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Research paper

# Identification of the spectrum of lymphomas, specific subtypes, and the limitations in the diagnosis; a descriptive study at two tertiary care centres in Sri Lanka

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**Abstract**

**Background:** According to the National Cancer Registry of 2019, lymphoma was the eighth commonest malignancy in males and the tenth commonest malignancy in females in Sri Lanka. There is only one previously published study on the distribution of lymphoma subtypes in the Sri Lankan population.

**Objectives:** The objectives of this study were to describe the morphological spectrum and the pattern distribution of subtypes of lymphomas in a cohort of Sri Lankan patients according to the latest World Health Organization (WHO) classification 2016, and to identify the limitations encountered in the process of laboratory diagnosis.

**Method:** This was a descriptive, cross-sectional study carried out in Departments of Histopathology of the National Hospital of Sri Lanka, Faculty of Medicine, University of Colombo and Apeksha Hospital, Maharagama.

**Results:** Four hundred and forty-four cases of lymphomas were analysed and studied. The majority of patients were in the seventh decade (21.8%,97) and the mean age at diagnosis was 49 (+/-19) years. There was a slight male preponderance (57.7%,256). Non-Hodgkin lymphomas comprised 76.6% (340), the commonest subtype being diffuse large B-cell lymphoma (39.1%,174). The commonest type of Hodgkin lymphoma was the nodular sclerosis type (13.7%, 61). Complete subtyping of 45.4% (202/444) of lymphomas, including diffuse large B-cell lymphoma, mantle cell lymphoma, high-grade lymphoma, (NOS), follicular lymphoma and classic Hodgkin lymphoma, were compromised due to limited availability of immunohistochemistry and molecular markers.

**Conclusions:** Although there were no major discrepancies in the diagnosis, the lack of immunohistochemistry markers and molecular and genetic studies led to diagnostic difficulties in some cases, and possibly underdiagnosis of certain lymphoma subtypes. Therefore, to maintain diagnostic accuracy, immunohistochemistry markers and molecular markers should be more widely and readily available in the government sector reference laboratories.

**Keywords:** lymphoma, morphological spectrum, cross-sectional study

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**Introduction**

Lymphomas are neoplasms arising from the lymphoid tissue. They are divided into two main groups: Hodgkin lymphoma (HL) and non-Hodgkin lymphoma (NHL) (1). The overall incidence of lymphomas has been increasing around the world at a rate of 3-4% over the last few decades (2). The aetiology and biology of lymphomas are influenced by genetic susceptibility, immune status, ethnicity, viruses, environmental and geographic factors (3,4).

According to the latest World Health Organization (WHO) classification, there are more than 60 subtypes of lymphomas (5). As the treatment protocols vary, subtyping is an essential part of the diagnosis, which depends on the morphological appearance, immunophenotype and molecular genetics of a lymphoma (1).

The National Cancer Registry of 2019 reports lymphoma as the eighth and tenth leading malignancy among Sri Lankan men and women, respectively (6). Although there are many published studies from other countries, only one previous study was published in Sri Lanka on lymphoma patterns, which was carried out in a limited cohort of patients in Kandy in 2014. A study with a large cohort representative of the Sri Lankan population and comparison of this data with the global pattern is useful to plan management strategies at the national level.

The primary objective of this study was to describe the morphological spectrum and the pattern distribution of subtypes of lymphomas in a Sri Lankan setting.

The secondary objectives were to compare the pattern of lymphoma subtypes with the data published in other countries, and to identify the limiting factors encountered in the accurate typing of lymphomas in the local setting,

**Methods**

This was a descriptive, cross-sectional study carried out at Departments of Histopathology of the National Hospital of Sri Lanka, Faculty of Medicine, University of Colombo and Apeksha Hospital, Maharagama, which provide histopathology diagnostic services to two tertiary care centres: the National Hospital of Sri Lanka and the Apeksha Hospital, Maharagama.

