

# Relative Frequencies and Patterns of Malignant Lymphoma in a Reference Centre in Khartoum, Sudan: A Descriptive Study Based on the WHO Classification of Lymphoid Neoplasms

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**Background:** The effective management and choice of appropriate treatment of lymphoma subtypes depend on an accurate diagnosis and differentiation, which require comprehensive haematology and pathology work.

**Methods:** A total of 134 cases of malignant lymphoma, newly diagnosed between January 2017 to January 2020, were selected. For each patient's samples, complete blood count, immunohistochemistry, and morphological evaluation were done.

**Results:** Clinical data showed that 81 patients (60.4%) were males and 53 (39.6%) females. The age range was 4 to 80 years. NHL lymphoma comprised 87.3% of cases, while HL comprised 12.7% of cases. Diffuse large B cell lymphoma was the most prevalent NHL subtype, representing 39.3% of cases. Among HL subtypes, mixed cellularity was present in 41.2% of cases. B cell lymphoma constituted 93.2% of cases. All HL patients and 74.4% of NHL patients had anaemia.

**Conclusion:** This is the first statistical report of malignant lymphoma patterns in Sudanese patients. These data suggest that malignant lymphoma in Sudanese patients is more frequent in males than females; its incidence increases with age. Further, B cell lymphoma is more common than T cell lymphoma. Diffuse large B cell lymphoma was the most frequent NHL subtype.

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## Introduction

Lymphomas comprise a heterogeneous group of clinically distinct neoplasms with varied aetiologies, outcomes, and treatment strategies [1-2]. The main types of lymphoma are non-Hodgkin lymphomas (NHL) and Hodgkin lymphomas (HL) and defined by the lymphoid cells from which the neoplasm originates, which can be either B-cell, T-cell or NK-cell neoplasms. The World Health Organization (WHO) classifies lymphomas in more than 90 subtypes, including provisional types [3]. According to reports of the International Agency for Research on Cancer (IARC), the incidence rate of lymphoma is 3.5% of all cancers, with 589,580 new patients worldwide in 2018 and 274,891 deaths from the disease [4].

Although several reports exist regarding the distribution of malignant lymphomas in different parts of the world, no studies have been previously reported on the pattern of malignant lymphoma in Sudan. In Sudan, lymphoma represents the fourth most prevalent type of cancer in adults and the second in children's neoplasms [5]. The effective management and choice of appropriate treatment of lymphoma subtypes depend on an accurate diagnosis and differentiation, which require comprehensive haematology and pathology work. Immunohistochemistry is an essential diagnostic

tool for distinguishing different lymphoma subtypes, besides histopathological evaluation of patient's samples. The presence of irregular margins in aggregated atypical lymphocytes is the main feature of malignant lymphoid cells [6]. However, indistinct cases are seen sometimes when biopsy samples are stained with routine stain only, i.e. haematoxylin and eosin (H&E), which has a significant impact on the treatment plan. Morphological evaluation, together with immunophenotyping and genetic studies, must be employed to achieve an accurate diagnosis for malignant lymphoma [7]. Immunohistochemistry is widely used for diagnosis and differentiation of malignant lymphoma [8]. The most commonly used biomarkers are CD3 and CD20 for T cell and B cell lymphoma, respectively [9]. The expression of biomarkers on lymphoid cells and subclasses of lymphoma are a dependent factor in the therapeutic intervention [10]. This study aims to investigate the relative frequency and pattern of different types of malignant lymphomas in Sudanese patients, according to the WHO classification of lymphoid neoplasms.

## Materials and Methods

The current cross-sectional descriptive study was conducted at Radioisotope Centre of Khartoum (RICK), which is the reference oncology centre in Sudan. The study subjects included 134 newly diagnosed patients with different subtypes of HL or NHL of three years duration, diagnosed between January 2017 and January 2020. Ethical approval was obtained from the institutional ethical board. Patients already on treatment were excluded from the study. The determination of ML sub-types was based on WHO criteria. About 3 ml of venous blood were collected in EDTA-containing tubes after obtaining written informed consent from each patient or their parents for those aged under 18 years to participate in this study. Personal and clinical data including age, sex, and past medical history were collected using a questionnaire. In all cases, the blood sample was used for complete blood counts using a five-part automated analyser (Sysmex XP-300™, Kobe, Japan).

