

Lymphomas in Ile-Ife, Nigeria: Immunohistochemical Characterization and Detection of Epstein-Barr virus Encoded RNA

IFEYINWA M. ONWUBUYA¹, KAYODE A. ADELUSOLA², MUHEEZ A. DUROSINMI³, DONATUS SABAGEH⁴, KEVIN N. EZIKE⁵

ABSTRACT

Background: The proper histopathological characterization of malignant lymphomas requires the use of immunohistochemistry along with other molecular pathology techniques.

Materials and Methods: Malignant lymphomas histologically diagnosed in our hospital were reclassified according to the WHO scheme using immunohistochemistry while in-situ hybridization was performed for the detection of Epstein-Barr virus encoded RNA.

Results: There were 83 cases of lymphoma. The male to female ratio was 1.9:1 while the overall mean age was 41.7 years. Non-Hodgkin lymphomas (NHL) constituted about 79.5% of cases. The majority of cases (98.8%) were B-cell lymphomas. Nine subtypes of lymphomas were identified with diffuse large B-cell lymphomas (56.4% of which were of the germinal centre type) constituting

the largest group (47.0%). Intermediate and high grade subtypes were more common. The majority of cases (72.3%) were nodal lymphomas with cervical lymph node being the commonest site (48.2%). Only classical Hodgkin lymphoma (HL) (20.5%) was seen of which the mixed cellularity subtype was the most common. Epstein Barr virus (EBV) encoded ribonucleic acid was detected in 7 cases (8.4%) including 4 cases of HL, 2 cases of Burkitt lymphoma and the only case of plasmablastic lymphoma. About five cases were reclassified as non-lymphoid malignant lesions.

Conclusion: Immunohistochemistry is vital to the proper classification of lymphomas even in a resource poor environment. Although nine subtypes of lymphomas were identified, diffuse large B-cell lymphomas formed the largest single group. Epstein-Barr virus probably plays an important role in lymphomatogenesis in this environment. A larger multicentre study is required to prove this.

Keywords: Classification, Immunohistochemistry

INTRODUCTION

Over the past two decades, molecular pathology techniques have assumed more important roles in the diagnostic work-up of lymphomas [1-3]. In fact, immunohistochemistry (IHC) now forms an integral part of diagnostic haematopathology and is required for the routine diagnosis and classification of virtually all lymphoid malignancies [3,4].

The review of literature reveals that the information available on the immunohistochemical features of lymphomas in Nigerians falls short of desirable [5-7]. Much of what is known today about lymphomas is the result of research work conducted outside Nigeria and Africa and these may only find limited applications in our local population. This has resulted in poor diagnosis, classification and management outcomes [7].

Although lymphomas account for approximately 3-4% of cancers worldwide, the incidence rates have been increasing, resulting in significant morbidity and mortality even among African, and especially, Nigerian populations [8-10]. These increases may not be unconnected with the changes being observed in contemporary diagnostic pathology practice in Nigeria which now require more the routine use of advanced techniques like immunohistochemistry among other molecular pathology methods [11,12]. It is expected that this will make the diagnosis and classification of lymphomas easier, more precise and in tandem with the new consensus World Health Organization (WHO) scheme with the attendant positive impact on the management and prognostication of affected patients [13,14].

It, therefore, becomes imperative to reappraise the biological characteristics of lymphomas in our local population and reclassify these according to the new WHO classification scheme with the aid of appropriate immunohistochemical markers.

Finally, the pathogenetic relationship between EBV and malignant lymphomas in Nigerians has not been clearly defined despite the endemicity of the virus in the population. Nevertheless, various reports seem to suggest that a possible association does exist [15-17].

MATERIALS AND METHODS

The demographic data and paraffin blocks from all cases histologically diagnosed as malignant lymphoma in the Department of Morbid Anatomy and Forensic Medicine of the Obafemi Awolowo University Teaching Hospitals Complex (OAUTHC) Ile-Ife, Nigeria over a ten year period (June 2002-May 2011) were retrieved and used for this study. Approval was obtained from the local ethics committee of the OAUTHC, Ile-Ife, Nigeria. These were classified according to the WHO scheme using immunohistochemistry [18].