According to the method of Lwanga and Lemeshow, a minimum of 248 cases were required for this study (7). A total of 572 cases, including both nodal and extranodal lymphomas, were diagnosed at the three centres during the study period. The cases where slides were not available for review were excluded from the study. The cases with faded haematoxylin and eosin-stained slides were re-stained. If the immunohistochemistry slides were faded, the stains were not repeated due to the high cost, and the immunohistochemistry results given in the initial report were considered. The cases with no conclusive diagnosis were reviewed, and if the diagnosis was still inconclusive, they were excluded from the study. Duplicate cases that were reported in more than one centre were considered as a single case. Leukaemia and plasmacytoma/multiple myeloma were excluded. Based on the above exclusion criteria, 128 cases were excluded, and 444 cases were included in the study.

The original haematoxylin and eosin-stained slides and the immunohistochemistry slides of the selected cases were re-evaluated. The confirmation of the subtype of lymphoma was made according to the revised WHO classification by double-reporting using a conference microscope. The clinical details were collected from the records. The data was analysed using SPSS statistics software version 22 (IBM, USA).

**Results**

The total number of cases reported per year was 98, 125 and 221 in 2014, 2015 and 2016, respectively. Of the 444 lymphoma cases, 57.7% (n=256) were males and 42.3% (n=188) were females. The ages ranged from seven months to 92 years. Most cases were diagnosed between 61-70 years (21.8%, 97), followed by 51-60 years (18.7%, 83). NHLs comprised 76.6% (n=340) of all cases and 23.4% (n=104) were HLs. NHL was commoner in all age groups, except 11-20 years and 21-30 years, where HL was more frequent. DLBCL, NOS was the commonest type, accounting for 39.2% (174/444) of cases in the study population. Nodal lymphomas were present in 70.5% (n=313) of cases, while 29.5% (n=131) were extranodal lymphomas. The distribution of lymphomas in different age groups is shown in Table 1.

**Table 1.** Distribution of lymphomas in different age groups

| Age group (years) | Frequency (%) | Frequency of HL (%) | Frequency of NHL (%) |
|---|---|---|---|
| 0-10 | 09 (2.0) | 1 (1.0) | 8 (2.4) |
| 11-20 | 33 (7.4) | 19 (18.2) | 14 (4.1) |
| 21-30 | 48 (10.8) | 30 (28.8) | 18 (5.3) |
| 31-40 | 53 (11.9) | 15 (14.4) | 38 (11.2) |
| 41-50 | 66 (14.9) | 16 (15.4) | 50 (14.7) |
| 51-60 | 83 (18.7) | 9 (8.7) | 74 (21.8) |
| 61-70 | 97 (21.8) | 11(10.6) | 86 (25.3) |
| 71-80 | 48 (10.8) | 3 (2.9) | 45 (13.2) |
| 81-90 | 05 (1.1) | 0 (0) | 05 (1.5) |
| 91-100 | 02 (0.4) | 0 (0) | 02 (0.5) |
| Total | 444 | 104 | 340 |

*NHL subtypes*

The distribution of NHL subtypes in the study population is shown in Table 2. Most cases of NHLs were of B-cell type (82.4%, 280/340), while T-cell NHLs were present in 15.9% (54/340) patients and 1.8% (6/340) were lymphomas of other lineage.

