

Pattern of Immunophenotype Among the Cases of Lymphoma Attending in A Tertiary Level Hospital

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Abstract

Background : To explore the relative frequency and different forms of lymphoma in tertiary level hospital. **Methods:** This descriptive observational study was carried out in the Department of Hematology at Dhaka Medical College Hospital, Dhaka. Patients attended with solid tissue lymphoma in Outpatient, Inpatient and Lymphoma Clinic services of Department of Hematology and Bone Marrow Transplant, Dhaka Medical College Hospital, Dhaka were taken as study population as per inclusion criteria. A total of 63 patients with lymphoma diagnosed by histopathologically were selected initially, among them 53 were confirmed by immunohistochemistry taken as study population finally. **Results:** Mean age was 39.2 ± 15.5 years, median age was 36 years within the range of 14 – 75 years. Males were predominant. Male female ratio was 4.3:1. Most of the samples were collected from cervical lymph node (84.1%). Most of the patients came with fatigue and significant weight loss. Maximum 42 (79.24%) cases were Non-Hodgkin's lymphoma and 11 (20.75%) cases were Hodgkin's lymphoma. Out of 42 non-Hodgkin's lymphoma, 27 (64.3%) were B-cell lymphoma and 15 (35.7%) were T-cell lymphoma. Among B-cell lymphoma, 19 (45.2%) were diffuse large B cell lymphoma, three (7.1%) were follicular lymphoma, three (7.1%) were mantle cell lymphoma, one (2.4%) was splenic marginal zone lymphoma and one (2.4%) was Burkitt lymphoma. Among T-cell lymphoma, nine (21.4%) were peripheral T-cell lymphoma and six (14.3%) were adult T lymphoblastic lymphoma. Out of 11 Hodgkin's lymphoma, 10 (90.9%) were classical Hodgkin's lymphoma and one (9.1%) nodular lymphocyte predominant. Among classical Hodgkin's lymphoma, five (45.5%) were mixed cellularity, three (27.3%) were lymphocyte predominant and two (18.2%) were Nodular sclerosis. Out of 42 non-Hodgkin's lymphoma, 13 (30.95%) were indolent, 21 (50.00%) were aggressive and eight (19.05%) were very aggressive. **Conclusion:** In our study, it was found that 79.3% were non-Hodgkin lymphoma of which 64.3% were B-cell lymphoma & 35.7% were T-cell lymphoma and 20.7% cases were Hodgkin lymphoma of which 90.9% were classical Hodgkin's lymphoma, 9.1% nodular lymphocyte predominant Hodgkin's lymphoma.

Key words: Immunophenotype; Immunohistochemistry; Lymphoma; Hodgkin's.

INTRODUCTION

In 1832, history of lymphoma begins with the description of Hodgkin's disease by Thomas Hodgkin and Dorothy Reed identify Reed-Sternberg cells¹. However Lymphomas are clonal hematopoietic lymphoid neoplasms of solid tissue naive Lymphoid cells. According to WHO Classification of Tumors of Haematopoietic and Lymphoid Tissue, Lymphoid Neoplasms are defined as B cell and T/NK cell neoplasms are clonal tumors of mature and immature B cells, T cells or Natural Killer (NK) cells at various stages of differentiation. Because NK cells are closely related, and share some immunophenotypic and functional properties with T cells, these two classes of neoplasms considered together².

On the basis of morphology and presence of Reed-Sternberg cells, obtained from histopathology of tissues sections; Lymphoma historically classified as Hodgkin and non-Hodgkin Lymphoma. Development of monoclonal antibodies and flow cytometry provides further evaluation of lymphoid neoplasms. Now lymphoid neoplasms can be further classified by the origin of cells like B cell or T/NK cells. Morphology and immunophenotype are sufficient to diagnose cases of lymphomas. Based on this principle with genetics, presentation, prognosis and non-overlapping entities WHO classify lymphoid neoplasm within Precursor Lymphoid Neoplasms (Which is further sub classified into B lymphoblastic leukaemia/ lymphoma and T lymphoblastic leukaemia/ lymphoma) Mature B-cell neoplasms, Mature T-cell neoplasms and Hodgkin Lymphoma in broad heading (Jaffe et al. 2008). This classification provides adequate information about the origin, presentation, aggressiveness and also about treatment outcome. Usually solid tissue lymphoma includes mature B-cell lymphomas, mature T-cell lymphomas and Hodgkin lymphoma².

