from paraffin block, stained with hematoxylin and eosin, Ziehl–Neelsen stain, and immunohistochemistry. Culture was done using Lowenstein–Jensen medium. Immunohistochemistry was interpreted as fine granular brownish cytoplasmic, coarse granular brownish cytoplasmic, and bacillus staining.

RESULTS: Out of the 30 samples studied, 12 (40.0%) were culture positive while 20 (66.7%) of them were IHC positive. Immunohistochemistry showed 17 granulomatous lesions of which 11 (55.0%) were well-formed granulomas. The sensitivity and negative predictive value were found to be high with immunohistochemistry, while specificity and positive predictive value were found to be on the lower side. Among the 20 positive IHC cases, the degree of staining was fine granular cytoplasmic staining in 13 cases (65.0%) and coarse granular staining in 7 cases (35.0%).

CONCLUSION: Immunohistochemistry is a reliable test with high sensitivity as well as high negative predictive value which can be done rapidly for establishing an etiological diagnosis of tuberculosis in histologic specimens.

KEYWORDS: Granuloma, tissues, immunohistochemistry, tuberculosis
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INTRODUCTION

Mycobacterium tuberculosis is responsible for one of the most infectious diseases known to man which is tuberculosis (TB). The estimated world prevalence of TB is 40 million, with approximately 10.6 million new cases reported each year. Also, nearly 3 million people die annually from the disease. The situation in India is shocking, where nearly 28.0% of the global burden exists, with approximately 1.8 million person developing TB every year.[1]

Due to various factors, including its high infectivity rate, intricate immunological response, progression which is lasting for many years, and the necessity for continuous management, TB has consistently been a significant health burden on healthcare system. Nowadays, the development of multidrug-resistant strains and the recent TB-HIV epidemic, associated with its severe social consequences have increased the burden. Laboratory diagnosis, treatment, and prevention of TB have embodied a permanent trial over the course of human history.[2] Clinically, TB presents in various forms, and tissue sections show very low yield of acid-fast bacilli, making diagnosis challenging.[3]

Taking into consideration the limitations in diagnostic accuracy of Ziehl–Neelsen (ZN) staining, mycobacterial culture, molecular, and serological methods, in a few cases of TB, histomorphological examination seems to be the only reasonable procedure for field diagnosis.[4] The detection limit for staining is more than 104 bacilli per slide or the presence of more than 104 bacilli per mL of specimen resulting in low sensitivity.[5] Culture of mycobacterium is considered to be one of the most sensitive techniques for identifying infections. Nevertheless, the time required for performing culture is several weeks, and its sensitivity is also low in paucibacillary conditions. Chronic granulomatous inflammation, which is a classical histological change seen in TB, is considered as the basis for the diagnosis of TB.[6]

Both immunocompromised as well as the immunocompetent patients are at high risk of developing infections and complications associated with mycobacterial infection. Hence, it is crucial to detect mycobacteria in all samples without

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fail.[7] Immunohistochemistry (IHC) is a novel and powerful method. In formalin-fixed, paraffin-embedded tissues, IHC focuses on specific antigens using antigen–antibody interaction. Immunohistochemistry not only highlights the presence of mycobacterial antigens for tissue diagnosis but also could morphologically localize its distribution in different cells.[3,8]

There is a dearth of studies looking at the immunohistochemistry findings of TB, especially in those countries that are great contributors to the burden. Hence, this study was conducted to understand the advantage of immunostaining over culture methods.

MATERIAL AND METHODS

This prospective cross-sectional study was carried out at Kempegowda Institute of Medical Science (KIMS), Bangalore and JJM Institute of Medical Science (JJMMC), Davangere Medical College, after obtaining necessary permissions from Kempegowda Institute of Medical Science Institutional Ethics Committee (aproval no: KIMS/IEC/D-13/2017, date: 7-11-2017). The study was conducted for a period of 3 years. Informed written consent was obtained before initiation of the study. The study included all the suspected cases of TB on histopathology, while any autolyzed or inadequate specimens were excluded. Tuberculosis was diagnosed with the help of thorough general and systemic examinations, as well as making use of routine blood tests, chest X-rays, ultrasonography, computed tomography/magnetic resonance imaging and fine-needle aspiration.