**Table 2.** Distribution of NHL subtypes in the study population

| Type of NHL | Frequency (%) (n=340) | Percentage (%) of all lymphomas (n=444) |
|---|---|---|
| CLL/SLL | 04 (1.2) | 0.9 |
| SMZL | 02 (0.6) | 0.5 |
| LPL | 05 (1.5) | 1.1 |
| MALTL | 04 (1.2) | 0.9 |
| NMZL | 04 (1.2) | 0.9 |
| FL | 45 (13.2) | 10.1 |
| MCL | 11(3.2) | 2.5 |
| DLBCL, (NOS) | 174 (51.2) | 39.2 |
| DLBCL and FL | 01 (0.3) | 0.2 |
| THRBCL | 09 (2.6) | 2.0 |
| PMLBCL | 02 (0.6) | 0.5 |
| PBL | 06 (1.8) | 1.4 |
| BL | 05 (1.5) | 1.1 |
| LGBCL | 01 (0.3) | 0.2 |
| DEL | 01 (0.3) | 0.2 |
| ENNK/TCL-NT | 01 (0.3) | 0.2 |
| MF | 15 (4.4) | 3.4 |
| PCCD30+LPD | 01 (0.3) | 0.2 |
| PCALCL | 03 (0.9) | 0.7 |
| PTCL, (NOS) | 10 (2.9) | 2.3 |
| AIBTCL | 01(0.3) | 0.2 |
| ALCL - ALK+ | 05 (1.5) | 1.1 |
| ALCL - ALK- | 01(0.3) | 0.2 |
| TCL | 02 (0.6) | 0.5 |
| B-LBL | 05 (1.5) | 1.1 |
| T-LBL | 17 (5.0) | 3.8 |
| MS | 04 (1.2) | 0.9 |
| HS | 01 (0.3) | 0.2 |

CLL/: Chronic lymphocytic leukaemia/small lymphocytic lymphoma, SMZL: Splenic marginal zone lymphoma, LPL:Lymphoplasmacytic lymphoma, MALTL:MALT lymphoma, NMZL: Nodal marginal zone lymphoma, FL: Follicular lymphoma, MCL: Mantle cell lymphoma, DLBCL: Diffuse large B-cell lymphoma, THRBCL: T-cell histiocytes rich large B-cell lymphoma, PMLBCL: Primary mediastinal large B-cell lymphoma, PBL: Plasmablastic lymphoma, BL: Burkitt lymphoma, LGBCL: Low-grade B-cell lymphoma, DEL: Double expressor lymphoma, ENNK/TCL-NT: Extranodal NK/T-cell lymphoma - nasal type, MF: Mycosis fungoides, PCCD30+LPD: Primary cutaneous CD30 positive lymphoproliferative disorders , PCALCL: Primary cutaneous anaplastic large cell lymphoma, SLL PTCL, (NOS):Peripheral T-cell lymphoma, (NOS), AIBTCL: Angioimmunoblastic T-cell lymphoma, ALCL-ALK+: Anaplastic large cell lymphoma - ALK positive , ALCL-ALK-: Anaplastic large cell lymphoma - ALK negative , TCL: T-cell lymphoma, B-LBL: B-lymphoblastic leukaemia/ lymphoma, T-LBL: T-lymphoblastic lymphoma, MS: Myeloid sarcoma , HS: Histiocytic sarcoma

Diffuse large B-cell lymphoma (DLBCL) was the commonest sub type (51.2%,174/340), followed by follicular lymphoma (FL) (13.2%,45/340).

Among the large B-cell lymphomas, the second commonest subtype observed in the sample was T-cell histiocyte rich B-cell lymphoma, which was seen in 2.6% (9/340). Mantle cell lymphoma (MCL) was present in 3.2% (11/340).

CLL/SLL was diagnosed in 1.2% (4/340) of patients in our cohort. The usual morphological appearance was observed with proliferation centres. Nodal and extranodal MZLs were present in 2.4% (8/340).

Mycosis fungoides (MF) was the commonest T-cell lymphoma in the study population (4.4%, 15/340) followed by peripheral T-cell lymphoma (PTCL) (2.9%, 10/340) and anaplastic large cell lymphoma with ALK positivity (1.5%, 5/340) with its usual morphological appearances. Among the blastic lymphomas, the T-cell subtype was seen more frequently (5%, 17/340) than the B-cell subtype (1.5%, 5/340).