Immunohistochemical Studies

Immunohistochemistry was performed by the indirect immunoperoxidase method using the tissue microarray (TMA) constructed from representative cores taken from the appropriate formalin-fixed, paraffin-embedded tissue blocks after the original H&E slides were reviewed. The microarray was constructed using a Beecher Instruments arrayer (Beecher Instruments, Wisconsin, USA). The sections were dewaxed and rehydrated while antigen retrieval was performed according to the protocol for the different antigens [Table/Fig-1]. Endogenous peroxidase was blocked by treating the hydrated sections with 3% H₂O₂ in methanol for 30 minutes. To prevent non-specific reactions, sections were incubated with 10% serum for 10 minutes. Antigen localization was carried out using TM immunohistochemistry detection system (Biogenex, California, USA). Immunostaining was done using the automated system developed by Biogenex. 3,3' diaminobenzidine was used

ANTIBODY	DILUTION	ANTIGEN RETRIEVAL	SOURCE
CD3	1:50	20MINS EDTA, PH 8.0	NOVOCASTRA
CD5	1:50	20MINS EDTA, PH 8.0	NOVOCASTRA
CD4	1:400	20MINS EDTA, PH 8.0	NOVOCASTRA
CD8	1:50	20MINS EDTA, PH 8.0	NOVOCASTRA
CD10	1:20	20MINS EDTA, PH 8.0	NOVOCASTRA
CD15	1:10	20MINS MW, CITRATE, PH 6.0	DAKO
CD20	1:250	20MINS MW, CITRATE, PH 6.0	DAKO
CD23	1:100	20 MINS MW, CITRATE, PH 6.0	NOVOCASTRA
CD30	1:50	20 MINS DAKO RETRIEVAL SOLUTION, PH 6.0	DAKO
CD45(LCA)	1:10	20 MINS EDTA, PH 8.0	DAKO
CD138	1:100	20 MINS MW, CITRATE, PH 6.0	SEROTEC
CD79a	1:20	20 MINS MW, CITRATE, PH 6.0	DAKO
BCL-2	1:7	20 MINS MW, CITRATE, PH 6.0	DAKO
BCL-6	1:20	20 MINS EDTA, PH 8.0	DAKO
CYCLIN D1	1:50	20 MINS MW, CITRATE, PH 6.0	DAKO
ALK	1:10	20 MINS DAKO RETRIEVAL SOLUTION, PH 9.9	DAKO
MUM-1	1:200	20 MINS MW, CITRATE, PH 6.0	SANTA CRUZ
HHV-8	1:50	20 MINS MW, CITRATE, PH 6.0	NOVOCASTRA
PANKERATIN	1:150	10 MINS TRYPSIN	DAKO
Ki-67	1:50	20 MINS MW, CITRATE, PH 6.0	NOVOCASTRA
MELAN A	1:50	20 MINS MW, CITRATE, PH 6.0	NOVOCASTRA

[Table/Fig-1]: List of antibodies, the dilutions and the antigen retrieval protocols used

to visualize the antigen-antibody reaction. Appropriate positive and negative controls were used. Staining was considered positive if more than 30% of the tumour cells stained with the antibody.

Detection of Epstein-Barr virus

Epstein-Barr virus (EBV) encoded ribonucleic acid (EBER) was detected by in-situ-hybridization (ISH) using an EBV probe-ISH kit according to the manufacturer's instruction (Novocastra, Newcastle Upon Tyne, UK) following proteinase-K digestion. The hybridization was done for four hours at a temperature of 37°C with fluorescein-conjugated oligonucleotide probes. Probe localization was carried out afterwards using rabbit F_{ab} anti-fluorescein isothiocyanate. 5-bromo-4-chloro-3-indolyl phosphate was used as substrate to produce the blue-black signal. For optimal mRNA preservation in these cases, a poly-dT probe on a separate section of the paraffin block was used as control. Appropriate controls were used in each case.

