ORIGINAL ARTICLE

Immunohistochemical Profile of Hodgkin and Non-Hodgkin Lymphoma

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Abstract

Objective: To analyze the frequencies of histological types of lymphoma, diagnosed with complete immunohistochemical profile in younger and older age group.

Study Design: Cross-sectional analytical study.

Place and Duration of Study: Dow Diagnostic Research and Reference Laboratory, Dow University of Health Sciences, Karachi, from January 2009 to September 2013.

Methodology: Consecutive cases of lymphomas, which were diagnosed using immunohistochemistry, were analyzed according to WHO classification. Frequency and percentages for different types of lymphomas were calculated. Hodgkin and non-Hodgkin lymphomas characteristics in two age groups of less than and more than 40 years were compared, applying chi-square test.

Results: Out of the 318 cases, 79 (25%) were Hodgkin Lymphomas (HL) and 239 (75%) were Non-Hodgkin Lymphomas (NHL). Mixed Cellularity Hodgkin Lymphoma (MCHL) was the commonest (n=48). Amongst the NHL, 215 (89.95%) were B cell lymphomas and 24 (10.05%) were T-cell lymphomas. Diffuse Large B-Cell Lymphoma (DLBCL) was the commonest lymphoma (n=165, 69.95% of NHL). Anaplastic T-Cell Lymphoma (ALCL, n=10) was the commonest T-cell lymphoma. The frequency of HL was significantly higher in the younger age group and that of NHL was higher in the older age group ($p < 0.001$). Primary lymph node involvement was reported in 175 (55%) and cervical lymph node was the most frequent site. Extra nodal involvement was seen in 93 (29%) of all cases and was reported in 87 (36.4%) of NHL and 6 (7.5%) of HL. The most common extra nodal site was the gastrointestinal tract.

Conclusion: Hodgkin lymphoma comprises 25% and non-Hodgkin lymphoma comprises 75% of all lymphomas. Both occur in younger age groups than reported in the West. B-cell NHL is three times more common than T-cell lymphoma. DLBCL is the most frequent lymphoma. ALCL is the most common T-cell, and mixed cellularity is the most common Hodgkin lymphoma.

Key Words: *Hodgkin lymphoma. Non-Hodgkin lymphoma. Diffuse large B-cell lymphoma. Anaplastic large cell lymphoma. Mixed cellularity Hodgkin lymphoma.*

INTRODUCTION

Lymphomas are classified into two major categories, Hodgkin Lymphoma (HL) and Non-Hodgkin Lymphoma (NHL). HL has five subtypes: nodular lymphocyte predominant (NLPHL), nodular sclerosis (NSHL), mixed cellularity (MCHL), lymphocyte rich (LRHL) and lymphocyte depleted (LDHL). Non-Hodgkin lymphomas are: B-cell and T-cell types, which are sub-classified into different subtypes using immunohistochemistry, according to WHO classification.[1]

According to global cancer facts and figures, the estimated age-standardized incidence and mortality rates (per 100,000), 2008, for NHL was 10.3 and 3.6 respectively for developed countries and 4.2 and 3 for developing countries. The estimated age-standardized incidence and mortality rates (per 100,000) for HL were 2.2 and 0.4 in developed countries and, 0.9 and 0.6 in developing countries.[2] The lowest incidence rates are found in Asia and Eastern Europe. NHL is the seventh leading cause of cancer death in the United States and represents 3.3% of all cancer deaths.[2] NHL incidence rate is highest in people aged 65 - 74 years and mortality rate is highest in people aged 75 - 84 years.[2] Incidence rate for NHL has been rising on average 0.5% each year spanning over the last 10 years.[2] Various factors such as improvement in diagnostic procedures, infections as Human Immunodeficiency Virus (HIV), Epstein Barr Virus (EBV), Hepatitis C Virus (HCV), Human T-Leukemia Virus (HTLV), *Helicobacter pylori*, exposure to chemicals and decreased immunity in old age have been implicated in the increased rate.[3]

Data from the West shows that HL comprises 11% of all lymphomas with a falling annual incidence. The highest recorded HL incidence rate is in Biella, Italy; intermediate rate in North America, and low in Japan and China.[4]

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Pakistan also lacks a national cancer registry for recording the incidence of the disease and much of the data of our population goes unreported. Data from a private sector hospital in Karachi shows that NHL is the fourth most common malignancy in males, accounting for 6.1%.[5] According to the published data from Karachi South Cancer Registry, there is a substantial increase in the age-standardized incidence rate of NHL in the last two decades. Children and adolescents, people belonging to low socio-economic status and ethnicities from the North and Northwestern Pakistan are at a higher risk of developing NHL.[6]

The purpose of this study was to report the subtype distribution of lymphomas in Pakistani population and to compare it with the national and international data.

