Diagnostic Utility of Immunohistochemistry in Lymphoma

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ABSTRACT

Introduction: The pattern of distribution of lymphomas varies across different regions. This study conducted at a tertiary care hospital in North east India has taken into account immunhistochemmical findings and subclassified the lymphoid malignancies according to the WHO/REAL classification.

Material and Methods: A total of 50 cases of lymphoidmalignancies over a period of one year (between March 2015to March 2016) were diagnosed in the Pathology Department of Assam Medica College and Hospital,Dibrugarh(Assam).Out of these,40 cases (80%) were Non Hodgkin's lymphoma and 10 cases were Hodgkin's lymphoma(20%). The cases were diagnosed based on light microscopy and confirmed by immunohistochemistry. Concordancebetween light microscopy and immune histochemistry was 100%. The NHL cases were subtyped based on the percentage of positive and negative markers according to the revised WHO/REAL system of classification.

Results: Out of all the Non Hodgkin's lymphoma cases,B cell lymphomas constituted 36% of the cases and Tcell lymphomas constituted 28% of the cases.Out of the B cell lymphoms, 16% diffuse large B cell lymphoma, 8% were follicular lymphomas, 8% each of mantle cell lymphoma, 4% constituted marginal zonelymphoma.Peripheral T cell lymphoma was found in 4% of the Tcell lymphomas, T cell lymphoblastic lymphoma constituted 8%, anaplastic large cell lymphoma(ALK positive),anaplastic large cell lymphoma (ALK negative) constituted 8% each.Out of 20% of the Hodgkin's lymphomas,18 % were classical and 2% were nodular lymphocyte predominant type.The remaining cases were unclassifiable.

Conclusion: The distribution of lymphoma subtypes in this part of India shows that Non Hodgkin's lymphomas are more common than Hodgkin's lymphomas.Out of the NHLs,B cell lymphomas are more common than Tcelllymphomas.Follicular lymphoma accounts for majority of the B cell lymphomas. Out of the Hodgkin's lymphomas classical subtype was more common than the Nodular lymphocyte predominant type'

Keywords: B Cell Lymphomas, T Cell Lymphomas, Hodgkin'slymphomas, Immunohistochemistry, WHO/REAL Classification.

INTRODUCTION

The lymphomas are broadly classified into Hodgkin'sandNon hodgkin's lymphoma according tothe WHO classification. The Non-Hodgkin's lymphomas are again classified into T cell, B cell and NK cell types.

The lymphomas are diagnosed based on their morphology under light microscope, changes it h lymph node architecture (center of thefollicle, interfollicular area, mantle and marginal zones), changes in the cortex as well as paracortex, sinuses.Whenever an abnormal morphology is suspected,the pattern is determined, for instance if it is diffuse or nodular in pattern,the size of the lymphocytes, nuclear characteristics and chromatin structure.

The lymphoms are defined as clonal proliferation of lymphocytes at different stages of maturation. Immunohistochemistry helps in the subtyping of the lymphomas into different caegories which have a therapeutic and prognostic importance.[1-3]

The panel of immunological markers essential forthesubtping of the lymphomas are CD45, Bcellmarkers (CD19, CD20,CD79a), T cell markers (CD3,CD5), CD23, cyclin D1, BCL2, anaplastic lymphoma kinase, CD15, CD30, Ki67 etc. Most important aspect is the type of positivity (membranous,ctoplasmic or nuclear) and the percentageof cells showing the positivity required in accurate categorisation and distinguishing from reactive processes.

Study aims and objectives were to find out the most common subtype of lymphoma in the north east part of india and to assess the pattern of lymphoid malignancies in different age groups.

MATERIAL AND METHODS

The present study was conducted in the Department of Pathology,Assam Medical college and Hospial,Dibrugarh.It was a non interventional descriptive study.A total of 50 lymph node specimens were received over a period of one year starting fromMarch 2015 to March 2016 after obtaning clinical information like age,sex,site of biopsy which were subjected to tissue processing according to the standard tissue processing protocol.The slides were stained with Haematoxylin and eosin stains and first observed under the light microscope and later immunohistochemistry was done using a panel of markers based on the suspected diagnosis at light microscopy.

Inclusion criteria: All cases suspected on clinical grounds and through cytological screening were taken into the study.

Exclusion criteria: All known cases which were undergoing treatment and badly preserved specimens were excluded

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How to cite this article: Mondita Borgohain, Kasturi Krishnatreya, Cheng Khow Weingken, Jayanta Kr. Das. Diagnostic utility of immunohistochemistry in lymphoma. International Journal of Contemporary Medical Research 2017;4(12):6-9.

from the study.

IHC panel employed in the study

The panel of IHC markers employed in the study were CD19,CD20, CD5, CD45, CD15, CD30, CD3, CYCLIN D1, Ki67, bcl2, Anaplastic lymphoma kinase,cytokeratin.

Concordance rate was 100% between light microscopy and IHC studies.

Standard immunohistochemistry protocol

3-5 Micro serial sections were taken in APTES (3 AMINOPROPYL TRIETHOXYSILANE) coated slides.

