Original Article

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Diagnostic Utility of PAX5 in Hodgkin and Non-Hodgkin Lymphoma: A Study from Northern India

Oncology Section

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ABSTRACT

Introduction: PAX5 is an immunomarker of B-cell origin and useful in the diagnosis of lymphoma. There is hardly any study on PAX5 expression in Indian patients with lymphoma.

Aim: To evaluate the diagnostic utility of PAX5 as an adjunct immunohistochemical marker in the diagnosis of Hodgkin Lymphoma (HL) and Non-Hodgkin Lymphoma (NHL).

Materials and Methods: Immunohistochemistry was performed against CD20, CD3, CD15, CD30, and PAX5 on formalin fixed paraffin embedded tissue of 71 cases of lymphoma and CD20, CD3 and PAX5 in control samples of reactive lymph nodes. Frequency, mean values, and percentage were calculated. Fisher's-exact test and test for analysis of variance were applied.

Result: For 24 cases of HL and 47 cases of NHL, the mean age of patients was 17.6±14.8 years and 44.1±21.6 years, respectively. The male: female ratio for both HL and NHL were 1.7:1. Among NHL cases, the numbers of B-cell and T-cell types were 39/47 (83%) and 8/47 (17%), respectively. In comparison to control samples, PAX5+ expression was seen in 23/24 (95.8%) cases of HL (p=1.000) and 32/39 (82%) cases of B-NHL (p=0.0834). All the cases of T-NHL showed negative expression of PAX5 (p<0.0001). Analysis of variance between NHL, HL and control samples was statistically significant (p<0.0001).

Conclusion: PAX5 staining between control samples and cases of classical HL and B-NHL was statistically not significant, whereas, statistically significant difference was observed with T-NHL. Thus, PAX5 may be used as an adjunct marker in the diagnosis of classical HL and B-NHL.

Keywords: B-cell specific activator protein, Hodgkin/Reed-Sternberg cell, Immunohistochemistry, Lymphoproliferative disorder

INTRODUCTION

Lymphoma is a heterogeneous group of malignant lymphoproliferative disorder; in which presence or absence of Hodgkin/Reed-Sternberg (HRS) cells determine nomenclature to HL or NHL, respectively. Broadly, the World Health Organization (WHO) classification subdivides NHL into B-cell, T-cell or Natural Killer (NK) cell types. This lineage determination of lymphoma cells can be done either by immunohistochemistry in tissue sections or rarely, using flow cytometry in lymph node aspirates. Immunohistochemical demonstration of B-cell markers (CD19, CD20, CD79a), T-cell markers (CD2, CD3) and/or rarely NK cell markers (CD16, CD56) identifies cell of origin in lymphoma [1].

Out of these types, B-cell NHL is the most common sub-type, seen in 80% cases followed by T-cell NHL and NK-cell NHL [2]. According to the Surveillance, Epidemiology, and End Results (SEER) stat fact sheet, the estimated incidence of NHL in 2016 in the USA is 72,580 with 27.7% mortality [3]. In comparison, the estimated incidence of HL for 2016 in the USA is 8,500 with 13.1% mortality [4]. So, the likelihood for the development of NHL will be 8.5 times more than HL in the USA in 2016. The incidence of NHL in the urban registries of India is 5.1 per 100,000 [5]. In a study from western India, the proportion of HL among lymphoma was found to be 20% [6]. Similarly, in a 10-years' study from Southern India, the proportion of HL was 21.3% among all the lymphomas [7]. In yet another study from Punjab, HL formed 39% of total lymphoma cases [8].