*HL subtypes*

The majority of HLs were classic HLs (91.3%, 95/104); 58.6% (61/104) were nodular sclerosis HLs (NSCHL), 30.8% (32/104) were mixed-cellularity classic HL and 1.9% (2/104) were lymphocyte rich classic HL. There were no cases of lymphocyte depleted classic HL. Only 8.7% (9/104) had nodular lymphocyte predominant HL (NLPHL).

*Extranodal lymphomas*

The commonest lymphoma subtype found in extranodal sites was DLBCL, (NOS). The skin, gastrointestinal tract and central nervous system were the commonest sites involved. Extranodal lymphomas were also diagnosed in tonsils, spleen, bones, salivary glands, thyroid gland, breast, ovary, retro-orbital tissue, testis and retroperitoneum.

Table 3 depicts the main lymphoma subtypes identified in this study and compares their pattern of distribution with that of different geographic areas in the world.

*Limitations in the laboratory diagnosis of lymphomas*

Table 4 highlights some of the important immunohistochemistry markers and molecular markers which were limited or not available in the study setting but should be available for accurate diagnosis with subtyping.

**Discussion**

The number of patients with the diagnosis of lymphoma increased from 2014 to 2016. The incidence of HL is consistently lower than that of NHL throughout the world. Of the studies reviewed, the highest proportion of HL were found in Pakistan, followed by Sri Lanka (22). NHL was more common than HL in most age groups in this study, except in the 11-20 and 21-30 year age groups. There was a male preponderance for both HLs and NHLs in the study population. The incidence of NHL is higher in men than in women worldwide with an age standardized rate of 6.1 for men and 4.2 for females (11). However, studies carried out so far have not found any statistically significant survival difference between males and females (12). NHL is found more commonly in older people, and advancing age is a strong risk factor (11). In this study, NHL was most commonly seen in the seventh decade.

*NHL B-cell type*

DLBCL was the commonest subtype of lymphoma involving both nodal and extranodal sites in this study. It is the commonest subtype of lymphoma diagnosed

