Original Article

Non –Hodgkin lymphoma in Sudanese Children

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ABSTRACT

Background: Non-Hodgkin Lymphoma is a very heterogeneous lymphoproliferative disease with clinical and histological pattern different from children to adults.

Objective: To characterize the clinical and pathological pattern of Non -Hodgkin`s lymphoma among Sudanese children.

Materials and Methods: This study was undertaken prospectively on paediatric cases (≤16 years) referred to histopathology department, Radio-Isotope Centre, Khartoum which is the main Centre for cancer management in Sudan from January 2008 to December 2012. The clinical and demographical data of these patients were recorded.

The H&E stained slides of each case were examined initially, then the confirmed cases of non-Hodgkin`s lymphoma were classified according to the 2008 WHO classification of neoplastic diseases of the haematopoietic lymphoid tissue following immunostaining of sections cut from formalin-fixed, paraffin embedded (FFPE) tissue blocks with the following panel of antibodies:- LCA, CD3, CD5, CD10, CD20, CD23, BCL2, CyclinD1, MUM1,CD15,CD30 andKi67.

Results: Age range was 9 months to 16years. Fifty percent of the cases occurred between the age 2-5 years and only one case below one year. Male to female ratio was 1.6. Extranodal lymphoma (60%) was higher than nodal type (40%).The most commonly affected site was the gastrointestinal tract. Most of the gastrointestinal lymphoma presented with abdominal mass. The most common histological type was Burkitt`s lymphoma. None of the cases were small lymphocytic, follicular or T- cell type. Bone marrow involvement was in 87.5% of the cases at the time of diagnosis.

Conclusion: Burkitt`s lymphoma is the predominant paediatric lymphoma in Sudan. The majority of the cases presented late with bone marrow involvement.

Key words: Non -Hodgkin`s lymphoma, immunohistochemistry, Paediatric, Sudan.

Non-Hodgkin`s Lymphoma (NHL) is a heterogeneous group of lymphoproliferative malignancies with differing patterns of behavior and responses to treatment[1]. Most of NHL (i.e., 80-90%) are of B-cell origin[1]. NHL usually originates in the lymphoid tissues and can spread to other organs. However, unlike Hodgkin disease, NHL is much less predictable and has a far greater predilection to disseminate to extranodal sites. Due to the realization that each entity exhibits a different clinical and pathological behavior, much effort has been exerted over the years in attempts to accurately characterize and classify NHL[2].As the treatment of NHL becomes more aggressive and type dependent, immunohistochemistry became more important in diagnosis and monitoring treatment.

NHL is the fifth most common diagnosis of paediatric cancer in children under the age of 15years and it accounts for approximately seven percent of childhood cancers in the developed world[3].The histological pattern of NHL in children differs from that in adults. While indolent lymphoma is common in adults, high grade lymphoma is the predominant lymphoma in children[3,4].

In this study we tried to describe the current situation in Sudan using cancer registry and immunohistochemistry in part of the study in attempt to classify paediatric NHL in

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Sudanese children according to the recognized WHO classification.

MATERIAL AND METHODS:

Study design and study area: This prospective hospital based cross sectional study was conducted at Radiation and Isotopes Centre Khartoum (RICK), which is the main oncology centre in Sudan, located in Khartoum state, serving almost all paediatric cancer patients in Sudan during this period.

A. Case selection:

All paediatric cases (age ≤16 years) referred to Radio-Isotope Centre, Khartoum with an initial histological diagnosis of lymphoma in a 4years period from January 2008 to Dec. 2012 were included. The clinical and demographical data of these patients were extracted from cancer registry of the centre; additional information was taken from the information provided on the request forms accompanying the biopsy specimen. Cases with frank leukaemia i.e. bone marrow blast count more than 20%, were excluded.

B. Histology and Immunohistochemistry:

This was undertaken on cases referred to histopathology department. The initial histopathology diagnosis was recorded. The H&E stained slides of each case were examined initially and the lesions categorized broadly into Hodgkin's lymphoma, non-Hodgkin's lymphoma, or possible non lymphoma groups. The last two groups subsequently were confirmed to be non-Hodgkin`s lymphoma, or excluded from the group with the aid of immunophenotyping. Only NHL cases were included for further studies.