The individual B- cell neoplasms vary in their relative frequency in different parts of the world. Mature B-cell neoplasms comprises over 90% of non-Hodgkin's lymphoid neoplasms worldwide. They represents approximately 4% of new cancers each year. They are more common in developed countries like US, Australia, New Zealand and Western Europe. The most recent survey from the Surveillance, Epidemiology and End results (SEER) program in United states indicated an incidence rate per 1, 00,000 persons per year of 33.65 for all lymphoid neoplasms, 26.13 for B-cell neoplasms, 1.79 for all T-cell neoplasms and 2.67 for Hodgkin lymphoma. Burkitt lymphoma is endemic in equatorial Africa, which is the most common in childhood².

Median age of all types of mature B-cell neoplasms is in 6th to 7th decades but mediastinal large B-cell lymphoma has median age around 35 years except Burkitt lymphoma and DLBCL which has significant frequency in childhood. Most of the lymphomas (52-55%) have male predominance, but mantle cell lymphoma has 74 % male predominance. Female predominance observed in follicular lymphoma (58%) and in primary mediastinal large B-cell lymphoma (66%). Primary mediastinal large B-cell lymphoma and nodular sclerosis type classical Hodgkin lymphoma commonly affect adolescent and young adult females².

Major risk factors observed in mature B-cell neoplasia is any type of abnormality of the immune system, either immunodeficiency of any cause or autoimmune disease. Major forms of immunodeficiency include Human Immunodeficiency Virus infection, iatrogenic immunosuppression or graft versus host disease and primary immunodeficiency which particularly observed in Burkitt lymphoma and DLBCL. B-cell lymphomas were observed in Hashimoto thyroiditis. Mature T-cell and NK-cell neoplasms are relatively uncommon and have potential geographical variation and racial population. Mature T-cell and

NK-cell are common in Asia eg. HTLV-1 endemic region of Japan, nasal NK/T-cell lymphoma common in Hong Kong².

Infectious agents have etiological contribution in development of lymphomas. Epstein-Barr Virus evaluated in nearly 100% of endemic Burkitt lymphomas and in several other cases of lymphomas. Human herpes virus-8 found in primary effusion lymphomas associated with HIV infection. HTLV-1 found in adult T-cell lymphoma/leukaemia. Evidences of presence of Hepatitis C virus observed in type- II cryoglobulinaemia, splenic marginal zone lymphoma, nodal marginal zone lymphoma and in DLBCL².

Among them morphological findings by Histopathology and Immunophenotyping by Immunohistochemistry or flow cytometry are sufficient to diagnose and classify according to WHO classification.

However, Immunophenotyping and Molecular diagnosis are relatively costly and requires special equipment. Immunohistochemistry represents the most effective method for Immunophenotyping lymphocytes on fixed paraffin-embedded material³. It denotes Immunohistochemical staining, the use of specific antibody probes on tissue sections or smears of blood and bone marrow (Kristi and Sherrie, 2014). Among the Immunophenotyping procedures Immunohistochemistry is more specific for solid tissue lymphoma like Mature B-cell lymphoma, Mature T-cell and NK-cell lymphoma and Hodgkin lymphoma. It allows the visualization of an antigen by means of primary monoclonal or polyclonal antibodies and a detection system. Monoclonal primary antibodies specific for the same antigens are assigned cluster differentiation numbers at international leukocyte typing workshops³.

MATERIALS AND METHODS

This study was designed as descriptive type of observational study. This study was carried out in the Department of Hematology at Dhaka Medical College Hospital and was conducted from April 2015 to October 2015 for a period of six (06) months. Patients attended with solid tissue lymphoma in Outpatient, Inpatient and Lymphoma Clinic services of Department of Hematology and Bone Marrow Transplant, Dhaka Medical College Hospital, Dhaka were taken as study population as per inclusion criteria. Inclusion criteria were patients with lymphadenopathy histopathologically diagnosed as lymphoma and exclusion criteria were patients with lymphadenopathy diagnosed as acute leukaemia by complete blood count with peripheral blood film and bone marrow study, patients with lymphadenopathy diagnosed as tuberculosis or other chronic diseases, patients with lymphadenopathy diagnosed as metastatic carcinoma of other malignancy, patients with reactive or any type of infective disorder eg. viral, bacterial, fungal or paralytic lymphadenopathy and patients refusing to do excision biopsy. A total number of 63 patients with lymphoma by histopathologically were selected initially, of them 53 were confirmed by immunohistochemistry taken as study population. Patients were selected by purposive sampling

method. Data capturing master sheet was maintained throughout. At enrollment patients demographic and baseline characteristics were recorded. Sixty cases of lymphoma were included using convenient sampling. Provisional diagnosis was made on the basis of clinical features and other standard tests. Before excision biopsy for histopathological and immunohistochemistry, in every particular case was investigated with complete blood count and peripheral blood film. If suspected as acute leukaemia or other diagnosis mentioned in exclusion criteria bone marrow study was performed for confirmation. Blocks of tissue sample obtained from tissue excision were only sent immunohistochemistry in case of histopathological diagnosis of lymphoma.

