Original Article

Non Hodgkin's Lymphoma

Significance of CD Markers in the Classification, Patterns and Sub Typing of Non Hodgkin's Lymphoma

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ABSTRACT

Objective: Aim of this study is Immunohistochemical analysis of H&E diagnosed cases of NHL for confirmation, classification and differentiation on the basis of phenotypic expression of CD3, CD30, CD45 and CD20 markers.

Study Design: Prospective study.

Place and Duration of Study: This study was conducted in the Pathology department of Liaquat University of Medical and Health Sciences Jamshoro (LUMHS) during October 2010 to March 2012

Materials and Methods: The sample consisted of all the specimens received in the Pathology department of Liaquat University of Medical and Health Sciences Jamshoro (LUMHS) during the above period. Immunohistochemical stains including CD3, CD30, CD20 and CD45 were used for classification and differentiation of cases of NHL.

Results: Out of one hundred and eighty (180) H & E diagnosed cases of Non Hodgkins Lymphoma, only 142 (78.8%) were positive for CD20 and were confirmed as B cell NHL; however 6 (3.3%) cases showed positivity with CD30 and were confirmed as large T-cell NHL. 38(21.2%) cases showed positivity for CD3 and all 180 (100%) cases were positive for CD45 and were confirmed as NHL.

Conclusion: It is concluded that Immunohistochemistry is helpful in differentiation of NHL. Cases of B cell NHL occur more frequently than T cell NHL. Furthermore NHL is more common in males and mostly presents with nodal involvement.

Key Words: CD markers, Immunohistochemistry, Non Hodgkin's Lymphoma.

INTRODUCTION

Malignant tumors are composed of abnormal cells having multiple genetic mutations thus resulting in the escape from cell cycle regulatory genes[1]. Non-Hodgkin's Lymphoma (NHL) is one of the many malignant tumors arising from lymphoid organs. The yearly incidence of non-Hodgkin's lymphoma is variable in different geographical location and is found to be around 3% to 4% in developed countries[2]. Non-Hodgkin's lymphoma is encountered more in males than the females and commonly encountered between sixth and seventh decade of life[3]. In Pakistan, NHL accounts for 4th commonest malignant tumor in males with the incidence of 6.1%[4]. WHO classifies NHL into many B cell and T cell subtypes, consisting of several distinct lymphoid neoplasm[5].

The classification of the NHL is based on various parameters including histomorphologic changes on biopsy specimen, immunohistochemistry and flow cytometry[6,7]. NHLs classification is based on the origin of neoplastic lymphocytic cells and is divided into the B-cell and T-cell lymphoma. Lymphoma of the B cells accounts about 90% of NHLs, whereas rest of the 10% are T-cell lymphoma[8].

The steps involved in the diagnosis of lymphoma includes hematoxlin and eosin stained biopsy slides, Immunohistochemical markers along with other diagnostic tools, some of which may be either invasive or non-invasive leads to final confirmation of the disease. This helps out to have a proper therapeutic plan[9]. The classification of both Hodgkins disease and NHL is continuing to evolve incorporating not only histopathologic data, but also immunophenotypic, genotypic and clinical characteristics to design a treatment plan using varying combinations of chemotherapy, radiotherapy and immunotherapy[10].

The principle of the Immunohistochemistry or IHC involves the use of antibodies for detecting antigens in a tissue sample. Lymphoid as well as hematopoietic diagnosis and classification can be done easily with the help of IHC[11]. Two different types of dyes including chromogenic or fluorescent means are used in IHC for detecting target antigens, however experimental design will be needed to decide the type of readout. In fluorescent method, fluorophore is used for the conjugation of primary and secondary antibody which is later detectable by fluorescent microscopy. In contrast chromogenic detection involves enzymes mainly horseradish peroxidase (HRP) or alkaline phosphatase (AP). This causes formation of colored, insoluble precipitates when substrates, such as DAB and NBT/BCIP are added respectively.[12]

So this study is planned for the Immunohistochemical analysis of H&E diagnosed cases of NHL for confirmation, classification and differentiation on the basis of phenotypic expression of CD3, CD30, CD45 and CD20 markers.

MATERIALS AND METHODS

This prospective study was conducted in the Pathology department of Liaquat University of Medical and Health Sciences Jamshoro (LUMHS) during October 2010 to March 2012. All sample received during above period were included in this study. All diagnosed cases of NHL on H&E staining of all ages and either gender were included in this study. Biopsies were taken from nodal or extranodal sites and were processed for gross and microscopic examination. After the routine processing and paraffin embedding, H & E and special staining; the immunohistochemical analysis was performed. The antibodies used in immunohisto chemical staining included CD3, CD30, CD20 and CD45. The antibodies were ordered from DAKO DENMARK. All cases were analyzed for age, sex distribution (male vs female), site (nodal vs extranodal) and subtypes of NHL. Seven cases (28 slides) of positive and negative control of H&E diagnosed NHL were included for the staining (performed in batches).

RESULTS

The sample size of this study consisted of one hundred and eighty (180) cases of H & E diagnosed NHL diagnosed on staining. Out of total 180 cases of NHL, patients with age below 60 years were 146 (81.1%) while the patients with age above 60 years were 34 (18.9%) and the mean age was 46 years. Table 1 shows age wise distribution of all these patients.

Out of total 180 cases of NHL, 110 (61.1%) cases were seen in male while 70 (38.9%) female were affected. Gender distribution is shown in Table 2.

