

Analysis of PAX5 expression of Hodgkin's and non-Hodgkin's lymphomas: in comparison with routine panel

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Abstract

Aim of the study: To assess the expression of PAX5 in different types and stages of lymphomas and to study its utility in comparison with the classical pan B and T cell markers.

Materials and Methods: Immunohistochemistry was done to detect the expression of PAX5, CD45, CD3, CD20, CD15 and CD30 in all cases of lymphoma reported for a period of two years.

Results: 59 cases were reported as malignant lymphomas giving an overall incidence of 6.3% in our hospital. The age group ranged from 3 years to 75 years with a male preponderance. Nineteen cases were excluded owing to inadequate tissue samples. Of the 40 cases, 28 were nodal and 12 extra-nodal lymphomas. 33 cases were non-Hodgkin's lymphoma and 7 were Hodgkin's lymphoma. 23 out of the 40 cases were positive for PAX5 and out of 21 cases of B cell non-Hodgkin's lymphoma, 18 were positive. Reed-Sternberg cells of Hodgkin's lymphoma were positive in 5 cases. PAX5 was positive in most of the B cell non-Hodgkin's lymphoma and Hodgkin's lymphoma regardless of the stage, age, sex and site. PAX5 was negative in all T cell non-Hodgkin's lymphoma and the cases of unclassified category.

Conclusions: This study suggests that B cell neoplasms and Reed Stenberg cells strongly expressed PAX5 in comparison to classical lymphoma panel and hence can be a useful marker. However a larger prospective study on PAX 5 expression in Hodgkin's Lymphoma is vital to look for an increased expression in subset of Indian population.

Keywords: B-cell Lymphoma, Hodgkin's lymphoma, Immunohistochemistry, PAX5, T-cell Lymphoma.

Introduction

Lymphomas are the malignant neoplasm arising from the cells of immune system or the lymphoreticular system.¹ Painless lymph node enlargement confined to one lymph node region or involving multiple lymph node regions is the commonest clinical presentation.⁽¹⁾

The lymphomas can be broadly categorised as Hodgkin's lymphoma (HL) and Non-Hodgkin's lymphoma (NHL). Diagnosing the type of lymphoma and subcategorizing them is challenging as most of them have an overlapping morphology. Immunohistochemistry (IHC) is mandatory to establish the cell lineage and PAX5 is one such marker used in diagnosis of specific subtype of lymphoma. PAX5 gene is a member of the paired box gene family and is located in chromosome 9p13. It encodes the transcription factor, PAX5, also known as B cell specific activator protein (BSAP).⁽²⁾ It is expressed in B lymphocytes from pro B cells to mature B cells, is essential for B cell development and differentiation and can reliably be detected by Immunohistochemistry.⁽³⁾ PAX5 expression is specific for B cells especially in the precursor stage where CD20 is negative.^(4,5)

Therefore, we did a retrospective study to assess the PAX5 immunoreactivity as a B cell lineage marker in the samples received as lymphoid malignancies and correlated with the other routinely used CD markers in cases of lymphoma.

Materials and Methods

All the cases diagnosed as lymphomas for a period of two years, in the department of pathology, PSG institute of medical science and research, Coimbatore were considered in the study. The clinical data required for the study like the age, sex, site and stage of the tumour were retrieved from the medical records department, after obtaining permission from the concerned authorities and institute human ethics committee clearance. The hematoxylin and eosin slides of these cases were analyzed for assessing the morphology and also typing the lymphoma. Paraffin blocks of those sections which had high tumour density were included for the study by reading the H and E slides. Blocks of slides which had less tumour material or extensive necrosis were excluded.

Immunohistochemistry was done using the supersensitive HRP detecting system. Paraffin blocks of the study population and controls (Table 1) were chosen, fresh sections 4µm in thickness were cut. After deparaffinising and dehydrating in graded alcohols, the slides were subjected to antigen retrieval in a pressure cooker for 10 minutes with EDTA buffer at pH 9. Primary antibodies available in liquid form, ready to use formulation (Table 1) were used and incubated for one hour. A standard HRP multimer-based hydrogen peroxide substrate without biotin, containing 3, 3'-diaminobenzidine tetrahydrochloride (DAB) chromogen was used. Harris hematoxylin was used as counter stain.