The confirmed cases of B-cell NHL were classified according to the WHO classification of neoplastic diseases of the haematopoietic and lymphoid tissue[5]following immunostaining of sections cut from formalin-fixed, paraffin embedded (FFPE) tissue blocks with the following panel of antibodies:

LCA, CD3, CD5, CD10, CD20, CD23, BCL2, CyclinD1, CD30, CD15, MUM1 and Ki67 (Dako).

Immunohistochemistry was performed on paraffin embedded tissue sections using the Envision method (Dako). Antigen retrieval techniques were applied as needed for each specific antibody. DAB was used as a substrate and the positive signal was dark brown in colour. The Ki-67 stain was assigned a percentage value that was calculated by positive nuclei staining per 1000 tumor cell nuclei in each case.

For Statistical Analysis: SPSS software was used.

RESULTS:

Clinical characteristics: The 60 paediatric patients with NHL were predominantly males (61.3%), females were (38.7%).The male to female ratio was 1:6. The median age was5 years (range, 9 months –16 years).Half of the cases occurred between ages of 2 to 5 years (Table 1). Paediatric NHL presented frequently as abdominal mass with bone marrow involvement in 87.5% of cases. Extranodal presentation was predominant (40 out of 60). The commonest affected site was gastrointestinal tract (41.7%) of all NHL cases and (69.4%) of extranodal cases (Figure1). Jaw came second after gastrointestinal tract (7%) in extranodal cases. In gastrointestinal tract, the small intestine was the most common site (46%) followed by large intestine (13%). One case presented with a rectal mass. Oesophagus and stomach were never affected. Forty one percent of cases presented with abdominal mass and the origin of the tumor couldn't be identified. Table 2 shows the blood indices at presentation. Anemia and thrombocytosis were found in 33(61 %) and 3 (5.5%) of cases respectively. Twelve cases had normal blood indices.

Histopathology and immunohistochemistry:

The majority of cases were classical Burkitt`s Lymphoma, followed by diffuse large B-cell NHL. None of the cases were small lymphocytic, follicular or mantle cell NHL (Figure2).

Table 3 summarize immunohistochemical findings. Burkitt's lymphoma exhibited the classical histological features with diffuse sheets of small tumor cells having round to

Table (1): Age distribution among the studied cases

Age/year	Frequency	Percent	Cumulative Percent
<2	1	1.7	1.7
2 -5	30	50.0	51.7
6 -10	14	23.3	75.0
10 -16	15	25.0	100.0
Total	60	100.0	

oval nuclei and several prominent basophilic nucleoli. The chromatin was coarse and nuclear membrane was thick. The majority of cases showed a prominent starry-sky pattern (Figure3 A). The tumor cells were positive for B-cell marker CD20(Figure 3 B), negative for CD3 (Figure 3 C), CD23, CD30 and CD5. CD10 showed variable expression. Ki 67 reflected high proliferative index (Figure 3 D), as Sixty percent of Burkitt`s cases scored 100%, 34% of cases scored between 90 to 99% and only two cases scored 85%and 80%.

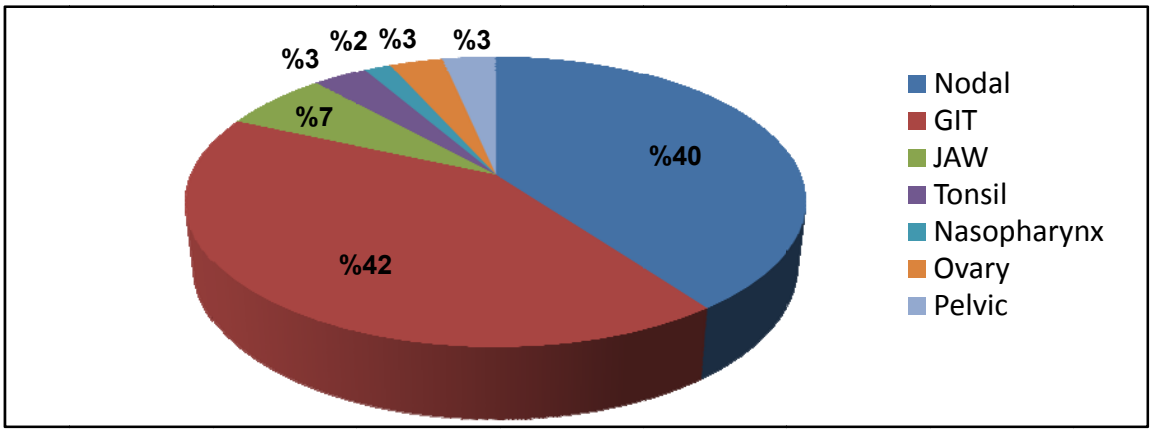

Figure (1): The Anatomic Distribution of the studied Paediaric NHL.