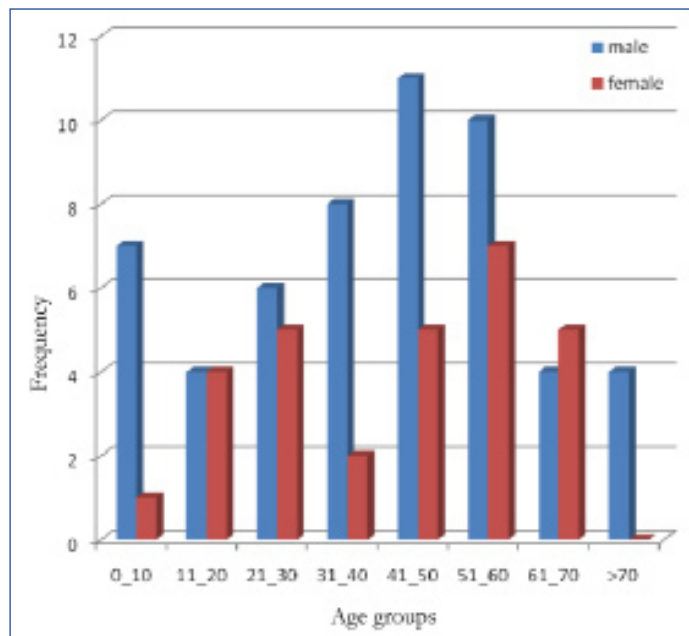
STATISTICAL ANALYSIS

The data were analysed using SPSS version 16.0 ($p \leq 0.05$) by SPSS version 16 for Windows. The level of statistical significance was set at $p < 0.05$. Cases with incomplete biodata, missing or inadequate archival tissue material were excluded from the study.

RESULTS

A total of 96 cases were identified and retrieved for this study. Of these only 83 cases were proven to be lymphomas using appropriate immunohistochemical markers. The 13 cases excluded from the study included 8 cases with inadequate tissue blocks and 5 cases ultimately diagnosed as non-lymphoid malignancies.

Of the 83 cases thus classified there were 54 (65.1%) males and 29 (34.9%) females with an overall male to female ratio of 1.9:1 [Table/Fig-2]. The overall mean age was 41.7 ± 21.0 years while the ages ranged from 4-96 years. The mean age for males was 41.3 years while that for females was 42.9 years. About 54 (65.1%) cases were found between the ages of 21 and 60 years with only 16 (19.3%)



[Table/Fig-2]: Age and sex distribution of cases of malignant lymphoma

and 13 (15.7%) cases below and above the ages of 20 years and 70 years respectively.

Eighty-two (98.8%) cases were B-cell lymphomas while there was only 1 (1.2%) case of T cell lymphoma [Table/Fig-3]. Seventeen (20.5%) cases were classified as Hodgkin lymphomas while 66 (79.5%) were categorized as Non-Hodgkin lymphomas. The tumours were primarily nodal in 67 cases. Of the 16 cases that presented at extranodal sites, 6(37.5%) primarily affected the gastrointestinal tract (GIT) while 3 (18.8%) were intra-abdominal but not related to the GIT. Two (12.5%) cases each presented primarily as splenic and jaw masses one case each was retroperitoneal, intraorbital and sinonasal.

According to [Table/Fig-3], only 9 subtypes of lymphomas were identified in this study. Their histopathological features and immunohistochemical profiles are summarized in [Table/Fig-4]. A summary of their salient features is given below:

Hodgkin Lymphoma

There were 17 cases of Hodgkin lymphoma and all were found to be of the classical type (CHL). These formed about 20.5% of all lymphoma cases. It was about 1.8 times more common in males than in females. It was found in all age groups with the highest frequencies occurring among individuals in the 21-40 year age group and those younger than 20 years old with 6 (35.5%) and 5 (29.4%) cases respectively. There were only 2 (11.8%) cases older than 60 years old. [Table/Fig-5a-c] shows photomicrographs of one case of CHL.

Diffuse Large B-Cell Lymphoma

Diffuse large B-cell lymphomas (DLBCL) formed the largest group with about 39 (47.0%) cases. The age range was from 6 to 69 years while the mean age was 45.6 years. It was found in all age groups although it was more prevalent in the 21-60 year age group with about 27 (69.2%) cases. It was 2.5 times more common in males. Diffuse large B-cell lymphomas accounted for about 51.9% of cases in males and 37.9% of female cases. The majority of cases (89.7%) were primarily nodal. The extranodal sites included one case each of an intra-abdominal, retroperitoneal, intraorbital and sinonasal mass. About 53.8% of these tumours were high grade while the remainder were low grade tumours. These lymphomas were further categorized into two recognisable prognostic subtypes based on their expression of germinal centre (CD10, BCL-6) or post-germinal centre (MUM-1) markers. About 22 (56.4%) of these