Table 1: Primary antibodies and controls used in the study

Markers	Manufacturer	Clone	Control
CD45	Biogenex Fremont, CA, USA	PD7/26/16	Lymphnode
CD3	Biogenex Fremont, CA, USA	PSI	Lymphnode
CD20	Biogenex Fremont, CA, USA	L-26	Lymphnode
CD15	Biogenex Fremont, CA, USA	BRA4F1	Kidney
CD30	Biogenex Fremont, CA, USA	HRS-4	Known case of Hodgkin's lymphoma
PAX5	Dako Carpinteria, CA, USA	DAK	Tonsil

These sections were than assessed for the immunoreactivity. All the CD markers were considered positive when they showed a membranous positivity in the neoplastic cells. Slides stained with PAX5 were considered positive when they show a brisk nuclear positivity. Less than 10% of cells showing positivity were considered negative. The intensity of the staining was also considered.

Results

The department of pathology, PSG institute of medical science and research reported 922 cases of malignancy during the study period of two years. Of these, 59 cases were reported as malignant lymphomas giving an overall incidence of 6.3%.

Of the 59 cases reported as lymphomas during the study period, 19 cases were not included in the study owing to inadequate tissue samples or unavailability of tissue blocks. The remaining 40 cases include 28 nodal lymphomas and 12 extra-nodal lymphomas of which 33 were NHL and 7 were HL. Most of the NHLs were of B cell type. 4 out of the 33 NHLs were not classified under a specific cell type and were reported as unclassifiable. Table 2 and Fig. 1 show the various types of lymphomas included in the study and its percentage incidence distribution.

Table 2: Distribution of lymphomas included in the study

Type	No of cases
Hodgkins Lymphoma	7
Nonhogkins Lymphoma	
Nodal	21
Extra Nodal	12
Total	40

The age group ranged from 3 years to 75 years, with a mean age of 43.5 years. Table 3 shows the age wise distribution of various lymphomas. Five out of seven cases of HL were in the second and the fifth decades. Eighteen out of the thirty three cases of NHL were in the sixth and seventh decade of life.

Table 3: Distribution of various lymphomas age wise

Age	Hl	Nhl		
		b cell	T Cell	Unclassified
0-10	1	0	1	1
11-20	3	1	1	0
21-30	0	0	2	0
31-40	0	0	0	1
41-50	2	5	1	0
51-60	0	6	1	0
61-70	1	8	2	1
>70	0	1	0	1
Total	7	21	8	4

Of all the 40 cases, 28 were males and 12 were females, giving rise to a male: female ration of 2.3:1. All the cases of HL were males. B cell type NHL was more common than the T cell type NHL in both the sexes. Fig. 2, 3 and table 4 depict the distribution of various lymphomas across different age groups and sex, which clearly shows a predilection for males.

Table 4: Sex wise distribution of various lymphomas across various age groups

Age	0-10	11-20	21-30	31-40	41-50	51-60	61-70	>70	Total
Male	2	4	1	0	4	7	9	1	28
Females	1	1	1	1	4	0	3	1	12

The lymphomas were staged based upon the Ann-Arbor staging into stage I, stage II, stage III and stage IV (Fig. 4). Four out of forty cases could not be staged due to unavailability of necessary data.

Results of immunohistochemical markers CD45, CD3, CD20, CD15 and CD30, used in the diagnosis of lymphomas were studied and determined as positive or negative. Areas staining the reactive lymphoid cells were excluded. All the cases except one were positive for CD45. This case had an immunoprofile of CD45-ve, CD3-ve, CD20-ve, CD15-ve, CD30+ve and EMA+ve. Based on the morphology and the immunoprofile, it was diagnosed as Anaplastic large cell lymphoma, a T/NK cell NHL.

The Reed-Sternberg cells of all the cases of HL were positive for CD15, CD30 (Fig. 5) and negative for CD3, CD20. This is the immunoprofile for classical Hodgkin's lymphoma.