Specimens were transported to the laboratory as soon as possible after collection. None of the samples were used if refrigerated for more than 2 days. Specimens were homogenized to free the bacilli from the mucus, cells, or tissues in which they may be embedded. All the specimens received were fixed in 10% formalin and routinely processed; 3-5 µm thick sections were made from paraffin-embedded blocks, stained with hematoxylin and eosin (H and E), Ziehl–Neelsen staining, and immunohistochemistry were performed. All samples were sent for culture using Lowenstein–Jensen (LJ) medium. For the culture process, 2 slopes per specimen were inoculated, each with one 5 mm loopful of the centrifuged sediment distributed over the surface. Measures were taken to minimize evaporation and drying of media. All cultures were inoculated at 35-37°C until growth was observed or discarded as negative after 8 weeks. Contaminated slopes were discarded. A total of 30 samples were studied. Tissue sections were deparaffinized, hydrated, and subjected to microwave antigen retrieval. The endogenous peroxidase activity was inhibited by incubating the sections with hydrogen peroxide.

Main Points

- Immunohistochemistry is a reliable test with high sensitivity as well as a high negative predictive value.
- Immunohistochemistry is capable of morphologically localizing antigen presence in various cells.
- Immunohistochemistry has advantages over polymerase chain reaction, with the former being robust and economical, and easily usable in routine laboratory settings.

The primary antibody used for IHC was the polyclonal anti-bacillus Calmette-Guérin antibody (pAbBCG) (Genxbio, India). This was done in a dilution of 1:50. One-step Envision method (HRP-streptavidin-biotin method; Dako, Germany) using diaminobenzidine as chromogen was employed for visualizing the system.

In the tissue samples collected, different areas and cells were assessed for the type of granuloma with the presence or absence of necrosis. Other findings studied included the visualization of multinucleated giant cells, lymphocytes, epithelioid cells, plasma cells, perigranuloma macrophages, necrotic zones, fibroblasts, and perigranuloma endothelial cells. The presence of staining in 10% of the epithelioid cells was considered positive staining. Comparisons were made between culture using LJ medium and immunohistochemistry. For each immunohistochemistry staining, one negative and one positive control (high leprosy bacillary positive section) were considered. Immunohistochemistry staining was interpreted as fine granular brownish cytoplasmic, coarse granular brownish cytoplasmic, and bacillus staining.

Statistical Analysis

The data were collected and entered into Microsoft Excel 365 and analyzed using the Statistical Package for the Social Sciences® version 20.0 (IBM Inc., Armonk, NY, USA). All study variables were analyzed using descriptive statistical methods such as frequencies and percentage for categorical variables, and mean with standard deviation or median with interquartile range for continuous variables. Chi-square test was performed to find the association between categorical variables. This study also assessed the specificity, sensitivity, as well as positive predictive value and negative predictive values of IHC. A P value of <.05 was considered statistically significant in this study.

RESULTS

The mean age of the study population was 32 years, with more males (17; 56.7%) than females (13; 43.3%). Regarding the nature of tissue studied, lymph nodes (30.0%) were the most common, followed by bone (20.0%), abscess (13.3%), and synovium (13.3%). Out of the 30 samples studied, 12 (40.0%) were culture positive, while 20 (66.7%) of them were IHC positive. Immunohistochemistry showed 17 granulomatous lesions, of which 11 (55.0%) were well-formed granulomas and 6 (30.0%) were ill-formed granulomas (Table 1).

There was a significant difference in the positivity rate between culture and immunohistochemistry in this study (Table 2).

Table 1. Distribution of the Samples in Terms of Organization

Organization	Frequency	Percentage
Well-formed granuloma	11	55.0%
Ill formed granuloma	6	30.0%
Necrotic tissue	3	15.0%
Total	20	100.0%

Table 2. Comparison of Ziehl–Neelsen stain with Immunohistochemistry

		Immunohistochemistry			
		Positive	**Negative**	**Total**	***P***
M. tuberculosis culture	Positive	12 (40.0%)	0 (0.0%)	12 (40.0%)	.002
	Negative	8 (26.7%)	10 (33.3%)	18 (60.0%)	
	Total	20 (66.7%)	10 (33.3%)	30 (100.0%)	

$P < .05$, hence statistically significant.

Table 3. Specificity, Sensitivity, Positive Predictive Value, and Negative Predictive Value of Immunohistochemistry in Diagnosing Tuberculosis Using Culture as the Gold Standard

	Immunohistochemistry
Sensitivity	100%
Specificity	40%
Positive predictive value	25%
Negative predictive value	100%

Table 4. Distribution of Samples in Terms of the Degree of Staining (n = 20)

Degree of Staining	**Frequency**	**Percentage**
Fine	13	65.0%
Coarse	7	35.0%
Total	20	100.0%

The sensitivity and negative predictive value were found to be high with immunohistochemistry, while specificity and positive predictive value were found to be on the lower side (Table 3).