## Morphological and immunohistochemical studies

From clinically suggestive lymphoma patients, consecutive excisional biopsies were obtained. The specimens related to lymph nodes (104 cases), abdominal tissues (13 cases), spleens (8 cases), soft tissues (5 cases), and head-neck tissues (4 cases). Using well fixed, processed, and embedded biopsy, multiple sections were selected and then stained with H&E, and wherever required, special staining was performed for the examination of histological pattern and infiltration. Three expert haematopathologists then reviewed all of the cases. Each expert independently reviewed all of the data available for each case. A consensus diagnosis was reached when two of the experts agreed on the diagnosis. H&E staining allowed sorting lymphomas between HL and NHL, according to the presence of different cells. Morphological diagnosis of HL cases was based on the effacement of nodal architecture by diffuse small lymphocyte cell, mononuclear, and multinucleated lymphocyte cell, prominent nucleus, a feature of Reed Sternberg cell, a feature of Hodgkin's cell, aggregated stroma with reactive histocytes and fibrosis. The included NHL features were effacement of sections by the mononuclear population of lymphocyte cells, dysplasia, increased N:C ratio, suppressing normopoiesis, and mixed cellular infiltration. DLCL presented with a diffused infiltrate of large noncleaved or transformed lymphocytes. Follicular lymphoma was characterised by effacement with mononuclear lymphocyte, and the centre of nodules were composed of pleomorphic lymphocyte with prominent nuclei as well as crowded, back-to-back neoplastic follicles, lack of zonation and tingible body macrophages, numerous cleaved cells, and loss of the mantle zones. BL sections presented with focal and effaced mononuclear cells, prominent nuclei and suppressing normal hemopoiesis. Malt lymphoma sections showed multiple fragments of gastric mucosa infiltrated by a mononuclear population of enterocyte-like lymphocyte destructive gastric gland (lymph epithelial lesion) adjusted showing intestinal metaplasia. T cell lymphoma presented with fragment mesenteric lymph node showing the suggestive feature of hyperplasia, no malignancy was observed in the section but extensive necrosis was found, visible cells showed a sheet of irregular lymphocytes with scattered macrophages.

Immunohistochemistry staining was prepared on specimens embedded with paraffin wax from the main tumours to confirm the morphological diagnosis by following the same techniques described in [11]. A panel of markers was selected based on morphologic diagnosis, including CD3, CD20, CD10, CD15, CD5, and CD30.

## Statistical analysis

Data analysis was performed using Statistical Package for the Social Sciences (SPSS version 20.0) and Microsoft Office Excel 2010 for Windows. Qualitative and quantitative variables were described. The criterion for statistical significance was  $P < 0.05$ .

## Results

The 134 patients diagnosed with lymphoma were distributed into the traditional categories of non-Hodgkin and Hodgkin lymphoma and are included, along with sub-type and gender in Table 1.

Lymphoma Sub-types/Gender	Male	Female	Frequency
Hodgkin's lymphoma (HL)	12	5	17 (12.7%)
Nodular sclerosis	3	1	4 (23.5%)
Mixed cellularity type	5	2	7 (41.2%)
Lymphocyte rich	2	2	4 (23.5%)
Lymphocyte depleted	2	0	2 (11.8%)
Non-Hodgkin's lymphoma (NHL)	69	48	117 (87.3%)
Diffused large B Cell Lymphoma (DLBCL)	27	19	46 (39.3%)
Burkett's Lymphoma (BL)	25	15	40 (34.2%)
Small lymphocytic lymphoma	5	4	9 (7.7%)
Extranodal marginal (MALT) lymphoma	3	1	4 (3.4%)
Follicular lymphoma	4	5	9 (7.7%)
T-cell-rich B cell lymphoma	1	0	1 (0.85%)
Peripheral T cell lymphoma	3	2	5 (4.3%)
Anaplastic Large Cell Lymphoma	0	1	1 (0.85%)
Precursor/lymphoblastic lymphoma	1	1	2 (1.7%)
Total	81	53	134

**Table 1: Frequency of Lymphoma and Lymphoma Subtypes within Studied Subjects Classified by Gender.**

Seventeen (12.7%) cases were diagnosed as HL, whereas 46 (87.3%) were classified as NHL. Regarding HL, four subtypes were identified: mixed cellularity was the most prevalent subtype (41.2%), followed by nodular sclerosis and lymphocyte-rich (4 case; 23.5% for each), and lymphocyte-depleted lymphoma (11.8%). Of the NHL subtypes, DLBCL was the most prevalent type, representing 46 of the 117 cases (39.3%). BL was the second most common type, with 40 diagnosed cases (34.2%), followed by follicular lymphoma and small lymphocytic with a rate of 9 cases (7.7%) for each. Males were predominant in both types of lymphoma with overall 81 (60.4%) males and 53 (39.6%) females, a male: female ratio of 1.5:1. Among HL patients, 12 out of 17 patients (70.6%) were males, and (29.4%) females. In NHL patients, males and females were (69 cases; 59%, 48 cases; 41%, respectively).