Among the NHL, 7 were positive for CD3, 21 were positive for CD 20 and 4 were negative for both CD3 and CD20. Lymphomas positive for CD20 were classified as B cell NHL (Fig. 6), those positive for CD3 were classified as T cell NHL (Fig. 7) and others were placed under unclassified category. Table 5 shows the number of cases in each immunoprofile.

Table 5: IHC analysis of commonly used antibodies in the diagnosis of lymphomas

Immuno profile	No of cases
HL (CD 15 +VE, CD 30 +VE)	7
NHL(CD 15 -VE, CD 30 -VE)	
CD 3 +VE	7
CD20 +VE	21
CD 45 +VE, CD 3 -VE, CD 20 -VE	4
CD 45 -VE	1

All the cases were stained with PAX5 antibody and its expression in different age groups, sex, site, stage and types of lymphomas. A strong nuclear staining was considered positive. 23 out of the 40 cases were positive for PAX5. The expression of PAX5 in females and males were assessed. Table 6 shows the expression of PAX5 in relation to sex. PAX5 was positive in 17 out of 28 male cases and 6 out of 12 female cases. All the B cell type NHL of females was positive for PAX5. PAX5 was negative in 2 cases of HL and 3 cases of B cell type NHL of male.

Table 6: Expression of PAX5 in relation to sex

Sex	HL	Pax 5+ve hl	B cell type nhl	Pax5 +ve b cell type nhl
Males	7	5	15	12
Females	0	0	6	6

Table 7 shows the expression of PAX5 in various lymphomas. PAX5 was positive in Reed-Sternberg cells, in five cases of HL (Fig. 5) and eighteen cases of B cell NHL (Fig. 6). PAX5 was negative in all cases of T cell NHL (Fig. 7) and the cases under the unclassified category.

Table 7: expression of PAX5 in various lymphomas

Type of lymphoma	No of cases	Pax 5 +ve	Pax 5 -ve
Hl	7	5	2
Nhl	33	18	15
-B cell type nhl	21	18	3
-T cell type nhl	8	0	8
-Unclassified	4	0	4

PAX5 was positive in 17 out of 28 nodal lymphomas. These 17 cases consist of 5 cases of HL and 12 cases of B cell type NHL. Among the 12 extra nodal lymphomas, seven were of B cell type NHL and PAX5 was positive in six of them (Fig. 6). Table 8 and 9 shows PAX5 expression in relation to site, type of lymphoma and across various age groups respectively.

Table 8: Expression of lymphomas in relation to site and type of lymphoma

		total no of cases	Pax 5 +ve cases
Nodal Lymphomas	HL	7	5
	B cell nhl	14	12
	T cell nhl	5	0
	Unclassified	2	0
Extra Nodal Lymphomas	HL	0	0
	B cell nhl	7	6
	T cell nhl	3	0
	Unclassified	2	0

Table 9: Expression of PAX5 in relation to age

Age	HL	Pax 5 +ve hl	B cell type nhl	Pax 5 +ve b cell type nhl
0-10	1	1	0	0
11-20	3	1	1	0
21-30	0	0	0	0
31-40	0	0	0	0
41-50	2	2	5	5
51-60	0	0	6	4
61-70	1	1	8	8
>70	0	0	1	1

PAX5 expression across various stages was assessed (Table 10) and found to be positive in most cases of B cell NHL and HL, regardless of the stage.