**Table 3.** The pattern of distribution of subtypes of lymphoma in different countries

| Country | | SL | In (21) | Pk (22) | Sg (20) | Jp (16) | Kor (09) | Chi (19) | UK (12) | Aus (18) | USA (17) | SA (23) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Study population size (n)** | | 444 | 2773 | 308 | 7131 | 2260 | 5318 | 6382 | 5796 | 128295 | 112380 | 487 |
| **Lymphoma sub type (% of study population)** | **CLL/SLL** | 0.9 | 4.8 | 1.6 | 4.5 | 1.4 | 1.8 | 4 | N/A | 16.1 | 14.8 | 8.4 |
| | **MZL** | 1.8 | 6.8 | 1.6 | 5.4 | 5.6 | 13.5 | 5.6 | 17 | 1.8 | 5.2 | 2.5 |
| | **FL** | 10.1 | 10.9 | 3.7 | 8.5 | 18.3 | 1.7 | 5.1 | 15.9 | 11.6 | 9.8 | 18.1 |
| | **MCL** | 2.5 | 2.9 | 1.6 | 1.7 | 2.7 | 1.8 | 2.7 | 4.3 | 1.8 | 2.3 | 1.8 |
| | **DLBCL** | 39.1 | 29.1 | 48.4 | 30.3 | 33 | 31 | 35.9 | 40.9 | 18.05 | 20.05 | 38.2 |
| | **BL** | 1.1 | 1.6 | N/A | 1.9 | 0.7 | N/A | N/A | 1.8 | N/A | 1.1 | 1.6 |
| | **NK/T CL** | 0.2 | 0.6 | 0 | 1.4 | 1.6 | 3.8 | 14.9 | N/A | N/A | N/A | 0.4 |
| | **MF** | 3.4 | 0.7 | 2.1 | 2.3 | 0.5 | 0.4 | 0.2 | 0.7 | 1.03 | 1.1 | 0.4 |
| | **CD30 LPD** | 0.2 | N/A | N/A | N/A | N/A | N/A | N/A | 0.6 | N/A | 0.2 | N/A |
| | **PTCL(NOS)** | 2.3 | 1.6 | 4.1 | 2.4 | 4.5 | 4 | 3.5 | N/A | 1.03 | 1.1 | 9.7 |
| | **AITL** | 0.2 | 0.9 | 0 | 1.3 | 5.1 | 0.8 | 2.9 | 0.9 | N/A | 0.2 | N/A |
| | **ALCL** | 1.1 | 3.5 | 1.6 | 0.9 | 2 | 1.4 | 3.1 | 0.8 | N/A | 0.5 | N/A |
| | **HL** | 23.4 | 14 | 26.7 | 6.9 | 7.3 | 4.3 | 13 | 14.4 | 6.5 | 7.8 | N/A? |
| | **CHL** | 21.3 | 12.3 | 26.7 | N/A | 7 | 4.1 | 12.5 | 12.7 | 6.7 | 7.8 | N/A |
| | **NSCHL** | 13.7 | 3.6 | 8.9 | 4.2 | 3.1 | 2.3 | 2.3 | 9.4 | 3.9 | 4.06 | N/A |
| | **MC-CHL** | 6.5 | 4.4 | 17.9 | 1.2 | 2.3 | 1.1 | 9.5 | 3 | 1.1 | 0.8 | N/A |
| | **LR-CHL** | 0.5 | N/A | N/A | 0.1 | N/A | N/A | N/A | 0.3 | N/A | 0.2 | N/A |
| | **NLPHL** | 2 | 1.7 | 0 | 0.4 | 0.3 | 0.2 | 0.5 | 1.7 | 0.2 | 0.5 | N/A |

HL: Hodgkin lymphoma, DLBCL: diffuse large B-cell lymphoma, MZL: marginal zone lymphoma, FL: follicular lymphoma, MCL: mantle cell lymphoma, BL: Burkitt lymphoma, CHL : classic HL, NSCHL: nodular sclerosis classic HL, MC – CHL: mixed cellularity classic HL, LR – CHL: lymphocyte rich classic HL, NLPHL : nodular lymphocyte predominant Hodgkin lymphoma, AITL : angioimmunoblastic T-cell lymphoma, ALCL: anaplastic large cell lymphoma. MF : mycosis fungoides, CD30 LPD : CD30 positive lymphoproliferative disease, CLL/SLL : chronic lymphocytic leukaemia/small lymphocytic lymphoma, PTCL (NOS) : peripheral T-cell lymphoma- not otherwise specified, NK/TCL :Natural killer/T-cell lymphoma
SL: Sri Lanka, In:India,Pk:Pakistan,Sg:Singapore,Jp:Japan,Kor:Korea,Chi:China,Aus:Australia,SA:South Africa, N/A: Not available

worldwide (Table 3). DLBCL is heterogeneous in nature, both clinically and morphologically, and demonstrates common and rare morphological appearances. Most DLBCLs show a centroblastic appearance (14). More than 90% of cases of DLBCL in this study were of common morphology with centroblastic, immunoblastic and mixed-cell subtypes.

According to the Hans algorithm, DLBCL is subdivided into germinal centre B-cell (GCB) and activated B-cell (ABC) subtypes depending on gene expression profiles (4). CD10, BCL6 and IRF4/MUM1 immunohistochemistry markers are used to classify DLBCL when the molecular and genetic studies are not available (5). Studies have shown that GCB subtype has a better prognosis than ABC subtype (15). Current clinical trials have shown that patients with ABC subtype benefit from the addition of bortezomib, lenalidomide and ibrutinib to the R-CHOP regimen. Therefore, it is recommended that accurate distinction between these two subtypes should be attempted in all DLBCL, (NOS) cases (14). Of the 174 cases of DLBCL in this study, only 20% were classified as GCB and ABC subtypes. The main reasons for not sub classifying all cases of DLBCLs were the lack of necessary