PAX5 gene, located on chromosome 9p13, expresses transcription factor PAX5 that is also known as B-cell Lineage Specific Activator Protein (BSAP), which is involved in the development of B-lymphocytes, central nervous system and adult testis [9]. Antibody against PAX5 is commercially available for immunohistochemistry, which has been evaluated as an excellent pan-B and pan-pre-B cell marker [10]. PAX5 is expressed in normal, reactive and neoplastic B-cells and its expression correlates well with the B-cell markers CD19 and CD79a [10,11]. In normal lymphoid tissue (tonsil and lymph node), PAX5 is expressed in the nuclei of lymphoid follicles and perifollicular mantle zone (stronger expression) but negative in T-lymphocytes, plasma cells, endothelial cells and macrophages [11]. Nuclear expression of PAX5 by immunohistochemistry is used to mark tumours of B-cell lineage, including B-cell acute leukaemia, B-cell NHL, and HL [10].

Worldwide, there are studies regarding PAX5 expression in lymphoma; however, we could not find a similar study from India. This study was done to evaluate the diagnostic utility of PAX5 as an adjunct immunohistochemical marker in the diagnosis of HL and NHL.

MATERIALS AND METHODS

Collection of Samples

This was an observational and analytical study, approved by the Institutional Review Board, and done between July 2012 and July 2015 in a university hospital of Northern India. Demographic data like age, sex, and site of biopsy was noted. Appropriate blocks of Formalin-Fixed Paraffin Embedded (FFPE) tissues were retrieved for immunohistochemistry. Histopathologically diagnosed cases of HL or NHL were included, for which FFPE blocks containing adequate tissue were available. After immunohistochemistry, diagnostic categories like, benign lymphoproliferative disorder, reactive lymphoid hyperplasia, etc. were excluded as cases. Thus, based on histopathology and immunohistochemistry, total 71 cases of lymphoma were retrieved. For comparison, 20 separate FFPE blocks of reactive lymph nodes were taken as control samples.

Immunohistochemistry

Immunohistochemical study was done against CD20, CD3, CD15, and CD30 for diagnostic confirmation and classification of cases as HL or NHL. Additional immunohistochemistry was performed on selected cases of lymphoma against CD5, CD10, Cyclin D1, bcl-6, Epithelial Membrane Antigen (EMA), and Anaplastic Lymphoma Kinase (ALK). CD20 and CD3 were done on control samples of reactive lymph nodes. PAX5 immunohistochemistry was done in all the cases of lymphoma and control tissue of reactive lymph nodes. Briefly, the procedure used for immunohistochemistry was as follows: 5µ thick sections were cut on 0.01% poly-L-lysine coated glass slides and baked at 60°C for minimum one hour on a standard histology hotplate. Sections were dewaxed in xylene and treated with three changes of alcohol and brought to water. Sections were subjected to heat induced antigen retrieval in a microwave oven (EZ Retriever, Biogenex Inc., USA) in citrate buffer (pH 6.0). For PAX5, Tris-EDTA buffer (pH 8.0) was used for antigen retrieval. Cooling at room temperature for 20 minutes followed this. Sections were then treated to block endogenous peroxidase, stained with ready to use primary antibodies and incubated overnight at 4°C. For PAX5, incubation with primary antibody was done at room temperature for 30 minutes. Detection was done by horseradish peroxidase polymer based detection system (Biogenex Inc., USA), diaminobenzidine chromogen and counterstain with haematoxylin.

Interpretation of Immunohistochemistry

Evaluation of all immunostained slides was performed without knowledge of the clinical data and histopathological diagnosis. Slides were considered assessable if the positive control and simultaneous internal controls were stained appropriately. Reactive lymph node sections were used as positive controls for CD20, CD3 and PAX5. Known positive case of HL was taken as a control for CD15 and CD30. Samples without primary antibody served as negative control. Regarding CD30 and CD15, membranous or granular cytoplasmic staining of any intensity in at least 10% of HRS cells was considered positive [12]. For CD20 and CD3 staining, any membranous or cytoplasmic staining in at least 10% tumour cells was considered positive [13].

Nuclear staining in greater than 10% of neoplastic cells was considered positive for PAX5 [13]. Nuclear staining of PAX5 was scored as weak, moderate or strong. The strong positivity of normal mantle zone cells and scattered (residual) small B lymphocytes was used to evaluate the relative staining intensity of PAX5 positivity in neoplastic cells as follows [14]: Strong expression when nuclear staining was similar to the staining of mantle zone cells of the reactive lymph node, moderate expression when weaker, but significant staining intensity was noted, and weak expression when detectable nuclear staining was present above the background level.