Table No.1: Age Distribution of Patients

Characteristic	No. of Cases	Percentage
Age < 60 years	146	81.1
Age ≥ 60 years	34	18.9

Table No.2: Gender Distribution

Characteristic	No. of Cases	Percentage
Male	110	61.1
Female	70	38.9

Out of total 180 cases of NHL, majority were found involving lymph node accounting 124 (68.8%) while 56 (31.2%) on extra nodal sides. Site of presentation is shown in Table 3.

Immunohistochemical staining of CD20 show that out of 180 cases of Non Hodgkin lymphoma 142 (78.8%) were seen positive for CD 20 and were confirmed as B cell NHL. Immunohistochemical staining of CD30 show that out of 180 cases of NHL, only 6 (3.3%) cases show positivity with CD 30 and were confirmed as large T-cell NHL. Immunohistochemical staining of CD3 show that out of 180 cases of NHL, 38(21.2%) cases show positivity and were confirmed as T-cell NHL. Immunohistochemical staining of CD45 showed that out of 180 cases of NHL all 180 (100%) cases show positivity with CD45 and were confirmed as NHL. Results of Immunohistochemical staining are shown Table 4.

Table No.3: Site of Presentation

Characteristic	No. of Cases	Percentage
Nodal	62	68.8
Extranodal	28	31.2

Table No.4: Immunohistochemistry Results

CD Markers	Positive cases	%age	Negative cases	%age
CD-20	142	78.9	38	21.1
CD-30	6	3.3	174	96.6
CD-3	38	21.1	142	78.8
CD-45	180	100	0	0

DISCUSSION

Diagnosis of Non-Hodgkin's Lymphoma on H&E staining seems to be quite helpful but presence of Reed-Sternberg like cells or round cells in NHL make it difficult to diagnose. In these circumstances, immunoassaying is very much helpful.

The present study included 190 lymph node biopsies, which were diagnosed on H&E by the Pathologists as Non-Hodgkin's Lymphoma, but remained doubtful and need confirmation and further sub typing of NHL, as is needed for the better management plans.

In this study 61.1% patients with NHL were male while 38.9% were female. Aftab et al in a study has also reported male predominance which is in agreement with findings of our study[13]. NHL affects males more when compared with females. However literature of the available data shows remarkable variation in the gender distribution. Male to female ratio of NHL in the developed and developing countries is reported as 1.4:1 and 4.5 to 3.1 respectively[14]. The study conducted by Zeba et al also reported male predominant and support our study[15].

In the present study 68% patients have nodal involvement while 31.2% patients have extra nodal involvement. Study conducted by Arora et al reported 33% of cases having extra nodal presentation and 67% have nodal presentation[16]. Another study conducted in china found that, tumors in lymph nodes were seen in 42.8% and extra nodal 57.2% and proposed that autoimmune etiology is thought to be involved in extra nodal marginal zone lymphomas and these are reported to be more common in East Asian countries like Japan and China than in Pakistan[17].

In our study out of 180 cases of Non-Hodgkin's Lymphoma diagnosed on panel of CD markers, 142

(78.8%) were seen positive for CD20 and confirmed as B-cell NHL and 38 (21.2%) showed positivity for CD3 and diagnosed as T-cell NHL. Study conducted by Yasmeen Bhurgri reported 80% of cases as B-cell NHL and 20% as T-cell NHL support our study[18]. Another study conducted in Saudi Arabia also revealed majority of cases of B-cell phenotype; while only 14% of the cases were T-cell lymphoma[19].

The CD20 marker is expressed in all the maturation levels of B cells except stem cells and plasma cells; however it is present in both the normal and malignant B cells[20]. CD3 is composed of at least five different polypeptide chains closely associated with T cell antigen receptor and with each other[21]. CD3 is a useful marker for the detection of T-cell lymphoma[22]. Polyclonal CD3 along with CD20 distinguishes between T-cell and B cell lymphomas[23].

The CD30 antigen is an isoglycoprotein whose expression is used for detecting the anaplastic large cell lymphomas (ALCL). This distinct variant of T cell NHL shows proliferation of large pleomorphic cells in a cohesive pattern and is strongly positive for CD30[24]. In the present study CD3 and CD30 were positive in 6 (3.3%) cases of ALCL, while the study conducted by Krol and Gulsah reported 8 (<1%) and 1.2% cases of ALCL respectively which is in accordance with our findings[25,26].

Immunohistochemical staining of CD45 showed that out of 180 cases of NHL all 180 (100%) cases show positivity with CD45 and were confirmed as NHL. CD45 glycoprotein expression is positive in all lymphocytic cells. However CD45 expression in low-grade B-cell NHL is not well reported in the literature[27]. CD45 is a transmembrane protein-tyrosine phosphatase and is found on the B and T cells, thymocytes and macrophages. Thus it is useful in differentiating lymphomas from non lymphoid tumors as CD45 shows positivity for malignant B and T cells. Furthermore expression of CD20 antigen is generally restricted to the B-cell lineage while CD3 stains only cells of T-cell origin[28]. Thus CD20 and CD3 only help in differentiating B and T cell Lymphomas.

CONCLUSION

It is concluded that Immunohistochemistry is helpful in diagnosing the types of NHL. Cases of B cell NHL occur more frequently than that of T cell NHL. Furthermore NHL is more common in males and mostly presents with nodal involvement.

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