Concerning age, as shown in Table 2, thirty nine (29.1%) were >60 years of age, and (70.9%) were <60 years of age. The youngest of these patients was four years old and the oldest 80 years old, with a mean age of 41 years.

Type of lymphoma/Age group		≤ 15 years	16-30 years	31-45 years	46-60 years	>60 years	Total
Hodgkin's lymphoma							
Mean ± SD	Range	3	3	2	4	5	17
47.14 ± 22.31 years	7-70 years						
Non-Hodgkin's lymphoma							
Mean ± SD	Range	33	10	18	22	34	117
40.22 ± 24.62 years	4- 80 years						
Total (%)		36 (26.90%)	13 (9.70%)	20 (14.90%)	26 (19.40%)	39 (29.10%)	134 (100%)
Overall mean of age =41.13 ±24.242 years/ Significance: $\chi^2 =0.69, P=0.952$							

**Table 2: Age Groups within Lymphomas Cases.**

There was no statistically significant difference within age groups (P=0.952). As shown in Table 3, 109 (93.2%) of B cell lymphoma cases were identified compared to 8 (6.8%) cases belonging to T cell lymphoma. The expression of CD20 was identified in all B cell lymphomas and was not detected in any T cell lymphomas. CD3 was expressed in all T cell lymphomas and was also seen in one case of diffuse large B cell lymphoma. All HL cases expressed CD30 and CD15, with no expression of CD3, CD5, and CD10. CD20 was detected in the lymphocyte-rich subtype and one case of mixed cellularity type.

Lymphoma Sub-types/Immuophenotype	CD 3	CD5	CD10	CD15	CD20	CD30
Hodgkin's lymphoma						
Nodular sclerosis	-	-	-	+	-	+
Mixed cellularity type	-	-	-	+	±	+
Lymphocyte rich	-	-	-	+	+	+
Lymphocyte depleted	-	-	-	+	-	+
Non- Hodgkin's lymphoma						
Diffused large B Cell Lymphoma (DLBCL)	± *	±	±	NA	+	NA
Burkett's Lymphoma (BL)	-	-	+	NA	+	NA
Small lymphocytic Lymphoma	-	+	-	NA	+	NA

Extranodal marginal (MALT) lymphoma	-	-	-	NA	+	NA
Follicular lymphoma	-	-	±	NA	+	NA
T-cell-rich B cell lymphoma	+	+	-	-	+	NA
Peripheral T cell Lymphoma	+	+	-	NA	-	NA
Anaplastic Large Cell Lymphoma	+	+	-	+	-	+
Precursor T lymphoblastic Lymphoma	+	+	+	-	-	NA

**Table 3: Markers of Expression within Lymphomas Cases.**

\*One case of DLBCL gave positive reaction with CD3.

Haematological changes in patients with lymphoma are listed in Table 4.

Haematological variables/ Type of Lymphoma	Hodgkin's lymphoma	Non- Hodgkin's lymphoma	P-value
Haemoglobin (Mean ±SD) g/dl	9.81 ±1.72	10.7 ± 2.17	0.312
Range of haemoglobin g/dl	7.30 - 11.60	6.00 - 15.20	--
Normal haemoglobin level N (%)	0 (0.00 %)	30 (25.6%)	0.084
Low haemoglobin level N (%)	17 (100%)	87 (74.4%)	0.082
Leucocyte count (Mean ±SD) cell/L	18.65±34.23	17.02±41.81	0.098
Range of leucocyte count, cell/L	2.30 -96.00	1.00 - 272.00	--
Normal leucocyte count N (%)	12 (70.6%)	49 (41.9%)	0.332
Leucocytosis N (%)	3 (17.6%)	56 (47.9%)	0.028
Leucocytopenia N (%)	2 (11.8%)	12 (10.3%)	0.849
Platelets count, cell/L (Mean ±SD)	389.17±239.46	261.88±161.45	0.097
Range of platelets count, cell/L	41.00 -762.00	15.00-636.00	--
Normal platelets count N (%)	5 (29.4%)	71 (60.7%)	0.021
Thrombocytosis N (%)	7 (41.2%)	18 (15.4%)	0.015
Thrombocytopenia N (%)	5 (29.4%)	28(23.9%)	0.624

**Table 4: Variables of Peripheral Blood Parameters within Lymphomas cases.**

The prevalence of anaemia was evaluated by measuring the haemoglobin concentration. At enrolment, it was observed that all HL patients had anaemia with a mean haemoglobin level of 9.81 g/dl, ranging from 7.3 to 11.6 g/dl. Eighty-seven NHL patients (74.4%) had anaemia, with an average haemoglobin level of 10.7 g/dl. Leucocytosis of a variable degree was observed in 56 (47.9%) of NHL cases. The mean platelet count among patients with HL and NHL was 389.17 and 261.88 x 10<sup>9</sup> cell/L, respectively. Thrombocytosis was observed in seven cases (41.2%) of patients with HL. The mean leucocyte count was 18.65 and 17.02 x 10<sup>9</sup> cell/L in HL and NHL patients, respectively.