Table 10: expression of PAX5 across various stages

Stage	HL	B cell type nhl	Pax 5 +ve cases
I	1	6	7
II	1	6	6
III	1	4	3
IV	4	3	6

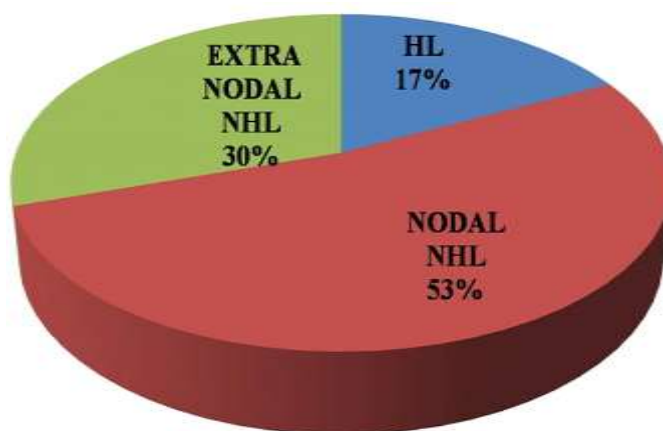


Fig. 1: Distribution of lymphomas with percentage incidence

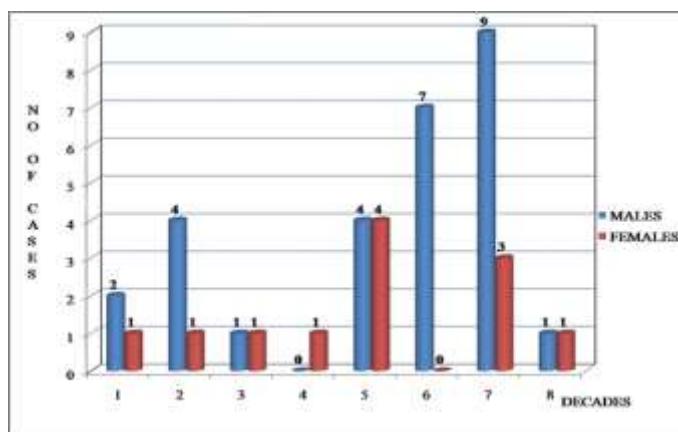


Fig. 2: Sex wise distribution of lymphomas across various age groups

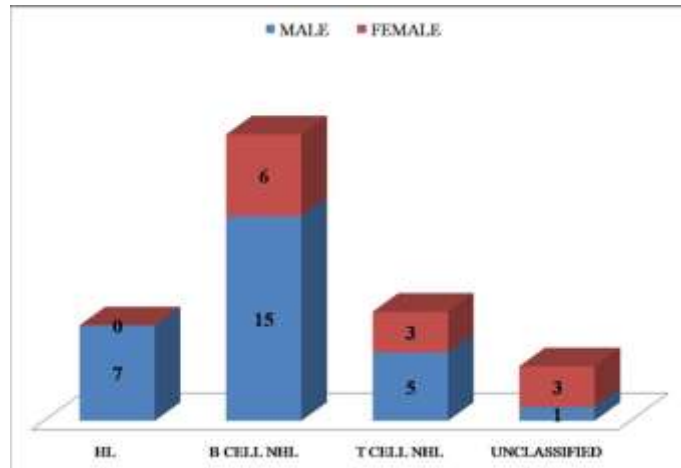


Fig. 3: Distribution of different types of lymphomas sex wise

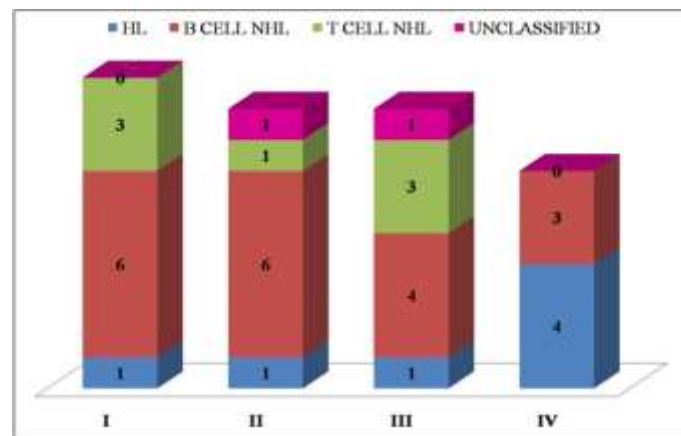


Fig. 4: Distribution of lymphomas across different stages

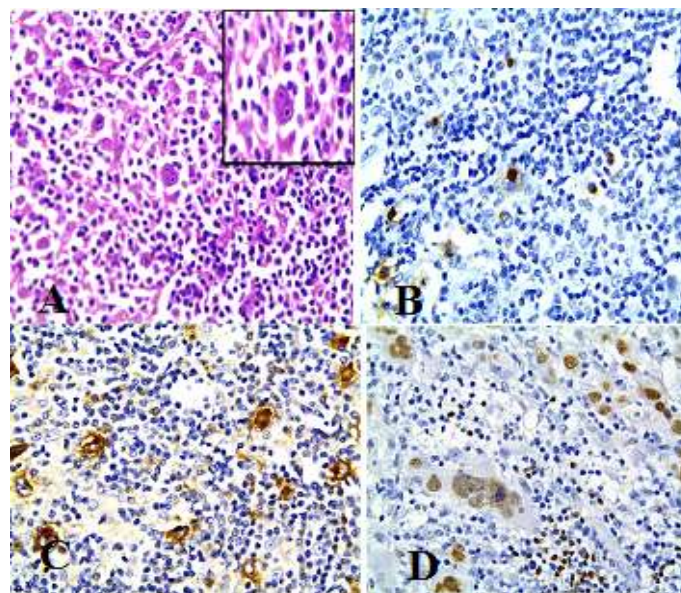


Fig. 5: A - Classical HL (H&E, 40X). Inset shows a classical Reed Stenberg cell. B - RS cells showing a faint membranous positivity and a paranuclear dot like positivity with