**Table 4.** Analysis of cases in which the diagnosis was limited, and additional immunohistochemistry /molecular markers were required

| Type of lymphoma | IHC marker/ markers | Cases needing this marker for the diagnosis | Number of cases in which the diagnosis was limited due to the lack of the marker (% of the total number of cases) | Comments |
|---|---|---|---|---|
| To separate GCB and ABC subtypes of DLBCL | BCL6<br>CD10<br>IRF4/MUM1 | Categorization as GCB and ABC subtypes was performed only in 26 out of 174 cases of DLBCLs | 148 (33.3%) | For therapeutic benefits, this subtyping must be performed in every case of DLBCL, (NOS). Therefore, these markers should be readily available. |
| HGL, (NOS), double-hit and triple-hit lymphomas | Molecular studies | 12 cases diagnosed as DLBCL (6.8% of DLBCL) showed high-grade morphology and a high Ki-67 index | 12 (2.7%) | These were not investigated further due to the lack of molecular studies. MYC, BCL2 and BCL6 molecular studies should be available to separate these specific sub types as required treatment regimens and hence the prognosis varies |
| FL | Ki-67 | Assessed in 14 cases (31% of FLs) | 31 (7.0%) | Ki-67 index is an important indicator of prognosis. Therefore, this must be performed in every case of FL and also in all NHLs. |
| MCL | Cyclin D1 | Used in the initial panel in 6/11 cases of MCL and in the secondary panel in the remaining cases. | 5 (1.1%) | Cyclin D1 should be available to confirm the diagnosis. |
| | SOX11 | There was a difficulty in the diagnosis in one cyclin D1-negative case, in which SOX11 would have been useful. | 1 (0.2%) | SOX11 is required to confirm MCL when cyclin D1 is negative. This was required in one case in this study. |
| CHL | PAX5 | It was required to confirm the diagnosis in 5 cases of HL. | 5 (1.1%) | CHL is a curable disease. Thus, this should be available, even though it was required in only 5% of HL cases. |

CHL: Classic Hodgkin lymphoma, DLBCL: Diffuse large B-cell lymphoma, GCB : germinal centre B-cell, ABC: activated B-cell, HGL: High-grade lymphoma

immunohistochemistry markers and their high cost. It is important that these markers are made available and performed in every case of DLBCL.

Of the DLBCLs, 5.7% (10/174) showed high-grade morphology and a very high Ki-67 index. Two of these showed a starry sky appearance. Due to the unavailability of necessary molecular studies, these cases were diagnosed as DLBCL, (NOS), and could not be investigated further to rule out the possibility of double-hit or triple-hit lymphomas. High-grade B-cell lymphoma with MYC and BCL2 and/ or BCL6 rearrangements is an aggressive B-cell lymphoma (14). These patients usually do not respond to induction therapy with the R-CHOP regimen, and therefore, if not diagnosed and managed correctly, they may present with early recurrences (14). This further highlights the importance of molecular studies, as the diagnosis of some lymphoma subtypes cannot be made on immunohistochemistry alone. Although high-grade lymphoma, (NOS) or double-hit or triple-hit lymphomas are not common, their aggressive nature necessitates the definitive diagnosis and management, and due to the unavailability of molecular studies, the incidence of these lymphomas among the Sri Lankan population is not known. It is important that reference laboratories of the government hospitals have adequate facilities to support optimal diagnosis. This is an issue that must be addressed at the national level.