Statistics

Calculation of frequency, mean values, and the percentage was done using online Graphpad software, which is freely available at: http://www.graphpad.com. Fisher's-exact test was used for comparison between control samples and lymphoma cases by Quick Calcs software. Test for analysis of variance (ANOVA) was performed by Microsoft Excel software. A p-value <0.05 was taken as statistically significant.

RESULT

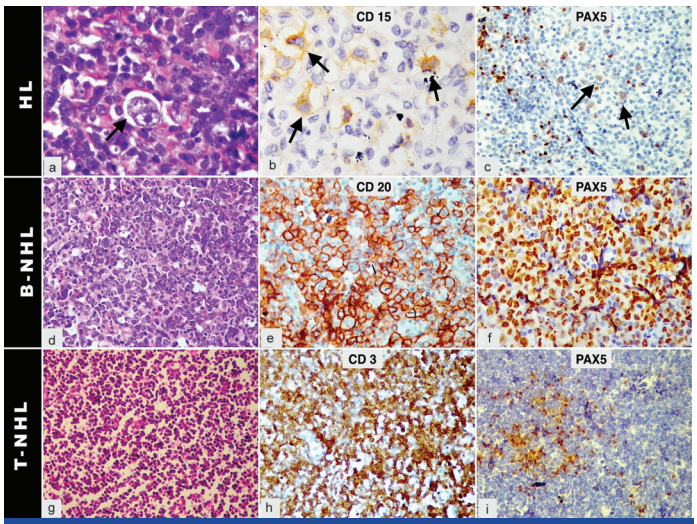
The demographic data on lymphoma cases of the study are given in [Table/Fig-1].

All control samples of reactive lymph nodes (n=20) showed the normal distribution of CD20 and CD3 staining and strong intensity of PAX5+ expression. All the 24 cases of HL were of classical type [Table/Fig-2a] and showed negative expression of CD3 and CD20 in Hodgkin/Reed-Sternberg (HRS) cells. Further, all the 24 (100%) cases of HL showed positive expression of CD30 in HRS cells, whereas, 14/24 (58.3%) cases showed positive expression of CD15 in HRS cells [Table/Fig-2b]. Weak nuclear staining of PAX5 in HRS cells was noted in 23/24 (95.8%) cases of HL [Table/Fig-2c]. On applying Fisher's-exact test, statistically significant result was not seen for PAX5 staining between control samples and HL (p=1.000) [Table/Fig-3]. The co-expression pattern of PAX5 staining with CD15 and CD30 in HL is given in [Table/Fig-4].

Based on immunohistochemistry, 39/47 (83%) cases were categorized as B-cell NHL (CD20+/CD3-) [Table/Fig-2d,2e]. Distribution among various subtypes of B-cell NHL was: large B-cell lymphoma (35/39, 89.7%), small B-cell lymphoma (2/39), small lymphocytic lymphoma (1/39) and follicle-centre cell lymphoma (1/39). PAX5+ expression was seen in the nuclei of neoplastic tumour cells in 32/39 (82%) cases of B-NHL [Table/Fig-2f]. Among 32 PAX5 positive cases of B-cell NHL, intensity

Parameter	Hodgkin lymphoma (n=24)	Non-Hodgkin lymphoma (n=47)
Age range (Years)	5-50	7-80
Mean age (Years)	17.6±14.8	44.1±21.6
No. of males	15	30
No. of females	9	17
Male: female ratio	1.7:1	1.7:1
Nodal distribution	24	41
Site of lymph node biopsy - Cervical - Submandibular - Preauricular - Supraclavicular - Axillary - Mediastinal - Inguinal	 15 1 1 0 4 0 3	 17 0 2 1 11 2 10
Extra-nodal distribution	0	6
Extra-nodal site - Duodenum - Stomach - Colon - Nose - Spleen	 0 0 0 0 0	 2 1 1 1 1

[Table/Fig-1]: Demographic characteristics of lymphoma cases of the study.