CD15 (CD15, 40X). C - RS cells showing a strong membranous positivity and a paranuclear dot like positivity with CD30 (CD30, 40X). D - RS cells showing a strong nuclear positivity with PAX5 (PAX5, 40X)

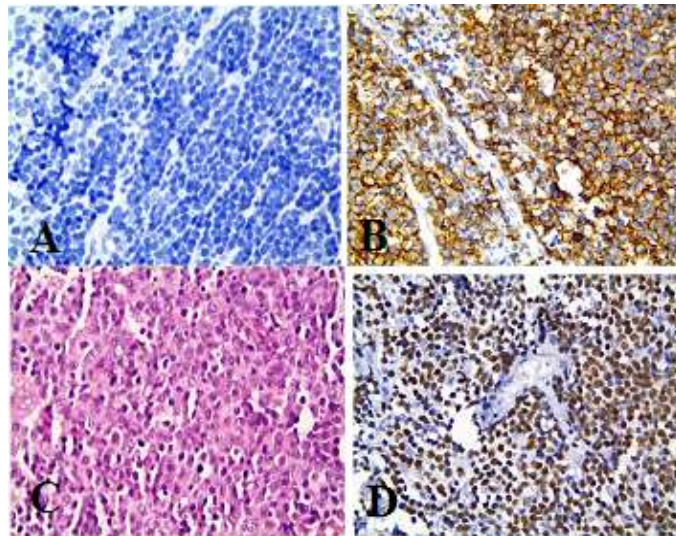


Fig. 6: A - Nodal B cell lymphoma (H&E, 40X). B - A strong membranous positivity with CD20 (CD20, 40X). C - Negative staining with CD3 NHL (CD3, 40X). D - A strong intense nuclear staining with PAX5 in a case of B cell NHL (PAX5, 40X)

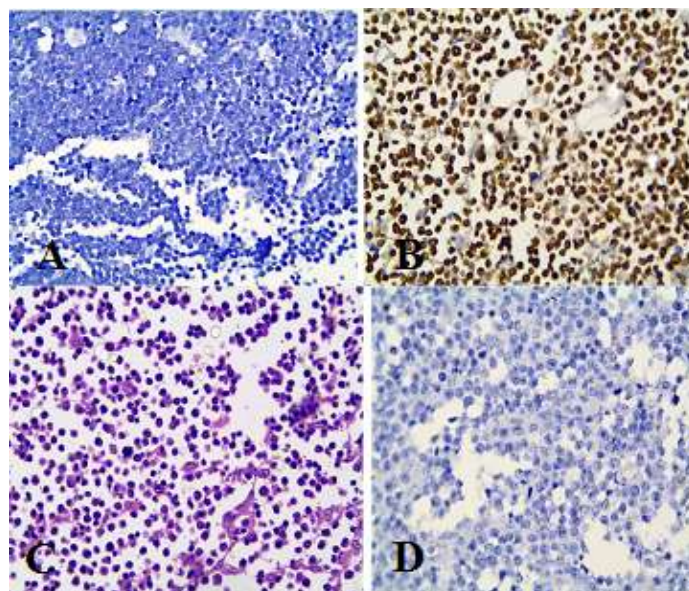


Fig. 7: A - T cell NHL (H&E, 40X). B - A strong membranous positivity with CD3 in a case of T cell NHL (CD3, 40X). C - Negative staining with CD20 in a case of T cell NHL (CD20, 40X). D - Negative staining with PAX5 in a case of T cell NHL (PAX5, 40X)