## Discussion

This study confirms that diffuse large B cell lymphoma as an NHL subtype, and mixed cellularity in HL subtype are highly prevalent in Sudanese malignant lymphoma patients. The current study was conducted to provide data that may contribute to better health planning and understanding of potential predisposing factors for ML in Sudan, by investigation the relative distribution of various types of ML not previously investigated. In developing countries the prevalence and incidence rates of lymphoma as well as the distribution of lymphoma subtypes may vary (12-14). In the current study, HL and NHL were diagnosed in 12.7% and 87.3% of cases, respectively, which is consistent with the results of a survey conducted among ML patients in the United Kingdom, [15] Poland,[16] and also in ML patients in the United States [17] and Chinese ML patients [18]. However, among NHL subtypes in the present study, diffused large B cell lymphoma was the most prevalent subtype, which was identified in 39.3% of patients. Similar findings were reported in Sudan [19], as well as in India [20]. DLBCL has also been documented to be the most frequent NHL subtype in most studies worldwide, and geographic variations in NHL subtypes are well documented. In Pakistan 66.1% of NHL cases were reported to be of DLBCL subtype [21]. In some African countries the distribution of NHL subtypes varies according to age group, with DLBCL accounting for 55% of all NHL cases among adults, [22] and BL comprising nearly 50% of childhood cases of NHL in Africa [23]. Regarding HL, mixed cellularity, was the more predominated type in the present study. Results were consistent with those found among Ethiopian ML patients [24]. In our study, the male gender group was more predominant in both types of lymphoma, with overall frequencies of 60.4% in males, and 39.6% in females. Among HL patients, 12 out of 17 patients were males. In NHL patients, males represented 59% of cases. These results are consistent with worldwide observations, which show that both ML and other haematological malignancies often occur more frequently in males than in females [17]. Concerning the characteristic patterns of lymphoma cells, B-cell lineage dominated in our current data (94%), compared to a low relative proportion of T cell lymphomas (6%). These findings are in agreement with previous studies [25]. However, the distribution of T cell lymphoma varies worldwide, ranging from a low frequency of about 4% such as in Korea [26], to more a frequent rate, such as 23% in Turkey [27]. In the present study, in ML cases expression of CD3 and CD20 were the most specific surface antigens for lymphocytes. CD20 was expressed in all B cell lymphomas and was negative in all T cell lymphomas while CD3 was expressed in all T cell lymphomas, and in one case of large B cell lymphoma. These results are consistent with previous studies in ML cases. Regarding the finding of one large B cell lymphoma case that gave a positive reaction for CD3, the same result has been reported in rare instances of mature B-cell neoplasms in some studies [28-30]. The explanation behind this odd finding remains unclear. However, several mechanisms have been suggested to clarify the aberrant expression of T-cell antigens by neoplastic B cells [31, 32]. Concerning the age of our study subjects, 29.1% were >60 years of age, and 70.9% were <60 years of age. The youngest of these patients was four years old and the oldest 80 years old, with a mean age of 41 years. These results were consistent with Caminha et al. study [33]. Evaluations of peripheral blood parameters are required as part of pre-treatment check-up in cases of lymphoma, parameters which are also reflective of prognostic inferences, especially if an abnormality is found [34]. In the current study, anaemia was the most frequent feature, found in all HL patients and the majority of NHL patients (74.4%). Leucocytosis was observed in 47.9% of NHL cases. Thrombocytosis was detected in 41.2% and 15.4% of patients with HL and NHL, respectively. Further, the frequency of leucopenia was observed in 11.8%, and 10.3% of patients with HL and NHL, respectively. These haematological variables did not show any statistically significant differences within studied lymphoma patients. These abnormalities, also observed in previous studies, may be initiated by the influence of cytokines released by malignant cells, and also as a consequence of bone marrow replacement by ML cells at the late stage of the disease [35-36]. ML in Sudanese patients was more frequent in males than in females; its incidence increases with age. B cell lymphoma is more common than T cell lymphoma. DLBCL was the most frequent NHL subtype.

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## Conflict of Interest

None

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