[Table/Fig-2]: (a) Reed-Sternberg cell (arrow) of HL (Haematoxylin and eosin stain, 1000×); (b) CD15+ expression in Hodgkin cells (arrows) of HL (Diaminobenzidine, 1000×); (c) Weakly positive nuclear expression of PAX5 in Hodgkin cells (arrows) (Diaminobenzidine, 400×); (d) Sheets of large atypical lymphoid cells in a case of NHL (Haematoxylin and eosin stain, 400×); (e) Strong membranous positivity of CD20 in NHL (Diaminobenzidine, 400×); (f) Strongly positive nuclear expression of PAX5 in neoplastic cells of B-cell NHL (Diaminobenzidine, 400×); (g) Sheets of monomorphic atypical lymphoid cells in NHL (Haematoxylin and eosin stain, 400×); (h) Strongly and diffusely positive membranous expression of CD3 in NHL (Diaminobenzidine, 400×); (i) Negative PAX5 expression in T-cell NHL. Note scattered strong PAX5 positive B lymphocytes in the background (Diaminobenzidine, 400×).

of PAX5 expression was noted as mild, moderate and strong in 9 (28.1%), 16 (50%), and 7 (21.9%) cases, respectively. All seven PAX5 negative B-cell NHL were large B-cell subtype. In this study, there were 8/47 (14.8%) cases of T-cell NHL; out of which seven were CD3+/CD20- [Table/Fig-2g,2h] and a single case was diagnosed as anaplastic large cell lymphoma (CD20-/CD3-/CD30+/ALK+/EMA+). All eight cases of T-cell NHL were negative for PAX5 [Table/Fig-2i]. For PAX5 staining between control samples and B-NHL, statistically significant result was not seen by Fisher's-exact test (p=0.0843), however, statistically highly significant result was obtained between control samples and T-cell NHL (p<0.0001) [Table/Fig-3]. For PAX5 staining, the test for analysis of variance between NHL, HL and control samples was statistically highly significant (p<0.0001) [Table/Fig-5]. The co-expression pattern of PAX5 staining with CD20, and CD3 in cases of NHL is given in [Table/Fig-6].

Groups	PAX5		Fisher's-exact test	
	Positive	Negative	Comparison between groups	p-value
1. Hodgkin lymphoma	23	1	1 Vs.4	1.000
2. B-cell non-Hodgkin lymphoma	32	7	2 Vs. 4	0.0834
3. T-cell non-Hodgkin lymphoma	0	8	3 Vs. 4	<0.0001
4. Reactive lymph node	20	0	--	--

[Table/Fig-3]: Comparison of control samples with Hodgkin lymphoma, B-cell non-Hodgkin lymphoma and T-cell non-Hodgkin lymphoma.

Sets of antibodies	Number	Proportion (%)
CD 30+/ CD 15+/ PAX5+	13	54.2
CD 30+/ CD 15-/ PAX5+	10	41.7
CD 30+/ CD 15+/ PAX5 -	1	4.2

[Table/Fig-4]: Co-expression pattern of CD30, CD15 and PAX5 in HRS cells of Hodgkin lymphoma (n=24).

Groups	Sample size	Sum	Mean	Variance		
Non-Hodgkin lymphoma	47	1,864	39.65	920.01		
Hodgkin lymphoma	24	900	37.5	217.39		
Reactive lymph node	20	2,000	100.0	0		
Analysis of variance						
Source of Variation	SS	df	MS	F	p-level	F crit
Between Groups	58,272.19406	2	29,136.09	54.18	4.594×10^{-16}	3.10

[Table/Fig-5]: Test result for analysis of variance among three groups of the study.