	Lymphoma subtype	CHL	DLBCL	BL	MZL	FL	SLL	ALCL	BCLU	PL	Total%
Number (%)		17 (20.5)	39 (47.0)	7 (8.4)	8 (9.6)	3 (3.6)	6 (7.2)	1 (1.2)	1 (1.2)	1 (1.2)	83 (100.0)
M:F (p=0.448)		1.8:1	2.5:1	2.5:1	1.7:1	1:2	1:2	All Female	All Male	All Male	1.9:1
Age range (yrs)		7-73	6-96	4-54	24-63	50-67	40-68	Not applicable	Not applicable	Not applicable	4-96
Mean age (yrs)		34.2	45.6	16.9	44.8	57.3	56.0				41.7
Age distribution (years)	0-20	5 (29.4)	5 (12.8)	6 (85.7)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	16 (19.3)
	21-40	6 (35.3)	11 (28.2)	0 (0)	2 (25.0)	0 (0)	1 (16.7)	0 (0)	1 (100.0)	0 (0)	21 (25.3)
(p=0.190)	41-60	4 (23.5)	16 (41.0)	1 (14.3)	5 (62.5)	2 (66.7)	3 (50.0)	1 (100.0)	0 (0)	1 (100.0)	33 (39.8)
	61>	2 (11.8)	7 (17.9)	0 (0)	1 (12.5)	1 (33.3)	2 (33.3)	0 (0)	0 (0)	0 (0)	13 (15.7)
Nodal site(%)		17(25.4)	35(89.7)	3(4.5)	1(1.5)	3(4.5)	5(7.5)	1(1.5)	1(1.5)	1(1.5)	67(100.0)
Extranodal site (p<0.001)	GIT	-	-	-	6	-	-	-	-	-	6(37.5)
	Spleen	-	-	-	1	-	1	-	-	-	2(12.5)
	Intraabdominal	-	1	2	-	-	-	-	-	-	3(18.8)
	Jaw	-	-	2	-	-	-	-	-	-	2(12.5)
	Retroperitoneal	-	1	-	-	-	-	-	-	-	1(6.3)
	Intraorbital	-	1	-	-	-	-	-	-	-	1(6.3)
	Sinonasal	-	1	-	-	-	-	-	-	-	1(6.3)
	Total(%)	-	4(25.0)	4(25.0)	7(43.8)	0(0.0)	1(6.3)	0(0.0)	0(0.0)	0(0.0)	16(100.0)
Tumour Grade (p<0.001)	Low(%)	-	0 (0.0)	0 (0.0)	8 (100.0)	3 (100.0)	6 (100.0)	0 (0)	0 (0)	0 (0)	17 (25.8)
	Intermediate(%)	-	18 (46.2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	18 (27.3)
	High(%)	-	21 (53.8)	7 (100.0)	0 (0)	0 (0)	0 (0)	1 (100.0)	1 (100.0)	1 (100.0)	31 (47.0)
	Total(%)	-	39(100.0)	7(100.0)	8(100.0)	3(100.0)	6(100.0)	1(100.0)	1(100.0)	1(100.0)	66(100.0)

[Table/Fig-3]: Some clinicopathologic parameters of the various subtypes of malignant lymphoma

CHL= Classical Hodgkin Lymphoma; DLBCL= Diffuse large B-cell lymphoma; BL= Burkitt Lymphoma; MZL= Marginal zone lymphoma; FL= Follicular lymphoma; SLL= Small lymphocytic lymphoma; ALCL= Anaplastic large cell lymphoma; BCLU= B-cell lymphoma unclassified; PL= Plasmablastic lymphoma

