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HISTOPATHOLOGICAL STUDY AND CORRELATION OF IMMUNOHISTOCHEMISTRY IN DETECTION OF MYCOBACTERIUM TUBERCULOSIS ANTIGEN IN SUSPECTED CASES OF TUBERCULOSIS IN TISSUE SECTIONS

Pathology

Tripathi Jayati Department of Pathology, MLB Medical College, Jhansi.

Singh Yogesh* Department of Pathology, MLB Medical College, Jhansi. *Corresponding Author

Srivastava Arvind Department of Pathology, MLB Medical College, Jhansi.

ABSTRACT

BACKGROUND: The diagnosis of Tuberculosis by ZN staining in tissue sections for AFB is challenging due to its low yield. Immunohistochemistry can prove to be highly useful for tissues while awaiting culture results and has great utility in cases in cases with low bacterial load or those partially treated.

AIM: To localise tubercle bacilli or their components using immunohistochemistry, which have lost the staining property with ZN staining .We also assessed advantage of immunostaining over conventional ZN staining.

MATERIALS AND METHODS: This retrospective study was conducted on 60 suspected cases of TB. Tissue sections from these cases were subjected to H&E ,ZN and IHC staining using polyclonal antibody to Mycobaterium Tubeculosis and comparing the results of both.

RESULTS : Using ZN stain, acid fast bacilli were identified in about 18% while IHC staining identified AFB in about 78% cases. We found that the sensitivity of IHC (81.03%) and positive predictive value of IHC (48.95%) were higher while specificity(20.96%) was exactly equal to that of ZN staining .This yielded p value <0.001 making our study statistically significant.

CONCLUSION: We therefore inferred that immunohistochemistry is a sensitive technique for localisation of Mycobacterium Tuberculosis and its components on tissue sections and therefore can be a useful diagnostic adjunct to conventional ZN staining for diagnosis of extrapulmonary TB granulomas.

KEYWORDS

Immunohistochemistry, Mycobacterium tuberculosis, Polyclonal antibody,Extrapulmonary TB , Tuberculous granuloma

INTRODUCTION

India is the country with highest burden of TB with about 2 deaths occurring every 3 minutes from TB .This means that TB continues to be not only a medical malady but also a social and economic tragedy in our country. 1.8 million people develop TB every year in India.

Although TB is primarily considered a pulmonary disease, it can affect almost any organ, with lymph node involvement being the commonest form of extra-pulmonary TB [1]. The gold standard for diagnosis is detection of **Mycobacterium tuberculosis**, by using Ziehl-Neelsen (ZN) staining or culture .However, fresh, unfixed tissue with live bacilli is usually not available for culture .Also keeping in view low sensitivity of conventional methods to confirm the presence of M.tuberculosis , we conducted present study to localise tubercle bacilli or their components by IHC which persist in the granulomas which have lost the property of staining with acid fast stain in tissues. We also assessed the advantage of immunostaining over conventional ZN staining . Moreover, it has a low sensitivity and may take weeks to months before organisms can be identified. In recent times, the use of polymerase chain reaction has been reported which in developing countries is limited by the expense, easy contamination and technical expertise.

The diagnosis of extrapulmonary TB in particular can be elusive because a negative smear for AFB, lack of granulomas on histopathology and failure to culture M.tuberculosis do not exclude its diagnosis. Thus a necessity for IHC staining arises which assumes even more significance in socially and economically backward areas like Bundelkhand region where TB load is significantly high and extrapulmonary TB cases are also on rise.

MATERIALS AND METHODS

This retrospective study was conducted in Department of Pathology, MLB Medical college, Jhansi on tissue specimens received from Department of Surgery ,MLB Medical college, Jhansi. Paraffin blocks of 60 patients of granulomatous lymphadenitis, enteritis and granulomatous lesions of other sites from October 2016 to October 2017 were collected. We assessed these cases for immunoexpression of polyclonal antibody (anti-BCG) using supersensitive IHC detection system by Biogenex. Our study included cases of granulomatous lymphadenitis (cervical ,submandibular ,axillary, inguinal ,submental lymph node biopsies) , granulomatous enteritis and other sites like chest wall ,breast lesion etc. But we **excluded** cases of skin TB.