METHODOLOGY

This was a cross-sectional study. Immunohisto-chemistry-based types were analyzed in cases of lymphomas, diagnosed at Dow Diagnostic Research and Reference Laboratory, Dow University of Health Sciences, Karachi, from January 2009 to September 2013. Variables recorded were the hospital patient-number, age, gender, site of involvement, morphology and type of lymphoma. Cases with complete immunohistochemical workup were included in the study. Cases without or incomplete immunohisto-chemical workup were excluded.

All the biopsy samples were fixed in 10% buffered formalin and grossed according to the guidelines[7] by the pathology residents. Paraffin embedded blocks were stained for Hematoxylin and Eosin. The pathologists studied the morphology, made an initial diagnosis of lymphoma and selected a panel of immunohisto-chemical stains, to be applied on the block showing well preserved and largest tumor area. The antibodies used were LCA, CD 20, CD3, CD30, CD15, Ki-67, ALK, EMA, CD10, CD5, Cyclin D1, Bcl-2, CD23, CD43, and Pax 5. These blocks were stained with the antibody manually, using 20 minutes heat induced epitope retrieval in target retrieval solution. After washing and blockage of endogenous peroxidase, the slides were incubated with the primary antibody for 30 minutes, followed by detection with a streptavidin-biotin immuno-enzymatic antigen system. Positive and negative controls were included with each batch. Two pathologists studied each case and reviewed difficult cases in Departmental Consultation Meeting, attended by all pathologists at service. The cases were diagnosed and classified in accordance with WHO Classification of Tumors of Hematopoietic and Lymphoid tissue 2008.[1]

Data was analyzed using SPSS version 20, and descriptive statistics were calculated as mean and median for age of patients and frequency and percentages for different types of lymphomas. Two age groups of less than and more than 40 years were made and chi-square test was applied using 95% confidence interval; p-value of less than 0.05 was taken as significant. The type of lymphomas were also divided into 15 years' strata; the common lymphomas in each stratum, and distribution of lymphomas in these age strata was also determined.

RESULTS

The number of cases analyzed were 318 in 4 years and 9 months duration. Of all the lymphomas, 214 (67%) of the patients were males and 104 (33%) were females; male to female ratio was 2:1. Male predominance was there in most of the lymphomas; except extra nodal marginal zone lymphoma, which showed female predominance. T and B ALLs and mantle-cell lymphomas showed an equal frequency in both the sexes. Age ranged from 2 - 100 years. Mean age for all NHL patients was 46.31 ±18 years with median being 50 years. The mean age for all HL patients was 30.18 ±17 years with median being 30 years.

Out of 318 cases of lymphomas, 79 (25%) were Hodgkin Lymphoma (HL) and 239 (75 %) were non-Hodgkin Lymphoma (NHL).

In HL (n=79), mixed cellularity (MCHL) was the commonest, 48 (60.75%of HL), followed by 27 cases of nodular sclerosis (NSHL, 34.17%), lymphocyte rich (LRHL, n=3) and Nodular Lymphocyte Predominant (NLPHL, n=1). No case of lymphocyte depleted HL (LDHL) was reported during the study period (Table I).

In the NHL, (n=239), 215 (89.95% of NHL) were B-cell lymphomas and 24 (10.05% of NHL) were T-cell lymphomas. Diffuse Large B-Cell Lymphoma (DLBCL) was the most common lymphoma (n=165) having a frequency of 69.03% of NHL (Table II). In the B-cell lymphomas, following DLBCL were Chronic Lymphocytic Leukemia/Small Lymphocytic Lymphoma (CLL/SLL, n=15, 6.27%), Follicular Lymphoma (FL, n=15, 6.27%) and Mantle Cell Lymphoma (MCL, n=8, 3.34%). The major bulk of T-cell lymphoma comprised of Anaplastic Large Cell Lymphoma (ALCL, n=10, 4.18%) followed by T-cell Acute Lymphoblastic Lymphoma/leukemia (TALL, n=8, 3.34%) and peripheral T-cell lymphoma (PTCL, n=5, 2.09%). Single case of Angio-Immunoblastic T-cell Lymphoma (AITL, 0.41%) was reported; whereas, no cases of NK/T cell and cutaneous T-cell lymphomas were reported (Table II).