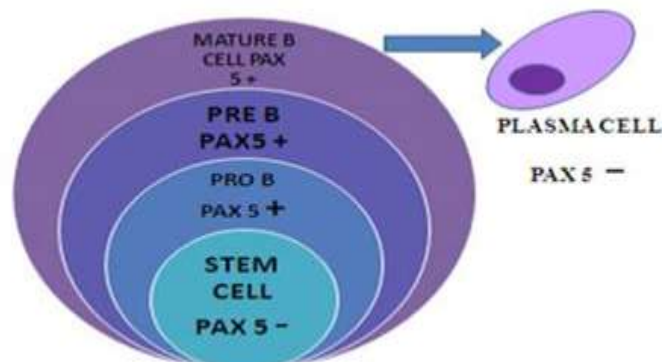


Fig. 8: B cell Maturation and Pax 5 Expression

Discussion

The incidence of lymphomas is very less in the south central Asia when compared to the rest of the world.⁽⁵⁾ The incidence of lymphomas is increasing throughout the world as well as in India. Yeole BB et al⁶ studied the incidence of NHL in all the major registries in India and observed a significant increase in the incidence of NHL over the years in all the registries. This increasing incidence rates, which in part is contributed by improving diagnostic methods, and poor survival rates make lymphoma a major concern.⁷ Lymphomas constituted 6.3% of all malignancies in this institute which is significantly higher than that reported in the western literature (3%) and from the Chennai (4.25%) as reported in the national cancer registry atlas.⁽²⁾

Lymphomas show a definite male predominance throughout the world.⁽⁵⁾ In our study, there was a slight male preponderance which is very similar to the literature.

HL shows a peak incidence between 11-30 years and between 51-60 years. In general, NHL is more common after 50 years of age.⁽¹⁾ The peak age incidence of lymphoma observed in our study is 5th to 7th decade and is similar to all other studies. PAX 5 expression does not have a significant correlation with the age at presentation.

A non-tender enlarged lymph node, involving a single or multiple groups is the most common presentation of lymphoma. Lymphomas involving sites other than lymph nodes are called extra-nodal lymphomas.^(1,8) They can secondarily involve the bone marrow or spill into the peripheral blood and present as leukemia.⁽⁸⁾ In addition to the lymphadenopathy they can have other symptoms like fever, weight loss, anorexia, dyspnoea, abdominal or chest pain, compressive symptoms, pruritus or even bleeding. There could be an enlarged liver or spleen and anemia associated with lymphomas.⁽⁹⁾ They are prone to infections due to loss of immunity. The patients also have predilection to develop auto immune diseases owing to immune modulation and the vice versa is also true.⁽⁸⁾

The first case of lymphoma was identified by Thomas Hodgkin while working at the Guys hospital, London in the year 1832. It was in 1898 and 1902, when Carl Sternberg and Dorothy Reed gave the most important diagnostic entity for HD by defining the classical Reed-Sternberg cell (RS cell). Variants of RS cells were later identified.⁽¹⁰⁾

Since 1944, Many classifications for lymphomas were proposed and accepted around the world. The Revised European American classification was introduced in 1994.⁽¹¹⁾

WHO classification of the hematopoietic and lymphoid neoplasm's proposed in the year 2003. The current WHO classification (2008) is a revision of the 2003 classification. The Ann-Arbor staging system has

been in use for staging both the HL and NHL.^(1,8) History, physical examination and a minimum of ultra-sonogram of abdomen has to be done in order to stage the disease efficiently.¹² The staging system stratifies lymphomas into four stages, from stage I to stage IV. A letter 'E' is added to this stage in case the primary tumor is in an extra-lymphoid organ. These stages are again sub-classified based upon the presence (B) or absence (A) of systemic symptoms. Once a diagnosis of lymphoma is made on Histological examination, it needs to be categorised as HL or NHL, followed by placing it under a particular subtype. The NHL project observed a significant increase in accurate diagnosis and sub-classification of lymphoma using IHC.⁽¹⁴⁾ IHC markers can also be used to assess the prognosis in lymphomas. Lymphomas with high proliferation index have a bad prognosis.⁽¹⁶⁾ Reinhard Von Wasilewski et al observed that cases of HL that lack the expression of CD15 showed a bad prognosis.⁽¹⁷⁾