RESULTS

This prospective observational study was conducted in the Department of Hematology at Dhaka Medical College Hospital, Dhaka from April 2015 to October 2015 for a period of six (06) months. Patients attended with solid tissue lymphoma in Outpatient, Inpatient and Lymphoma Clinic services of Department of Hematology and Bone Marrow Transplant, Dhaka Medical College Hospital, Dhaka were taken as study population as per inclusion criteria. The results are as follows:

Table 1 : Distribution of patients according to age (n=53)

Age	Frequency	Percentage
≤20	6	11.3
21 - 30	13	24.5
31 - 40	11	20.8
41 - 50	11	20.8
>50	12	22.6
Total	53	100.0
Mean ± SD	39.2 ± 15.5	
Median	36	
Range (Min – Max)	14 - 75	

Table 2 : Patients pattern according to gender (n=53)

Gender	Frequency	Percentage
Male	43	81.13
Female	10	19.87
Total	53	100.0

Table 2 Male were predominant than female. Male female ratio is 4.3:1

Table 3 : Stratification according to site of sample collection for pathological evaluation (n=63)

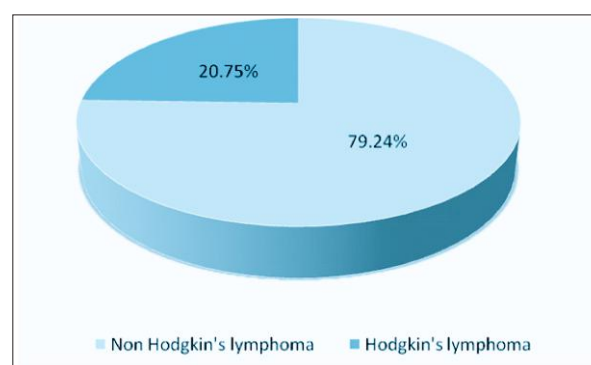
Site	Frequency	Percentage
Cervical lymph node	53	84.1
Neck mass	1	1.6
Chest wall tissue	1	1.6
Rectal tissue	1	1.6
Para pharyngeal tumor	1	1.6
Soft tissue	2	3.2
Tissue from right eye	1	1.6
Inguinal swelling	1	1.6
Nasopharyngeal growth	1	1.6
Iliac group	1	1.6
Total	63	100.0

Table 4 : Distribution of patients according to clinical features (n=53)

Clinical features	Frequency	Percentage
B symptoms	37	69.8

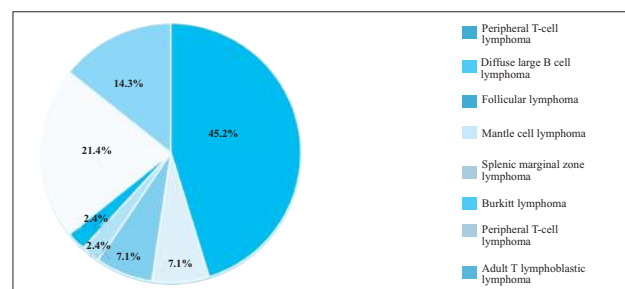
Table 4 Most of the patients reported with fatigue and significant weight loss. Fever and night sweat were also seen but patients could not described briefly.

Figure 1 : Pie chart shows distribution of patients according to combined immunophenotypically and histopathologically confirmed lymphoma



Maximum 42 (79.24%) cases were non-Hodgkin's lymphoma and 11 (20.75%) Hodgkin's lymphoma.