Table 2: Blood Indices at diagnosis of the studied cases

	Haemoglobin	TWBC	Platelets
High*	0	19(35%)	23(42.5%)
Normal	21 (39%)	34(63%)	28(52%)
Low*	33(61 %)	1(2%)	3 (5.5%)

*High TWBC ≥ 10.000 $X10^3$, High Platelets ≥ 450.000X109
*Low TWBC ≤ 3.000$X10^3$,Low Platelets ≤ 150.000$X10^9$ Low Hb. ≤ 12g/dl

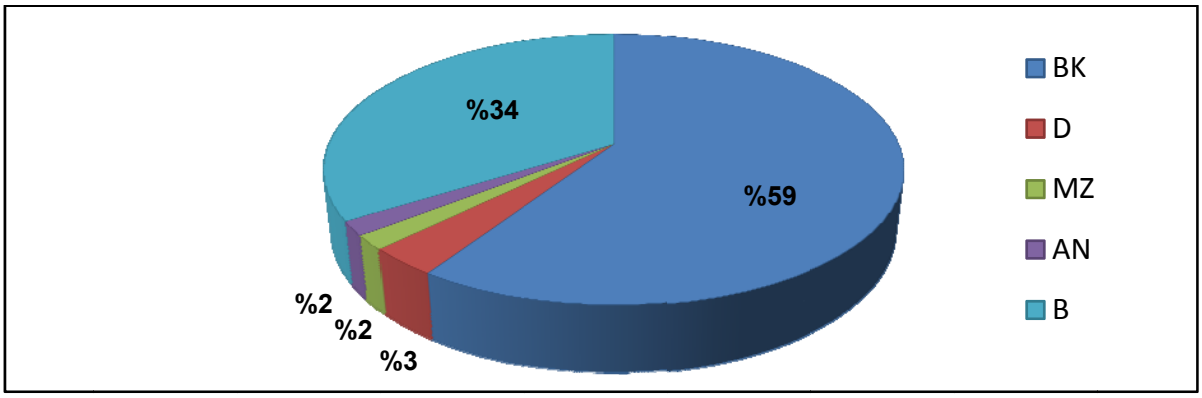

Figure (2): The Histological classification of the studied cases. (BK Burkitt`s lymphoma, D Diffuse large B cell lymphoma, MZ marginal Zone lymphoma AN anaplastic Lymphoma and B B-cell NHL)

Table 3: The immunohistochemistry findings in the studied cases

	LCA	CD20	CD5	CD3/CD15	CD30	CD23	CD10	BCL2	Ki67%
Burkitt`s	++	+	-	-	-	-	++/-		90-100
Diffuse	+	+	-	-	+/-	-	+/- -	+/-	40-80
Anaplastic	+	+	-	-	-	-		-	50
MZ	+	+	-	-	-	-		+	<20