A panel of markers is almost always necessary for the diagnosis of lymphoma.⁽¹⁵⁾ The panel of markers commonly used in a case of lymphoma include: CD45 – to confirm the diagnosis of lymphoma (CD 45 is negative in classical HL and NK cell phenotype of ALCL), CD3 – pan T cell marker, CD20- pan B cell marker, CD15 and CD30 – for diagnosing HL.

CD45, also referred to as leucocyte common antigen (LCA) is a tyrosine phosphatase present on the surface of the leucocytes. CD45 is lost in plasma cells.⁽¹⁸⁾ CD45RB is the commonly used isoform as it is present in all leucocytes.

CD3 is the frequently used pan T cell marker.⁽¹⁵⁾ CD 3 is preferred because of its specificity and easy, reliable detection on paraffin embedded sections.⁽¹⁸⁾

CD20 also known as Leu26 and B1⁽¹⁹⁾ is a non-glycosylated phosphoprotein seen on cell membrane surface of all the mature B cells. It is expressed in a part of classical HL and is seen in all NLPHL.^(10,20)

CD15 is also referred to as Lewis X antigen, X haptan, Leu M1 and myelomonocytic marker^(18,19) is a cell adhesion molecule expressed on the cell membrane of RS cells of classical HL.⁽¹⁸⁾ CD15 is positive only in a part of HL.

CD30 is also referred to as K1, Ber H2 and lymphocyte activation antigen.⁽¹⁹⁾ It belongs to TNF receptor superfamily and is always positive in classical HL and ALCL. CD30 mediated NFkB activation is believed to be the pathophysiology behind classical HL^(22,23).

PAX stands for paired box. The PAX proteins are all transcription factors that usually determine the fate of the cells during the early stages of development and maturation mostly during embryogenesis and sometimes even in the adult life.⁽⁴⁾ PAX5 is otherwise called B cell specific activator protein (BSAP). It is named so because of its exclusive expression in B lymphoid lineage. Its expression is noted as early as pro B cell stage and is lost during the plasma cell stage.⁽⁴⁾

PAX5 drives the cells into B cell lineage and hence plays an essential part in B cell development. The genes that encode the PAX5 are situated in the chromosome 9p31. The role of PAX5 in B cell differentiation and maturation is mainly due to its ability to regulate the CD19 gene. CD19 is involved in B cell proliferation in addition to its functions in regard to the immune system.⁽²⁴⁾ The other genes that are activated by PAX5 are CD79a and B lymphoid kinase (Blk).

PAX5 suppresses the J chain and XBP1, thereby preventing the cells from maturing into plasma cells. Thus BSAP is not only essential in driving the cells towards B cell lineage, but also retaining its identity.⁽²⁵⁾ PAX5 along with PAX2 and PAX8 has been demonstrated to influence the apoptosis and have a role in survival of the B cells.⁽²⁶⁾

The suppression of PAX5 is mandatory for plasma cell differentiation. This is achieved through increased expression of B lymphocyte induced maturation protein 1 (Blimp 1), which suppress the expression of PAX5 in addition to other genes like C-myc.⁽²⁵⁾

Though immunohistochemical expression of PAX5 is noted in all stages of maturation the intensity of staining varies in subsets of B cells. Krenacs et al⁽²⁷⁾ observed a strong PAX5 immunoreactivity in the cells of the marginal zone as opposed to the follicular centre cells and monocytoid B cells.