Figure 2 : Pie chart shows Immunophenotypic pattern of non-Hodgkin's lymphoma (n=42)

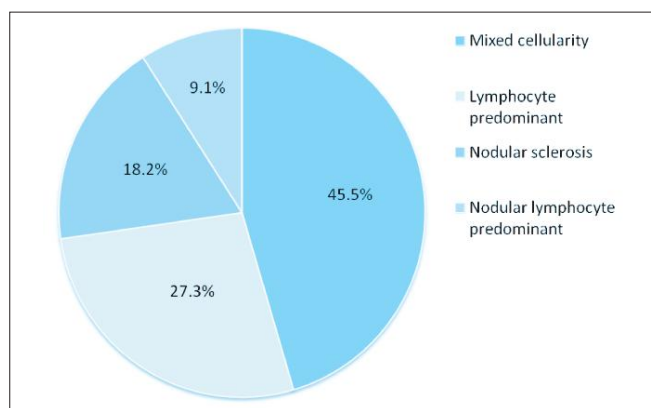


Out of 42 non-Hodgkin's lymphoma, 15 (35.7%) were T-cell lymphoma and 27 (64.3%) were B-cell lymphoma. Among B-cell lymphoma, 19 (45.2%) were diffuse large B cell lymphoma, 3 (7.1%) were follicular lymphoma, 3 (7.1%) were mantle cell lymphoma, 1 (2.4%) was splenic marginal zone lymphoma and 1 (2.4%) was Burkitt lymphoma. Among T-cell lymphoma, 9 (21.4%) were peripheral T-cell lymphoma and 6 (14.3%) were adult T lymphoblastic lymphoma.

Table 5 : Distribution of patients according to aggressiveness in non-Hodgkin's lymphoma by proliferation rate index

Ki-67	Frequency	Percentage
Indolent (<35.0%)	13	30.95
Aggressive (35.0% – 85.0%)	21	50.00
Very aggressive (>85.0%)	8	19.05
Total	42	100.0

Figure 3: Pie chart shows immunophenotypic pattern of Hodgkin's lymphoma



Out of 11 Hodgkin's lymphoma, 10 (90.9%) were classical Hodgkin's lymphoma and one (9.1%) nodular lymphocyte predominant. Out of 10 classical Hodgkin's lymphoma, five (45.5%) were mixed cellularity, three (27.3%) were lymphocyte predominant and two (18.2%) were Nodular sclerositis.

DISCUSSION

The unique feature of lymphomas is the fact that these are considered as clonal proliferation of lymphocytes arrested at different stages of differentiation, thereby recapitulating stages of normal lymphocyte differentiation. Immunohistochemistry (IHC) with various antibodies identifies the specific lineage and developmental stage of the lymphoma. This prospective study was carried out on 53 patients in the Department of Hematology, Dhaka Medical College Hospital, Dhaka where Male were predominant than female. Male female ratio is 4.3:1. Mean age of the patients was 39.2 ± 15.5 years, median was 36 years within the range of 14 – 75 years. In comparison, in the UK between 2010 and 2012, an average of 10% of cases were diagnosed Hodgkin's lymphoma in men and women aged 75 years and over, and more than a fifth (22%) were diagnosed in children, teenagers and young adults aged 24 and under. Incidence rates are higher for males than for females in many age groups, with the male female incidence ratio of age-specific rates varying between 14:10 and 22:10⁴. Male has significant predominance in lymphoma than female⁵. Non-Hodgkin lymphoma incidence among males is significantly higher than in females⁶. In this study male was more comparing other studies mentioned above. This may be due to small sample size. Moreover we give more preference to male healthcare.

In this study, most of the samples were collected from cervical lymph node (81.1%). Apart from this, two samples were collected from soft tissue of back and one sample collected from each of the following sites: neck mass, chest wall tissue, rectal tissue, para pharyngeal tumour, tissue from right eye, inguinal swelling, nasopharyngeal growth and Iliac group.

Regarding Immunohistochemistry pattern of lymphoma, maximum 42 (79.24%) cases were non-Hodgkin's lymphoma and 11 (20.75%) cases were Hodgkin's lymphoma. About 90% of lymphomas are the non-Hodgkin's type while about 10% are

Hodgkin's (Shankland et al 2012). Out of 42 non-Hodgkin's lymphoma confirmed by histopathology and Immunohistochemistry, 27 (64.3%) were B-cell lymphoma and 15 (35.7%) were T-cell lymphoma. Among B-cell lymphoma, 19 (45.2%) were diffuse large B cell lymphoma, three (7.1%) were follicular lymphoma, three (7.1%) were mantle cell lymphoma, one (2.4%) was splenic marginal zone lymphoma and one (2.4%) was Burkitt lymphoma. In comparison, In UK, 25.0% follicular lymphoma, 5-10% Mantle cell lymphoma, 12.0% marginal zone lymphoma, 6.0% small lymphocytic lymphoma, 2.0% lymphoplasmacytic lymphomas and 3.0% Burkitt's lymphoma (Cancer Research UK, 2015a). As per WHO, diffuse large B-cell is 37.0%, Follicular is 29.0%, Mantle cell lymphoma is 7.0% and Burkitt is 0.8%². Diffuse large B-cell is more common in our study as well as WHO and UK study and also B-cell lymphoma has higher incidence than T cell lymphoma.