Sets of antibodies	Number of B-NHL	Proportion (%)	Number of T-NHL	Proportion (%)
CD 3+/ CD 20-/ PAX5 -	0	0.0	7	14.8
CD 3-/ CD 20+/ PAX5+	32	68.0	0.0	0.0
CD 3-/ CD 20+/ PAX5 -	7	14.8	0.0	0.0
CD 3 -/ CD 20-/ PAX5 -	0	0.0	1	2.1

[Table/Fig-6]: Co-expression pattern of CD3, CD20 and PAX5 in non-Hodgkin lymphoma (n=47).

DISCUSSION

PAX+ expression in HL and B-NHL of the present study was found to be statistically similar to PAX5+ staining in non -neoplastic B-cells of the control samples. To the best of our knowledge, this type of study is not available in the published literature from India. PAX5 is a member of the highly conserved paired box (PAX) domain family of transcription factors, located on chromosome 9p13 and it is expressed in all the developmental stages of the B-cells, but not in the plasma cells or the T-cells [11]. We have observed PAX5+ expression in CD20+B-cell follicles and mantle zone of reactive lymph nodes, while negative PAX5 expression was seen in CD3+T-cells. In this study, PAX5+ expression was seen in 95.8% cases of HL, 82% cases of B-cell NHL and none of the cases of T-cell NHL.

In various studies, PAX5+ expression was observed in 100% cases of B-cell haematolymphoid malignancies [13,15,16]. Krenacs et al., and Mhawech-Fauceglia et al., have found PAX5+ expression in 81% and 91.5% of B-cell NHL, respectively [14,17]. In current study, PAX5+ expression was observed in 81% cases of B-cell NHL. Now, it may be argued that as PAX5 is expressed through all the developmental stages of B-cell, its expression should have been seen in all the cases of B-cell NHL. However, PAX5 expression is not seen in certain subsets of B-cell NHL where the malignant clone of B-cells are derived from post-follicular transformed B-cells representing the transition from mature B-cells to plasma cells [14]. Though, in present study, we did not observe plasmacytic differentiation in cases of HL or NHL. In contrast, a study on CD20 negative lymphomas showed PAX5 positivity in 100% cases of CD20- precursor B-cell lymphoblastic leukaemia/lymphoblastic lymphoma, 88% cases of CD20- recurrent mature B-cell lymphoma after rituximab therapy and negative staining in all cases of CD20- Diffuse Large B-Cell Lymphoma (DLBCL) [13]. Similar results were seen in another study with additional PAX5 positivity in CD20- DLBCL without plasmacytic differentiation [18]. The findings of these studies indicate that malignant clones of lymphoma cells, which lack CD20 expression with or without therapy, may retain PAX5 expression. Thus, PAX5 may be a better marker of B-lineage than CD20. However, till date, there is no therapy available targeting PAX5.

Expression of the presence or absence of PAX5 is also useful in distinguishing classical HL from nodular lymphocyte predominant HL and anaplastic large cell lymphoma. PAX5 is expressed in 90% to 98% of HRS cells in classical HL and 100% of HRS cells in lymphocyte predominant HL [19]. Similarly, in another study, varying intensity of PAX5 expression was seen in 93% cases of classical HL [18]. This observation of PAX5 staining in HRS cells of classical HL also confirms their B-cell origin, despite the frequent absence of other B-cell antigen [19]. In classical HL, the staining intensity varies from weak to moderate and is rarely strong compared with the normal reactive small lymphocytes. In nodular lymphocyte predominant HL, the staining of PAX5 is as strong as seen in the small B cells in at least some of the tumour cells. There is a negative expression of PAX5 in anaplastic large cell lymphoma, which was also observed in a single case of this study.

LIMITATION

We studied expression of PAX5 in Indian patients of lymphoma, however, we could not correlate PAX5 expression with other parameters like stage, treatment response, and survival of patients.

CONCLUSION

PAX5 is a promising marker for diagnosis of classical HL and B-cell NHL. PAX5 staining remains negative in T-NHL. However, larger studies from different parts of India are required to observe staining pattern of PAX5 in patients with lymphoma and its correlation with outcome of disease.

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