In addition to B cell development, PAX5 is thought to play a role in CNS and urogenital development.⁽²⁸⁾

PAX 5 show a sharp nuclear reactivity making the assessment of immunoreactivity easy since they are devoid of background staining. Being a nuclear antigen, their immunoreexpression is altered with poor tissue preservation.⁽⁵⁾

The main utility of PAX5 is its expression in B cell lymphomas which lack or show equivocal immunoreactions with the commonly used IHC markers in paraffin embedded sections like CD20. CD20 expression is usually absent in classic HL and precursor lymphoid neoplasms. The surface CD20 expression is lost in patients following Rituximab therapy as it is a targeted therapy against CD20. In case of relapse in these patients, the cells may remain to be negative for CD20. PAX 5 helps in situations like these to establish the cell lineage.

Though PAX5 expression is seen in most of the cases of both classical HL and NLPHL, the intensity of staining is weak in a majority of the cases. Some cases of HL can be negative for PAX5 especially in cases of nodular sclerosis type. This reduced expression is due to the down regulation of PAX5 along with other transcription factors and surface antigens. Blimp1 has been observed to be expressed in certain classical HL. This is thought to be the reason behind the reduced expression of PAX5 in some cases and even for the absence of PAX5 reaction in some cases.⁽⁵⁾ Though, PAX5 is negative in few cases of DLBCL, it is equivalent to CD20 in its ability to recognize DLBCL.

The weak or absent immunoreactivity with PAX5 in DLBCL may be because they represent the post follicular cells in normal B cell development.⁽²⁷⁾

T cell lymphomas are consistently negative for PAX5. In our study, all the CD3 positive cases (T cell NHL) were negative for PAX5. Nevertheless PAX5 expression in T cell lymphomas was observed very rarely by different authors.^(30,31,32) These cases are thought to represent the aberrant expression or over expression of PAX5.

Problems can arise in differentiating between DLBCL, ALCL and HL as all these lymphomas can show large cells closely resembling RS cells. The routinely used IHC markers can add on to the confusion as these tumours can have similar immunophenotypes. Browne et al used PAX5 along with other B cell transcription factors oct2, BOB1 and other pan B cell antigens in known cases of ALCL, DLBCL and HL. PAX5 was observed to be negative in all the cases of ALCL and positive in all cases of DLBCL and NLPHL. It was positive in about 91% of classical HL.⁽³³⁾ Similarly, we also observed weak expression of PAX5 in DLBCL and strong expression in RS cells of HL. The literature states that, HL usually shows a weak positivity with PAX5, but in our study four out of the five cases which expressed pax5 showed a strong intense reaction and in greater number of cells. As in most of the cases, the entire node is submitted for routine analysis; sampling error is highly unlikely in these cases. The strong reaction could be due to a neoplastic clone, which expresses increased PAX5 transcription factor in our population. However the number of samples in our study is minimal, and hence a further larger study exclusively in HL is essential.

The translocation t (9;14) involving the PAX5 region is found to be linked to lymphoplasmacytic lymphoma, further implying the role of PAX5 in tumorigenesis.⁽³⁴⁾ Balasenthil et al⁽³⁵⁾ postulated that regulation of PAX5 by metastasis associated protein (MTA1) lead to its over expression and lymphoma genesis.

Gilles A Roubischad et al⁽⁴⁰⁾ identified about five isoforms of PAX5 from normal and lymphoma cells. They observed that all these isoforms had different sequence and transactivation properties. PAX5 FL isoform was noted predominantly in the lymphomas and this altered form may be the reason behind the tumorigenesis.

In spite of its expression in various non haematolymphoid tissues and tumors, PAX5 is still an excellent marker for B cell lineage. Paulette et al⁽⁶⁾ and Kirsten et al⁽³⁷⁾ observed a highly specific immunoreexpression of PAX5 in B cell lymphomas.

Conclusions

The analysis from our study showed there is a twofold increase in incidence of lymphoma (6.3%) in this institute which is 3% higher than that reported in

the literature. NHL was more common than HL and the B cell phenotype was the commonest. We also infer that the B cell neoplasms expressed PAX5 as reported in the literature. Pax5 expression had no significant correlation with age, sex or stage of the disease. Three of the CD20 positive cases did not express PAX5, which could be due to a terminal B cell differentiation.

We also observed that the RS cells of HL exhibited a strong intense nuclear staining for PAX5, when compared to other studies in the literature. However a larger prospective study on PAX 5 expression in HL is vital to look for an increased expression in subset of Indian population.

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