BL is an aggressive B-cell lymphoma (1), which has a low incidence worldwide. It is most common in equatorial African countries and is associated with EBV infection (13). Both Sri Lankan studies showed a very low prevalence of BLs (13). All five cases in our study were diagnosed using only immunohistochemistry markers, which included CD3, CD20, BCL2, CD 10, nuclear TdT, CD99, BCL6 and Ki-67. However, there may be some underdiagnosis of BLs in the local setting because CD10 and Ki-67 were not performed in all cases of high-grade B-cell lymphomas. As discussed above, molecular markers are required to distinguish between high-grade lymphoma (NOS), BL, and double-hit and triple hit lymphomas.

As a South Asian country, a considerable number of lymphomas in Sri Lanka are likely to be associated with Epstein-Barr virus (EBV) Further, plasmablastic lymphoma is aetiologically associated with EBV and needs to be differentiated from DLBCL because it has an aggressive clinical course and poor survival rate (8). However, immunohistochemistry markers for the diagnosis of EBV are not readily available in Sri Lanka.

Low-grade B-cell lymphomas (LGBCL) accounted for 17% (76/444) of all cases, the commonest being FL (10.1%, 45/444), which was the second commonest lymphoma subtype in this study. FL is more common in the Asian population, with the highest numbers reported in Japan (16) followed by South Africa (23). In the western part of the world and Australia, CLL/SLL is the commonest LGBCL followed by FL (17, 18). CLL/SLL was the third commonest lymphoma in the previous Sri Lankan study (13). However, it accounted for only 0.9% (4/444) of cases in this study. Low numbers of CLL/SLL were reported in the reviewed South Asian and East Asian countries (Table 3). The immunohistochemistry panel used in the diagnosis of LGBCL in our setting was CD3, CD20, BCL2, BCL6, CD10 and CD21/CD23, CD5, Cyclin D1 and Ki-67. However, unavailability of CD43, light chains and clonality studies were identified as a major limitation, especially in confirming MZLs.

The majority (80%, 36/45) of FLs were grade 1-2 FLs and the rest were grade 3 FLs. One patient was diagnosed as having DLBCL and grade 2 FL in the same lymph node. Generally, in FLs, grade 1-2 cases show a Ki-67 proliferation index of <20%, while grade 3 cases demonstrate an index of more than 20% (14). Some studies have highlighted a subgroup of morphologically low-grade FL with a proliferative index >20% that behave more aggressively than those having a low-

proliferative index (14). Therefore, estimation of Ki-67 proliferative index could be regarded as an adjunct to histological grading in FL. In our study population, the assessment of Ki-67 proliferative index was performed only in 35% of patients. This reiterates the cost factor and unavailability of reagents in the local setting.

MCL accounted for 2.5% (11) and was the fifth commonest lymphoma in this study. In the previous Sri Lankan study, MCL was the fourth commonest lymphoma. MCL accounted for 1.6% - 4.3% of the lymphoma cases studied worldwide (Table 3). Most MCLs in this study demonstrated the usual morphology, while two cases showed a blastic morphology. All cases were diagnosed using CD3, CD20, CD5, CD23, CD10 and BCL6. Cyclin D1 was included in the first panel in >50% (6/11) of the cases. A rare variant of MCL over expresses cyclin D2 or D3, instead of cyclin D1 (14). This variant also expresses the neural transcription factor SOX11, maintaining the classic immunophenotype seen in MCL (14). A diagnostic difficulty arose in a single case due to the unavailability of SOX11. However, this was not a major limiting factor. Cyclin D1 on the other hand is an essential marker to confirm the diagnosis. Additionally, the pleomorphic variant of MCL may be underdiagnosed in our setting because CD5 is not performed routinely on DLBCLs due to the cost factor.

When all MZL were categorised together they comprised the third commonest LGBCL (1.8%, 8/444)). Extranodal MZLs are commonly seen in East Asian countries with the highest incidence reported in Korea (9). This could be due to the high prevalence of *Helicobacter pylori*-associated gastritis, which in turn is associated with extranodal MZL/MALT lymphoma in the gastrointestinal tract (9).