In children (upto 15 years), HL (n=17) was more common than NHL (n=12), MCHL being the commonest, followed by lymphoblastic lymphoma with 2 cases each of B-ALL and T-ALL. ALCL Alk positive and DLBCL had 3 cases each. Two cases of Burkitt lymphoma were noted. ALCL Alk negative cases were not reported in this age group.

Table I: Frequencies of different types of Hodgkin lymphoma (n=79).

Type of Hodgkin lymphoma	Male	Female	Total	Percentage
Mixed cellularity	33	15	48	60.75
Nodular sclerosis	16	11	27	34.17
Lymphocyte predominant	3	0	3	3.79
Nodular lymphocyte predominant	1	0	1	1.26

Table II: Frequencies of different types of non-Hodgkin lymphoma (n=239).

Type of non-Hodgkin lymphoma	Male	Female	Total	Percentage
Diffuse large B-cell lymphoma	113	52	165	69.03
Follicular lymphoma	10	5	15	6.27
Small lymphocytic lymphoma	10	5	15	6.27
Mantle cell lymphoma	4	4	8	3.34
MALT lymphoma	1	2	3	1.25
Plasmacytoma	1	0	1	0.41
Burkitt lymphoma	3	1	4	1.67
B-cell lymphoblastic lymphoma/ leukemia	2	2	4	1.67
T-cell lymphoblastic lymphoma/ leukemia	4	4	8	3.34
Anaplastic large cell lymphoma	7	3	10	4.18
Peripheral T-cell lymphoma	5	0	5	2.09
Angio-immunoblastic T-cell lymphoma	1	0	1	0.41

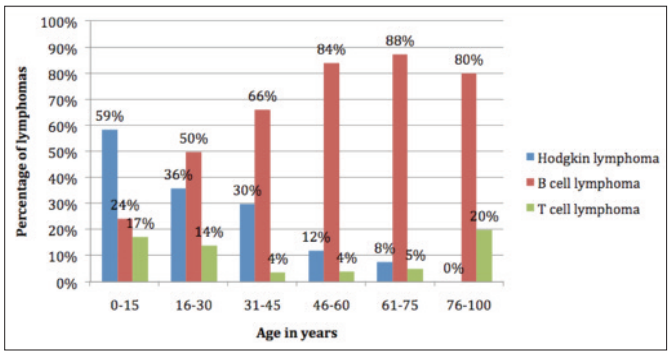

Figure 1: Percentage of lymphomas in different age groups.

Lymphoblastic lymphoma, ALCL, PTCL and Burkitt lymphoma were seen in younger age group (16 - 30 years). Follicular lymphoma, mantle cell lymphoma and CLL/SLL were more common in older age group (46 - 60 years). DLBCL was the commonest lymphoma in patients aged more than 60 years.

Primary lymph node involvement was reported in 175 (55%) and extra nodal involvement as the primary site was seen in 93 (29%) cases. Site was not known for 50 (15.7%) cases. Cervical lymph node enlargement was the most frequent site of presentation of lymphomas. Extra nodal involvement was seen in 87 (36.4%) of NHL, and the most common lymphoma to have an extra nodal involvement was Burkitt, showing gastro-intestinal tract (GIT) involvement in most cases. In DLBCL, extra nodal involvement was seen in about half of the cases. The most common extra nodal site was GIT, followed by head and neck.

The patients with lymphoma were divided into two age groups, one of less than 40 years and the other greater than or equal to 40 years. Out of total 318 patients, 125 (39.3 %) were less than 40 years and 193 (60.7 %) were more than 40 years of age. Fifty (63.3%) of the patients with Hodgkin lymphoma were less than 40 years of age; whereas, 164 (68.8%) of the patients with non-Hodgkin lymphoma were more than 40 years. The frequency of HL was significantly higher in the younger age group and the frequency of NHL was higher in the older age group ($p < 0.001$, Figure 1).

DISCUSSION

The present study is aimed at documenting the statistics of lymphomas diagnosed with complete immunohistochemical workup in Pakistani population and underscores the demographic variation in the distribution of subtypes of lymphomas in different parts of the world.