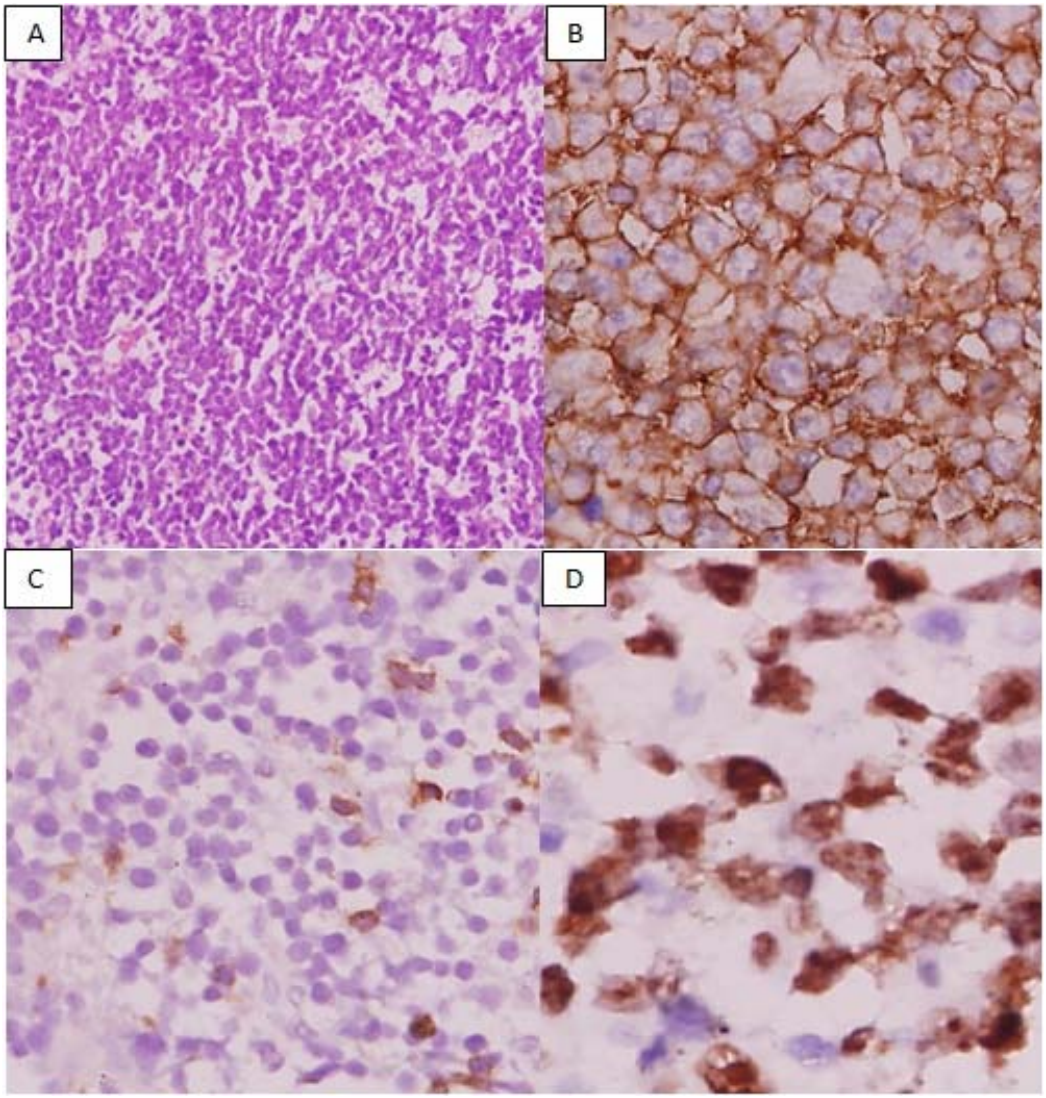

Figure (3) : Hematoxylin and Eosin stain and immunostain of Burkitt`s lymphom (A: H&E x40 showing sheets of dark monomrphic cells. Note the starry-sky pattern, imparted by numerous benign macrophages that have ingested apoptotic tumor cells. B: CD20 staining showing characteristic membranous Staining. C: CD3 stain showing scattered positivity only in reactive T-cells. D: Nearly 100% of tumour cells showing the nuclear positivity of Ki 67.)

DISCUSSION:

NHL is a heterogeneous group of malignant diseases with different biologic and clinical characteristics. Previous studies have reported a considerable geographic variation among histological subtypes of NHL, as well as significant differences between paediatric and adult NHL[6]. This study gives recent insight of NHL in Sudanese children using advanced techniques. In this study group male predominance was clear as male to female ratio was 1.6:1. Most of the cases aged between ages of 2 to5 (Table1).The age and gender distribution in our study group is consistent with worldwide trend[67].In this study we are reporting only one case of NHL below one year. This might be due to the fact that most of the cases were presenting late and also to our exclusion of cases with frank leukemic phase. Unlike adults with non-Hodgkin's lymphoma, who most often present with lymph-node disease, children typically have a prediction to extranodal sites (60% of the studied cases) than to nodal sites. When arising from nodes, cervical lymph node was the commonest site followed by axillary and inguinal sites. Axillary and inguinal lymph nodes were equally affected. The majority of extranodal NHL arose from gastrointestinal tract; the same have been reported by many other studies[8,9]. This is not surprising as gastrointestinal tract is harboring a large amount of lymphoid tissues that makes it the most common site of extranodal lymphoid malignancies. In gastrointestinal tract, small intestine ranked first followed by large intestine. The stomach and oesophagus were never affected. The anatomical distribution is similar to international figures although isolated gastric lymphoma in children has been recently described in conjunction with infection by *Helicobacterpylori*[8]. Thirty percent of the studied cases presented in late stages with abdominal mass and the origin of tumour could not be identified due to advanced extensive disease. As noted previously, that the tumor may act as a lead-point for ileocolic intussusception and a small bowel obstruction[10]. This can explain intussusceptions as presenting feature in abdominal lymphoma.

Extranodal involvement other than gastrointestinal tract included jaw where all cases were Burkitt's type. Tonsils were only affected in 3%of cases. The low percentage of tonsillar involvement in children is expected as the tonsils are very rare sites of NHL in children unlike adult. The rarity of tonsillar lymphoma lead to conclusion; that in routine tonsillectomy, gross pathological evaluation for tonsil specimen is enough and no need for histopathology examination unless there is clinical suspicion based on preoperative findings[11, 12].Ovary and pelvic were much less affected than GIT, this is the same as mentioned previously that ovarian infiltration in paediatric non-Hodgkin lymphoma (NHL) at presentation is rare[13].

Bone marrow was involved at the time of diagnosis in 87.5%.Bone marrow involvement occurred in most cases of paediatric NHL by the time the diagnosis is made, this have been attributed to the often rapidly growing nature of the tumuor, and the disease tendency to spread by blood-borne dissemination[14].Blood indices at presentation are normal in 20% of cases (Table 2).Although there is a significant association between the bone marrow involvement and abnormal blood indices but still there are cases with bone marrow involvement and have normal indices. This indicates bone marrow infiltration cannot be assessed reliably from blood indices findings only and a bone marrow biopsy for staging is mandatory even if the blood indices are normal. Sixty one percent of the studied cases were anaemic at the time of presentation. Anemia has been identified as an important adverse prognostic factor for the outcome of lymphoma patients, particularly in some histologic subgroups and in patients with bone marrow involvement[15]. Thrombocytopenia in NHL patients was not common at the time of diagnosis unlike the case with acute leukemia patients. In our study only three cases were thrombocytopenic at the time of diagnosis and majority have normal white blood count. Low platelets and

Lymphopenia have been also identified as of a poor prognostic value in NHL. Lymphopenia has been associated with poor prognosis especially with diffuse large B-cell type[16, 17, 18].

Burkitt`s lymphoma (BL) was the main histological subtype in our study. This was the same as in Egypt as BL accounted for 39% of NHL in a study done by Naresh[19], but in the present study the percentage was even higher (59%).Although most of African countries exhibit the endemic BL pattern[20], our cases follow clinically the sporadic pattern, with high frequency of abdominal involvement and infrequent jaw involvement (the hallmark of endemic BL). Endemic BL presents as a jaw mass in 50 percent of cases[21]. The sporadic pattern of BL in Sudan have been observed by Noon et al but the cause of change of pattern from endemic to sporadic BL need to be addressed[22].The sporadic pattern had been reported in Ethiopia and some regions in Kenya[23,24]. Very few of our cases exhibit other histological types in the form of Diffuse Large B cell Lymphoma (3%), Marginal Zone Lymphoma (2%) and Anaplastic Lymphoma (2%). This is the same pattern in Africa and western countries. Few studies in Japan showed higher percentages of Diffuse Large B- cell Lymphoma[19, 25].

CONCLUSION:

Childhood lymphomas were predominantly BL with extranodal presentation. The majority of the studied cases presented late with disseminated disease. Future studies will require careful attention to Burkitt's lymphomas, to investigate the possible aetiological factors such as infectious agents, including malaria.

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