Lymphoma subtypes	Histopathological features	Positive IHC stains
CHL	Various patterns were observed. Common to all, however, is the presence of a few neoplastic Reed-Sternberg (RS) cells in a mixed reactive inflammatory background of eosinophils, lymphocytes, histiocytes and neutrophils.	CD15, CD30 positive RS cells
DLBCL	Diffuse proliferation of large lymphoid cells with vesicular nuclei and prominent nucleoli, and relatively scant cytoplasm.	CD10, CD19, CD20
BL	Diffuse growth of medium sized round lymphoid cells with round to oval nuclei and several prominent basophilic nucleoli. The cytoplasm is amphophilic. Numerous mitotic figures are present. The cells are admixed with tangible body macrophages giving a starry sky pattern	CD10, CD20, BCL6, EBER, Ki-67 >95%
MZL	An admixture of small round lymphocytes, monocytoid B cells, cells with slightly irregular nuclei, plasmacytoid cells, plasma cells.	CD20 (negative for CD5, CD23, & CYCLIN D1)
FL	Nodular growth pattern characterized by a mixture of different proportions of small and large lymphoid cells. Small cells with scanty cytoplasm and irregular elongated cleaved nuclei and prominent indentations admixed with large cells with vesicular nuclei, one or more nucleoli and a thin rim of cytoplasm.	CD20, BCL-2 (negative for CD5 & CD45)
SLL	Monotonous proliferation of small round lymphocytes with clumped nuclear chromatin, scant cytoplasm and scanty mitotic activity. Presence of variable numbers of larger cells (prolymphocytes) with vesicular nuclei and distinct nucleoli, singly and in small aggregates.	CD5, CD23
ALCL	Polymorphic infiltrate of a variable admixture of neutrophils, lymphocytes, histiocytes and large neoplastic cells showing marked pleomorphism. The large cells have multilobed or horseshoe nuclei with prominent nucleoli and abundant eosinophilic cytoplasm.	CD30
BCLU	Features intermediate between diffuse large B cell lymphoma and Burkitt lymphoma	CD10, CD20
PL	Monomorphic population of large cells with vesicular nuclei, single centrally placed prominent nucleolus, abundant basophilic cytoplasm and paranuclear hoff. Presence of immature plasma cells noted	CD38, CD138, MUM-1 (negative for CD20)

[Table/Fig-4]: Histopathological features and immunohistochemical profiles of the various subtypes of malignant lymphoma

were categorized as germinal centre DLBCL with only 1 (2.6%) case of post-germinal DLBCL and 16 (41.0%) cases which did not fit into any of these prognostic subgroups. [Table/Fig-6a,b] shows the photomicrographs of one of the cases.

Burkitt Lymphoma

There were seven cases of Burkitt lymphoma constituting 8.4% of all lymphoma cases. Six of these were seen in children below the age of 20 years while only one case was seen in an adult aged 54-year-old. It was 2.5 times more common in males. About 4 cases occurred at extranodal sites including two jaw masses and two primary intra-abdominal tumours. The three cases with primary nodal disease were found in the cervical lymph node. [Table/Fig-7a-d] shows the photomicrograph of one of the cases.

Marginal Zone Lymphoma

About 8 cases of marginal zone lymphomas were found in this study. The male to female ratio was 1.7:1. These were only seen

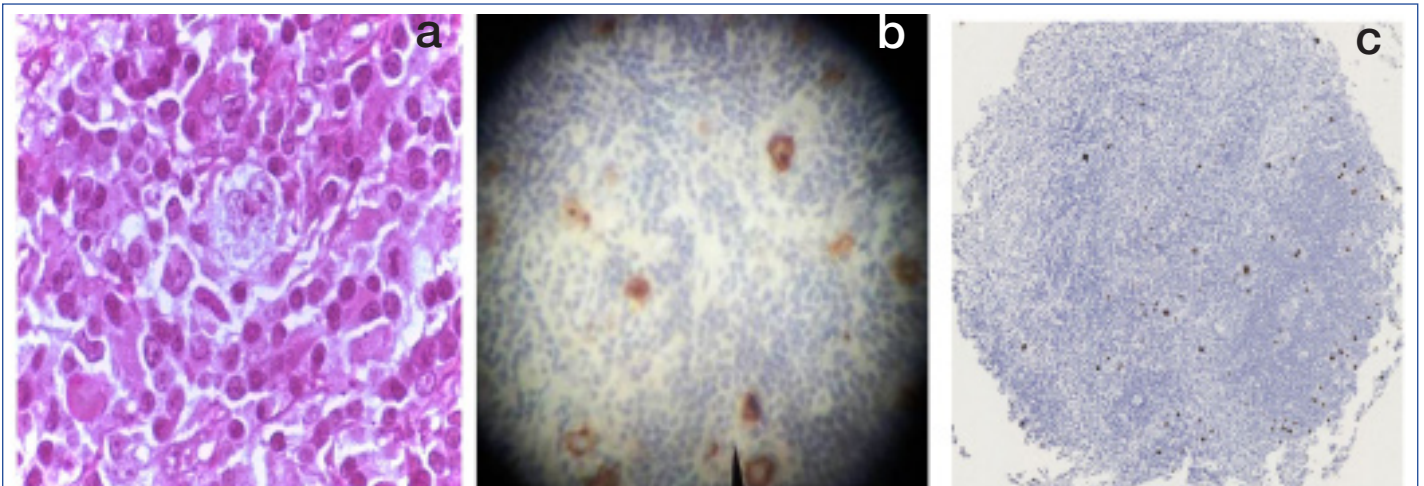
in individuals older than 21-year-old being most prevalent (62.5%) in individuals between 40 and 60 years old. The age range was 24 to 63 years and the mean age was 44.8 years. All cases were low grade lymphomas. Seven (87.5%) of these were extranodal with the gastrointestinal tract accounting for six of those cases. There was also a case of splenic marginal zone lymphoma. The only case with primary nodal disease affected the cervical node.