Tissue sections from these cases were subjected to H&E staining , Ziehl Neelson staining and IHC staining. Expression of anti-BCG antibody was determined by immunohistochemical reaction performed with supersensitive IHC detection system by Biogenex. This detection system uses the STREPTAVIDIN –BIOTIN Technology. Tissue sections of diagnosed cases of lepromatous leprosy were used as positive controls. Microscopic examination was done to study pattern of Mycobacterial antigen distribution in IHC positive cases. A comparison was then made between ZN staining and IHC.

We then did statistical analysis using Microsoft Excel and Microsoft Access .All data were exported to SPSS for Windows for analysis.

RESULTS

Most cases in our study conducted on 60 patients of extrapulmonary TB were found to be in the age group of 21-30 years with the mean age of presentation being 28.4 years with slight female preponderance . 70% patients in our study , belonged to rural areas and 30% to urban areas . Majority were hindus (96.66%) .Cervical lymphadenopathy was found to be the most common presentation (50%) followed by submandibular and axillary lymphadenopathies.

Table -1

CLINICAL PRESENTATION	NO. OF CASES	PERCENTAGE
Cervical LAD	30	50
Submandibular LAD	5	8.34
Axillary LAD	3	5
Supraclavicular LAD	2	3.34
Abdominal pain (intestine stricture)	11	5.45
Fallopian tube(salpingitis)	1	1.65
Others	8	13.33

(*LAD: LYMPHADENOPATHY

***OTHERS : PERIANAL SINUS ,ANAL ABSCESS ,L3 L4 DERMAL SINUS, ANTIBIOMA BREAST ,CHEST WALL ABSCESS ETC.)**

ZN staining was found to be positive in only 18.33 % cases and negative in 81.66 % cases.(TABLE-2)

We saw that ZN stain is most sensitive for lymph node sections (8/11). The sensitivity being 72.73% for lymph node tissue.

Table-2 Anti-bcg Antibody Stain Results (n=60)

RESULT	NO. OF CASES	PERCENTAGE
POSITIVE	47	78.34
NEGATIVE	13	21.66

Lymph node sections showed more positivity than other tissues for anti-BCG immunostain, which is comparable with other studies.

38/47 lymph nodes were positive for anti-BCG,which means the sensitivity of anti-BCG for lymph node tissue in our study was 80.85%, while sensitivity for intestine was 10.63 % only while for others ,it was just 8.5%.

We also found the antibody stain to be more intense in well organised necrotic granulomas.

STATISTICS OF ZN STAIN:
Table-3

METHOD		
ZN	TP=11	FP=49
NON-ZN	FN=47	TN=13

Thus , Sensitivity =18.96%
Specificity=20.96%
Positive Predictive Value=18.33%

STATISTICS OF IHC:
Table-4

METHOD		
IHC	TP=47	FP=49
NON-IHC	FN=11	TN=13

Thus,Sensitivity=81.03%
Specificity=20.96%
Positive Predictive Value=48.95%

COMPARISON OF IHC AND ZN STAIN STATISTICS

METHOD	SENSITIVITY	SPECIFICITY	PPV
ZN	18.96%	20.96%	18.33%
IHC	81.03%	20.96%	48.95%

Thus we found chi (p –value) <0.001, clearly indicating the statistical significance of our study.

DISCUSSION

The increasing burden of TB in India that too, of extrapulmonary TB ,especially in backward areas like Bundelkhand region, necessitated this study. Extrapulmonary sites of infection [1] commonly includes lymph nodes(most common site) , pleura and osteo articular areas ,although any organ can be involved. A negative smear for AFB, a lack of granulomas on histopathology and failure to culture , do not exclude the diagnosis.