Baking: The slides were put in hot air oven (37 degree centigrade for overnight or at 60 degree centigrade for 1 hour)

STEP 1: Deparafinization; the slides were put in xylene for 10 minutes for 2 changes.

STEP 2: Rehydration.
1. The slides were put in absolute alcohol for 5 minutes.
2. 70% alcohol for 5 min.
3. 50% alcohol for 5 min.
4. Running tap water for 5 min.

STEP 3: ANTIGEN RETRIEVAL: The slides were put in microwave at 450 watt for 5 min for 1 cycle then 600 watt for 5 min for 2 cycles and then the slides were brought out and cooled at room temperature.

STEP 4: The slides were washed in buffer (TRIS) three times 1 min each.

STEP 5: EROXIFREE BLOCK was added on the section for 10 min

STEP 6: The slides were cleaned.

STEP 7: PRIMARY ANTIBODY was added in the respective slides and incubated for 60 min in humidity chamber at room temperature.

STEP 8: Slides were washed as before.

STEP 9: Amplifier was applied and incubated for 10 min at room temperature,

STEP 10: Slides were washed as before.

STEP 11: HRP POLYMER was applied and incubated for 10 min at room temperature.

STEP 12: DAB wass added on the section and waited for 7 – 10 min.

STEP 13: Slides were washed with distilled water

STEP 14: Slides were counterstained with IRON FREE HAEMATOXYLENE for 30 seconds to 2 min.

STEP 15: Running tap water was added for 5 min.

STEP 16: Dehydration –
(1) 50% alcohol for 1 min.
(2) 70% alcohol for 1 min.
(3) Absolute alcohol for 1 min.

STEP 17: The slides were air dried for 20 min.

STEP 18: The slides were mounted with D.P.X and labeled.

STATISTICAL ANALYSIS

SPSS version 2007 was used for the analysis.

RESULTS

Out of 50 cases, 4% were below 10 years of age, 17% in the 10-19 years age group, 8% in the 20-29 years age group, 10% in the 30-39 years age group, 31% in the 40-49 years age group, 22% in the 50-59 years age group and 8% were above 60 years of age. 81% were males and 19% were females. Male to Female ratio was approximately 4:1. Table 1 shows the percentage of Hodgkin's and Non Hodgkin's lymphoma. Table 2 shows the percentage os different lymphomas found in the study. Immunohistochemical Markers are shown in figure 1 and 2.

In 85% of the cases the size of the swellings ranged from

Age group (years)	Non-Hodgkin's Lymphoma (%)	Hodgkin's Lymphoma(%)
<10	4	0
10–19	4	13
20–29	8	0
30–39	10	0
40–49	31	0
50–59	15	7
≥ 60	8	0
Total	80	20

Table-1: Percentage of Hodgkin's and Non Hodgkin's lymphoma

Final diagnosis	Percentage (%)
Classical Hodgkin's Lymphoma	20.00
Follicular Lymphoma	8.00
Anaplastic Large Cell Lymphoma (anaplastic lymphoma kinase negative)	8.00
Anaplastic Large Cell Lymphoma (anaplastic lymphoma kinase positive)	8.00
B Cell Lymphoblastic Lymphoma	8.00
Mantle Cell Lymphoma	8.00
Nodular Lymphocyte Predominant Hodgkin's Lymphoma	8.00
Diffuse Large B Cell Lymphoma	16.00
B Cell Non Hodgkin's Lymphoma	4.00
Unclassifiable	4.00
Marginal Zone Lymphoma	4.00
Peripheral T Cell Lymphoma NOS	4.00
Total	100.00

Table-2: Percentage os different lymphomas found in the study.

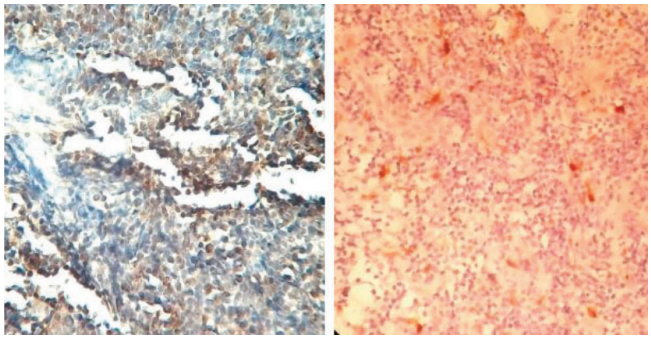

Figure-1: Immunohistochemical marker ki 67 positive in more than 30% of cells in a case of aggressive lymphoma. **Figure-2:** Immunohistochemical marker cyclin d1 positive in tumour cells of mantle cell lymphoma.