*NHL T-cell type*

Peripheral T-cell lymphoma subtypes are significantly low in incidence compared to their B-cell counterparts (12). In our study population, 8.7% (37/444) were diagnosed as mature T-cell lymphoma subtypes, the commonest was MF, which accounted for 3.4% (15/444) of lymphomas in the total study population. The second commonest was the peripheral T-cell lymphoma, not otherwise specified (PTCL, NOS). In the previous Sri Lankan study, PTCL, NOS was the commonest and MF was the second commonest T-cell lymphoma. Worldwide studies highlight that PTCLs are an uncommon group of lymphoproliferative disorders that are difficult to treat and manage (12). South Asian countries report a higher prevalence of MF compared to the other countries included for comparison (16,9,19), for which the exact reasons are not apparent. Among the other T-cell lymphomas, ALK-positive ALCL was diagnosed in 1.1% (5/444) and primary cutaneous ALCL was diagnosed in 0.7% (3/444). Among the reviewed countries, ALCL was observed more commonly in Asian countries than the western countries.

There was one case of angioimmunoblastic T-cell lymphoma (AITL) in the study population. However, there were no cases in the previous Sri Lankan study. This low diagnosis rate of AITL can be partially attributed to the lack of immunohistochemistry markers (e.g.PD1) which are needed to confirm the diagnosis of AITL in the local setting. Compared to other T-cell lymphomas, it is an uncommon subtype, and Japan shows the highest incidence among the countries considered (16). There was only one case of extranodal NK/T-cell lymphoma in our study and no cases were reported in the previous Sri Lankan study. China shows the highest incidence rate which could be related to the high prevalence of EBV infection (19).

*HL*

The commonest subtype of HL in the study group was the NSCHL, which accounted for 58.6% of HLs and 13.7% of all lymphomas. The

mixed-cellularity CHL was diagnosed in 30.7% of HLs. However, this type was the commonest HL reported in the previous Sri Lankan study (13). Since Apeksha Hospital receives a large number of referrals from all over the country, referral bias might have contributed to this difference. However, further studies are needed to confirm the true incidence of HL in our population. Although the lymphocyte depleted CHL is more commonly seen in Southeast Asian countries (14), none of the patients in this study or the previous Sri Lankan study had this sub type (13).

The commonly used immunohistochemistry markers in this cohort for the diagnosis of HL were CD15, CD30, CD3, CD20 and PAX5. Although Hodgkin and Reed/Sternberg (HRS) cells are positive for CD30 in all cases, CD15 is positive only in 75-85% cases (14), indicating that CD15 negativity does not preclude the diagnosis of CHL. Studies have shown that 15-25% cases of HLs are negative for CD15, in which case PAX5 and other immunohistochemistry markers must be used for confirmation (14). PAX5 was required for the diagnosis of 5% of HLs in this study. Although this is a small proportion, this marker should be readily available to diagnose CHL, which is curable with modern therapies.

## Conclusion

This study adds to the present limited pool of studies published on lymphoma all over the world. The main strengths of our study include the large catchment area and the fact that all cases were reviewed and diagnosed using the latest WHO classification of lymphomas.

As this study was carried out in tertiary care centres, there could be a referral bias. All three pathology departments receive cases as referrals, especially the Apeksha Hospital. This might have caused some differences in the pattern of distribution of the subtypes of lymphoma. A population-based study with a larger cohort is necessary to assess the true prevalence of lymphoma subtypes in the Sri Lankan population.

There were no significant discrepancies in the diagnoses. However, there were major limitations in the diagnostic process of lymphomas due to the high cost and unavailability of certain immunohistochemistry markers and molecular markers, and therefore, subtyping of some lymphomas could not be attempted according to the WHO classification.

In order to optimize the management of patients with lymphomas, it is important to establish a referral centre equipped with state-of-the-art immunohistochemical and molecular diagnostic facilities for the accurate diagnosis of lymphoma subtypes.

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