The frequency of NHL was three times greater than HL. A lower incidence of HL is reported in the West. According to the latest SEER data, HL made up 11% of all lymphomas. In the present observation, the frequency of HL was 25%, which is comparable to the high incidence as reported in studies from Pakistan[8] and India.[9] It is higher than US, China, Japan and South Korea.[10-13] Median age for HL in the study was 30 years, which is 6 - 8 years younger than the age reported in the West.[10] The median age for NHL is 50 years, and is about 16 years younger than American Cancer Society estimates for the US, which shows a median age of 66 years at diagnosis.[2] Dividing Hodgkin and non-Hodgkin lymphomas into two age groups of less than 40 and greater than or equal to 40 years, showed an increasing frequency of NHL with age; and a decreasing frequency of HL was seen with age, and the results were statistically significant. Males of all ages were more affected with the disease than the females.

MCHL was the most common HL in all age groups, with a frequency of 60.75%, followed by NSHL 34.17%, comparable to the local data,[14-16] and data from China[11] and Korea;[13] whereas, data from India[9] and the western countries[17] show that NSHL is much more frequent. A study from Pakistan has shown that MCHL is the most common type of HL in Pakistan and in most of the developing countries, and has the highest rate of EBV association; whereas, NLPHL has the lowest rate of EBV.[18] Studies have shown that childhood HL are predominantly of MCHL type, with a higher rate of EBV presence, as opposed to adolescent and adult onset HL, which are NSHL.[4] Elderly patients also show increased prevalence of MCHL type.[4] It is known that patients with LDHL and MCHL have a significantly worse prognoses compared to the patients who have NSHL, whereas patients with LPHL have the best prognosis.[19,20]

In the non-Hodgkin lymphoma, the frequency of B-cell lymphomas in this study is approximately 90% and that of T-cell lymphomas is 10%. The frequency of T-cell lymphoma is intermediate as reported in the West and Far Eastern countries, whereas the frequency of B cell lymphoma is significantly higher.[11-13,17]

ALCL was the most frequent T-cell lymphoma reported, followed by TALL and PTCL. Reports from India[9] and China[11] quote T/NK-cell lymphomas to constitute 30% of all NHL. Japan also reports a high rate of adult T-cell leukemia/lymphoma, attributed to high prevalence of human T-lymphocytic virus-1.[12] Lower rates of T-cell lymphoma are seen in Malaysia.[21] In US, mature T/NK-cell lymphoma and lymphoblastic leukemia/lymphoma account for 5% of all lymphomas.[22]

In this study, DLBCL was the commonest lymphoma, in all age categories greater than 15 years, followed by HL. The frequency of DLBCL was 69.03% of NHL, consistent with high rate in Asians[22] and analogous to an earlier study from the study center[23] and other studies from Pakistan.[24,25] The highest number of cases was seen in the age stratum 46-60 years. A local study has also reported a rate as high as 75% of NHL.[25] Data from USA shows a declining incidence of DLBCL in age group 16 - 54 years, whereas a rising incidence in the elderly population.[22]

In children, HL was the commonest, followed by lymphoblastic leukemia/lymphoma. Although the frequency of T-ALL was double that of B-ALL, but B-ALL was commoner in childhood and T-ALL was seen in all age groups. Frequency of pediatric lymphomas is variable in different parts of the world. In USA, lymphoblastic lymphoma is the leading pediatric lymphoma followed by HL.[22] Incidence of lymphoblastic leukemia/lymphoma is reportedly higher in areas with higher socio-economic development, with rates among United States whites four times higher than rates in sub-Saharan Africa. In contrast, Burkitt lymphoma/leukemia is the most common childhood cancer in tropical Africa, where incidence is related to EBV and malaria.[22] The present statistics are close to that reported from China with MCHL being commonest pediatric lymphoma followed by ALL.[11]

CLL/SLL and FL were seen in the elderly, comparable to the data in literature. Among B-cell lymphomas, the frequency of plasma cell neoplasms was significantly lower; its higher rate has been reported in Africans.[22]

Gastrointestinal tract (GIT) was the most common extra nodal site, followed by the head and neck, according to the reported local data.[16,24] In Far Eastern countries, Waldeyer's ring is the most common reported site due to high prevalence of extra nodal NK-T cell lymphoma.[10]

Some variations are noted in this study compared to international data, however, more comprehensive and descriptive studies need to be conducted to analyze the incidence and patterns of subtype distribution in this developing part of the world.

CONCLUSION

Hodgkin lymphoma comprises 25% and non-Hodgkin lymphoma comprises 75% of all lymphomas, both occurring at a younger median age than in the West. B-cell NHL is three times more common than T-cell lymphoma. DLBCL is the most frequent lymphoma. ALCL is the most common T-cell, and mixed cellularity is the most common Hodgkin lymphoma. The results highlighted may prove helpful to the future researches.

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