2cm to 4cm whereas in 7%, the size was below 2 cm, and in 8% of the cases the size was above 4 cm.Neck constituted the most common site of involvement being involved in 52% followed by axillary and inguinal regions both being involved in 14.67%.Supraclavicular and abdominal regions were involved each in 8% cases and face was involved in one case whereas thoracic region was affected in none of the cases. The average age for lymphoma cases was 36.8 years. With regards to the occupation history, it is seen that history of working in pesticides industry had 4 times excess risk of lymphoma (odds ratio – 4.03 and 95% confidence interval – 1.20 to 13.52, p value< 0.05 which is insignificant). And those working in the paint industry had 2 times increased risk of lymphoma (odds ratio -2.04 and 95% confidence interval – 0.51 to 8.12, p value>0.05 which was statistically insignificant)

DISCUSSION

In a study conducted by Aggarwal et al., REAL classification has advantage over working formulation classification[1] whereas in a study conducted by Kalyan K et al, the working formulaton classification of Non Hodgkin's lymphoma has preference over REAL/WHO[2].

The incidence of lymphomas was higher in males than females. This has been clearly shown in other studies and inconcordance with our study too[2-7].

Non Hodgkin's lymphoma was found to be more prevalent among older people and hodgkin's lymphoma in younger age group in our study. This is in accordance with other studies where incidence of NHL were highest in the 31-40 age group (21.3%)[8-10]. In a study conducted by Roy et al.,it was found that after the age of 40 years,the patients reported to have NHL were 63.6%, mostly in the age group of 51-60 years[4]. Padhi et al., conducted a study according to which the most commonly affected age group was between 30-50 years[6]. According to Vallabhajosyula et al.,the peak incidence of NHL was between 41-67 years[5]. Age and gender have prognostic significance in lymphoma and their treatment outcomes

In our study B cell lymphomas were more common than T cell lymphomas. This is in concordance with many studies as well.

In a study conducted in Amritsar, it was found that DLBCL was the most common subtype (46.8%)[13]. In studies conducted by Kalyan et al., Padhi et al., and Roy et al., being 26.5%, 69% and 29.3% respectively. This study is in concordance with two different studies conducted in Mumbai, India in 2000[9] and 2011[10], where the incidences of DLBCL were 34% and 42% respectively. Mushtaq et al., had found 76% of DLBCL in his study[8]. Tilly and Dreyling[11] showed that the incidence of DLBCL increases with age from 0.3/100000/ year int he age group 35-39 years to 26.6/100000/year in the age group of 80-84 years.

Follicular lymphoma was the next common entity in our study accounting for 8% of the cases along with mantle cell lymphoma.This finding is in correlation with other studies conducted in the Europe and U.S.In a study conducted in Amritsar, India,follicular lymphoma,mantle cell lymphoma, B cell lymphoblastic lymphoma and accounted for 4.3%,12.8%,17% respectively[13]. Roy et al. Found follicular lymphoma to be 6.8%, 2.8% marginal zone lymphoma 2.8%,small lymphocytic lymphoma 3.7%, mantle cell lymphoma 4%[4]. In a study conducted by Naresh et al,small lymphocytic lymphoma,mantle cell lymphoma,follicular lymphoma,marginal zone lymphoma accounted for 5.7%, 3.4%, 12.6% and 8.2% respectively[9]. In a study done by Aschebrrok-Kilfoy and colleagues on the incidence of mantlecell lymphoma in America, it has been found that mantle cell lymphoma incidence shows an upward trend regarding age being more common in the elderly age group[12]. Among the lymphomas in our study, T cell lymphomas accounted for 20% of the Non Hodgkin's lymphomas out of which Anaplastic large cell lymphomas accounted for 16% of the cases, peripheral T cell lymphomas 4% of the cases.In a study conducted by Federico et al.,[14] angioimmunoblastic T cell lymphoma accounted for 18.5% of the T cell lymphomas, whereas in our study we did not find any case of Angioimmunoblastic T cell lymphoma. Angioimmunoblastic T cell lymphoma has got very bad prognosis and without treatment,the outcome is dismal. In the study conducted by Manisha et al.in Amritsar, India, Angioimmunoblastic T cell lymphoma,anaplastic large cell lymphomas,T cell lymphoblastic lymphoma accounted for 2.1%, 2.1% and 6.4% respectively[13]. Senger et al.[7] found 18% T lymphoblastic lymphomas, 9%anaplastic large cell lymphomas,alk positive and 6% anaplastic large cell lymphomas alk negative.Kalyan et al.[2] had the findings of peripheral T cell lymphoma to be 6% and T lymphoblastic lymphoma to be 15%.Roy et al[4].and Naresh et al.[9] had the findings of anaplastic large cell lymphoma to be of 10.5% and 6% respectively.

CONCLUSION

The incidence of lymphomas is showing an upward trend in recent years worldwide.Non Hodgkin's lymphomas are more common than Hodgkin's lymphomas. The pattern of lymphomas in India is different from that in the western countries.Diffuse large B cell lymphomas are more common in India whereas Follicular lymphoma and Mantle cell lymphomas are less common as compared to that in the Europe and U.S.A.Anaplastic Large cell lymphoma is more prevalent in India. Histopathology along with immunohistochemistry play a very important role in making a proper and accurate diagnosis of lymphoma and help in subtyping. Our limitation was we lost many cases to follow up and the sample size was small because the duration of the study was just one year.However, we plan to increase the number of cases in the years to come and improve in the followup of the patients.

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Source of Support: Nil; **Conflict of Interest:** None

Submitted: 03-12-2017; **Accepted:** 01-01-2018; **Published:** 10-01-2018