In our study, we employed immunohistochemical staining procedure which is a simple and sensitive technique. The result of IHC staining in our study was similar to some other studies. Some studies have reported higher positivity(even about 87%), but the slightly lower positivity of our study (78.33%) than some other studies using polyclonal antibody, could be due to smaller sample size used in our study.

Author,year	Antibody used	No of cases	Results (positivity)
Humphrey et al ,1987	Polyclonal anti BCG	59	77.7
Barbolini et al ,1989	Monoclonal antibody	23	100
Wiley et al ,1990	Anti BCG	34	94.1
Radhakrishnan et al 1991	Ig G Antimycobacterial antibody	10	100
Mukherji et al ,2002	Polyclonal anti BCG	50	87
Oliviera et al ,2004	Anti MPT	3	100
Padmavathy et al ,2005	Polyclonal anti BCG	50	68
Mustafa et al,2006	Polyclonal anti BCG	55	64
Goel and budhwar 2007	Monoclonal antibody	36	100
Purohit et al ,2007	Anti mpt ,anti bcg	152	80 ,76.6
Baba et al ,2008	Anti mpt ,anti bcg	25	80, 48
Present study,2017	Polyclonal anti BCG	60	78.33

In a study conducted by **Goel and Budhwar[1]** , 16/16 cases of lymph nodes specimens, 2/2 cases of GIT specimens, 7/7 cases from female genital tract and 6/6 cases from bones and joints were found to be positive for IHC, i.e. a 100% positivity was seen. This 100% positivity could be because of the use of monoclonal antibody.

In our study ,38/40 (i.e. 95%) cases of lymph node specimens ,5/11 (i.e.45.55%) GIT specimens and 1/1 (100%) cases from female genital tract were positive for IHC.

In a study by **Mustafa et al[2**], the positivity range of ZN staining was found to be ranging from 0-44% while that of IHC ranged from 48 - 100%. This low AFB positivity could be because only the intact bacilli take the stain. The concentrated debris derived from Mycobacterium retained antigenic property ,therefore showing IHC positivity, but lost its AFB staining property. The similar observation was drawn by us also wherein 36 cases which were positive by IHC, showed no AFB positivity.

A similar study by **Purohit et al** [3] , showed ZN stain to have sensitivity of only 10-45%, which again proves that immunostaining can offer major improvement in diagnostic potential especially in cases of paucibacillary extrapulmonary tuberculosis.

Baba et al [4] in their study found that IHC with anti- MPT64 has better specificity ,sensitivity and predictive values than anti-BCG but it is costly and not easily available.Their study found that anti-BCG showed only 45.55 % specificity as compared to 76-80% for anti-MPT antibody.

In a study by **Wiley et al [5],** it was postulated that this lower specificity of anti-BCG could be due to its cross reactivity with other with other infectious organisms.

The sensitivity of anti-BCG , although ,is very high and not very different from anti-MPT64.Another explanation for the false positive results

CONCLUSION

We conducted this study at Department of Pathology with a view to study immunohistochemical staining pattern in suspected TB cases' tissues by applying rabbit polyclonal anti-BCG antibody and we did a comparative analysis with most widely used easily available ZN stain for Mycobacterium.

We found that the mean age of presentation of extrapulmonary TB cases in Bundelkhand region is 28.4 years with slight female predominance.

The sensitivity of ZN stain was found to be quite low(18.96%) as compared to polyclonal anti-BCG whose sensitivity was found to be 81.03%. We also found that 8/11 lymph node sections showed positivity with ZN stain indicating its higher sensitivity for lymph node tissue(72.73%). IHC also showed higher sensitivity for lymph node tissue (80.85%).

Also, IHC showed moderate to strong intensity of staining for well formed granulomas with central caseous necrosis, while, mild intensity of staining was seen for non-organised, non-necrotic granulomas. This observation is comparable to previous studies as well.

We also studied staining pattern of granulomas with anti-BCG antibody and observed that this antibody stained epithelioid cells at the periphery of granuloma more intensely than at the centre of granuloma.

Thus , we can say that IHC can be applied as a routine laboratory diagnostic technique for granulomas of Mycobacterial etiology as IHC

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