Among T-cell lymphoma, nine (21.4%) were peripheral T-cell lymphoma and six (14.3%) were adult T lymphoblastic lymphoma. In UK, among T cell lymphoma 6.0% were peripheral T cell lymphoma. (Cancer Research UK, 2015b). According to WHO, peripheral T-cell lymphoma was 25.9%, adult T lymphoblastic leukemia was 9.6% (Jaffe et al. 2008). Result of this study is consistent with stratification of WHO.

This study was a single centered study, moreover sample size was small. But the information of Cancer research UK was nationwide. If we had data from different region of Bangladesh, our result could be differ from present result.

Out of 11 Hodgkin's lymphoma confirmed by histopathology and Immunohistochemistry, 10 (90.9%) were classical Hodgkin's lymphoma and 1 (9.1%) nodular lymphocyte predominant. Out of 10 classical Hodgkin's lymphoma, 5 (45.5%) were mixed cellularity, 3 (27.3%) were lymphocyte rich and 2 (18.2%) were Nodular sclerositis. In comparison, 5.0% were nodular lymphocyte and 95.0% classical types. Among classical, 60.0% nodular sclerositis, 15.0% mixed cellularity and 20.0% lymphocyte predominant in UK (Cancer Research UK, 2015). This study was a single centered study, moreover sample size was small. But the information of Cancer research UK was nationwide. If we had data from different region of Bangladesh, our result could be differ from present result.

Aggressiveness of non Hodgkin's lymphoma was defined by proliferative index Ki 67 activity, out of 42 cases of non-Hodgkin's lymphoma, 13 (45.3%) were indolent, 21 (39.6%) were aggressive and 8 (15.1%) were very aggressive. Though indolent lymphoma is more common presentation in developed country, aggressive lymphoma is more common in this study because peoples of this country are not health conscious, no screening test is performed until they become disabled.

In our study, it was found that out of 53 lymphoid neoplasms 42 (79.3%)were non-Hodgkin lymphoma of which 27 (64.3%) were B-cell lymphoma, 15 (35.7%) were T-cell lymphoma and 11(20.7%) cases were Hodgkin lymphoma of which 90.9% were classical Hodgkin's lymphoma, 9.1% nodular lymphocyte predominant Hodgkin's lymphoma.

CONCLUSION

In our study it was found that no single investigation is sufficient enough to reach a pin point diagnosis but immunohistochemistry is more preferable to stratify and diagnosis of lymphoma. However it can guide the clinicians to select the treatment modality and follow up the cases of lymphoma. Immunohistochemistry is not widely used in our country but where it is available it should be performed for the betterment of patient.

DISCLOSURE

All the authors declared no competing interest.

REFERENCES

1. Macon WR, Kurtin PJ, Dogan A. Diagnosis and Classification of Lymphomas. In: Greer JP, List AF, Rodgers GM, Arber DA, Means RT (Eds) Wintrobe's Clinical Hematology 13th edn. China, LIPPINCOTT WILLIAMS & WILKINS.2014.
2. Jaffe ES, Harris NL, Stein H, Campo E, Pileri SA, Swerdlow SH. Introduction and overview of the classification of the lymphoid neoplasm. In: Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H et al. editors. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues, 4th edition. Lyon: International Agency for Research on Cancer. 2008; 11-166.
3. Jaffe ES, Harris NL, Stein H, Vardiman JW, eds. World Health Organization Classification of Tumours: Pathology and Genetics of Tumours of Haematopoietic and Lymphoid Tissues. Lyon, France: IARC Press. 2001.
4. Cancer Research UK. 'Different types of non Hodgkin lymphoma' Available at: <http://www.cancerresearchuk.org/about-cancer/type/hodgkins-lymphoma/about/types-of-hodgkins-lymphoma> (Accessed February 2, 2016). 2015.
5. Smith A, Crouch S, Lax S, Li J, Painter D, Howell D, et al. Lymphoma incidence, survival and prevalence 2004–2014: sub-type analyses from the UK's Haematological Malignancy Research Network. *British Journal of Cancer*. 2015;112:1575-1584.
6. Horesh N and Horowitz NA Does Gender Matter in Non-Hodgkin Lymphoma? Differences in Epidemiology, Clinical Behavior, and Therapy. *Rambam Maimonides Med J*. 2014;5(4): e0038.