Among the 20 positive IHC cases, the degree of staining was fine granular cytoplasmic staining in 13 cases (65.0%) and coarse granular staining in 7 cases (35.0%) (Table 4).

DISCUSSION

Considering morbidity and mortality among the various infectious diseases affecting adults in developing countries, TB still holds the top rank as the number one killer. In the case of TB, we usually reach a diagnosis based on the classical histomorphology of chronic granulomatous inflammation, which is pathognomonic of TB. A variety of techniques already exists to detect the pathogen responsible, *M. tuberculosis.* Even though quick and economical, the ZN stain is a procedure that shows positivity only in instances where there is a high bacillary load. Another drawback of ZN staining is its inability to distinguish between various Mycobacterium species. Culture is considered the gold standard for diagnosing TB. Other techniques such as enzyme-linked immunosorbent assay test and polymerase chain reaction (PCR) are intricate, requiring advanced equipment and trained individuals, costly, tardy, and not readily available.[9]

The factors that led to the preference of immunohistochemistry staining procedure in various settings are its simplicity and versatility, which are useful in the identification of mycobacterium in sputum, cultures, smears, and tissue segments.

In this study, the majority were males with a ratio of 1.2:1. This is similar to the study conducted by Mustafa et al,[6] which showed a similar ratio. Majority of the tissues were obtained from lymph nodes (30.0%), followed by bone (20.0%), abscess (13.0%), and synovium (13.0%) in this study. In the study conducted by Geol et al, lymph nodes (44.4%) were the predominant sample studied,[10] while in the case of Kohli et al,[11] most of the samples were from the gastrointestinal tract (24.0%) and bones (24.0%).

This study showed predominantly well-formed granulomas in IHC, while in the Karimi et al[3] study, ill-formed granulomas were the predominant. Immunohistochemistry positivity was found to be 66.7% in this study. Immunohistochemistry positivity ranged from 72% to 100% in various published studies by multiple authors.[9-11] Our results showed that IHC using polyclonal (anti-BCG) antisera has better sensitivity (100.0%), but lower specificity (40.0%) and high predictive value.

In the study conducted by Goel and Budhwar,[10] the sensitivity for IHC was found to be 64.0%-100.0%. They had used a monoclonal antibody instead of a polyclonal antibody for IHC. In the study conducted by Mustafa et al, both sensitivity and specificity were found to be 100% for IHC using a polyclonal antibody.[6] Barbolini et al[12] used a monoclonal antibody for IHC and found that the sensitivity and specificity were both 100.0%. In their study, they obtained tissues from extrapulmonary TB sites. In a study conducted on samples obtained from aspirates of lymph nodes, CSF, and effusion by Purohit et al,[13] both sensitivity and specificity were found to be 96.0%. In the study conducted by Prapanna et al,[14] using IHC, the sensitivity and specificity were 96.9% and 95.0%, respectively. In the study conducted using pAbBCG for IHC by Karimi et al,[15] both sensitivity and specificity were 100.0% each.

The main asset of this IHC technology is that it is readily available in routine surgical pathology laboratories and is a robust technology. Immunohistochemistry can detect fragmented tubercle bacilli with high sensitivity compared to ZN staining, which has a sensitivity of 25.0%-44.0% and requires an intact cell wall. Logani et al, in their study, concluded that the use of IHC with the help of pAbBCG generates positive outcomes in specimens with 10 bacilli per slide.[16]

The main limitation of this study was that the sample size was small. Various factors can contribute to the sensitivity of IHC, including the clinical stage of the disease and the duration of antitubercular treatment received prior to biopsy. These factors were not addressed in this study.

Immunohistochemistry was found to be dependable in diagnosing TB even in specimens with fragmented tubercle bacilli. Immunohistochemistry is a reliable test with high sensitivity as well as negative predictive value, which can be rapidly performed to establish an etiological diagnosis of TB in histologic specimens. Hence, this study has a place in the differential diagnosis of tuberculous and non-tuberculous mycobacterial diseases. Immunohistochemistry has advantages over PCR of being robust and economical, and it can easily be used in a routine laboratory.

Ethics Committee Approval: This study was approved by the Ethics Committee of Kempegowda Institute of Medical Science Institutional Ethics Committee (approval no.: KIMS/IEC/D-13/2017; date: 7/11/2017).

Informed Consent: Written consent was obtained from the patients who agreed to take part in the study.

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