Follicular Lymphoma

There were only three cases of follicular lymphoma, two of which were seen in females. All cases were seen in patients older than 40 years. All cases were low grade and primarily occurring in the cervical lymph nodes [Table/Fig-8a-d].

Small Lymphocytic Lymphoma

Six cases of small lymphocytic lymphoma were seen with five (83.3%) occurring above the age of 40 years. It was twice as common in females as in males. All were low grade lymphomas.



[Table/Fig-5a-c]: Photomicrographs of Classical Hodgkin's lymphoma showing: (A) The histological features on haematoxylin and eosin (X400), (B) CD 30 positive Reed-Sternberg cells (X40), (C) Reed-Sternberg cells with nuclear staining for EBV encoded RNA

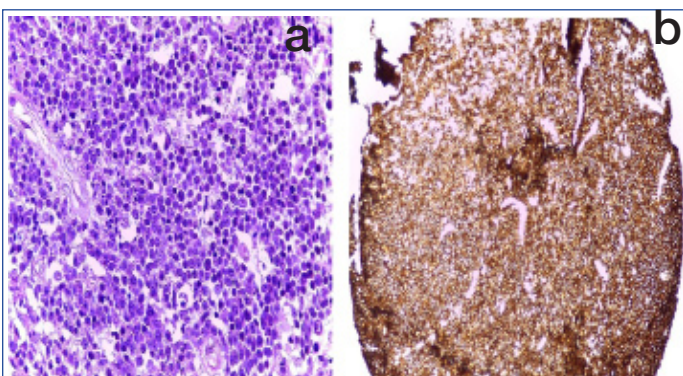
About five (83.3%) were primarily nodal in distribution all presenting as cervical lymph node masses.

Anaplastic Large Cell Lymphoma

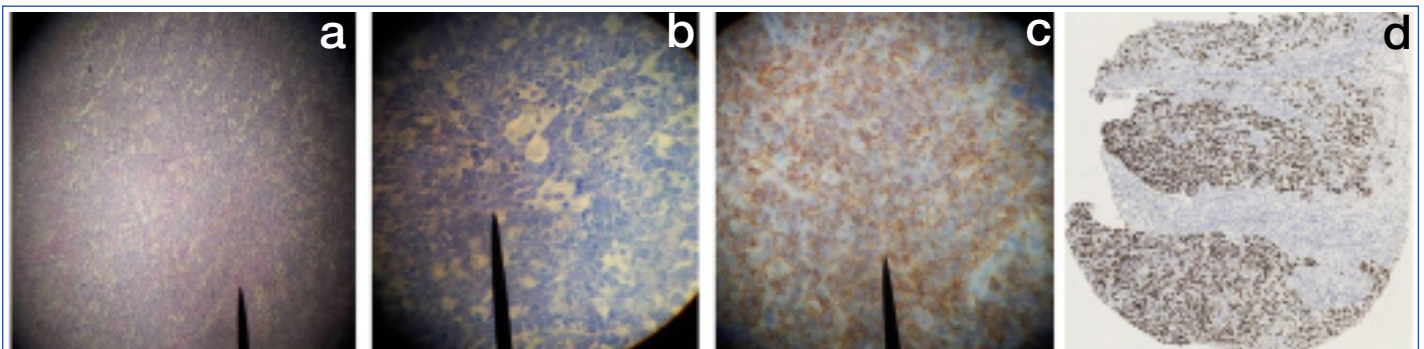
There was only one case of anaplastic large cell lymphoma which occurred in a 45-year-old woman [Table/Fig-9a-d]. This was a high grade T-cell lymphoma. The tumour cells were strongly positive for CD30 with 80% of them positive for Ki 67, but negative for ALK. Protein by immunohistochemistry. The histomorphology essentially consisted of paracortical infiltration by sheets of large lymphoid cells with poor staining horse-shoe shaped nuclei and multiple nucleoli.

B-Cell Lymphoma Unclassified

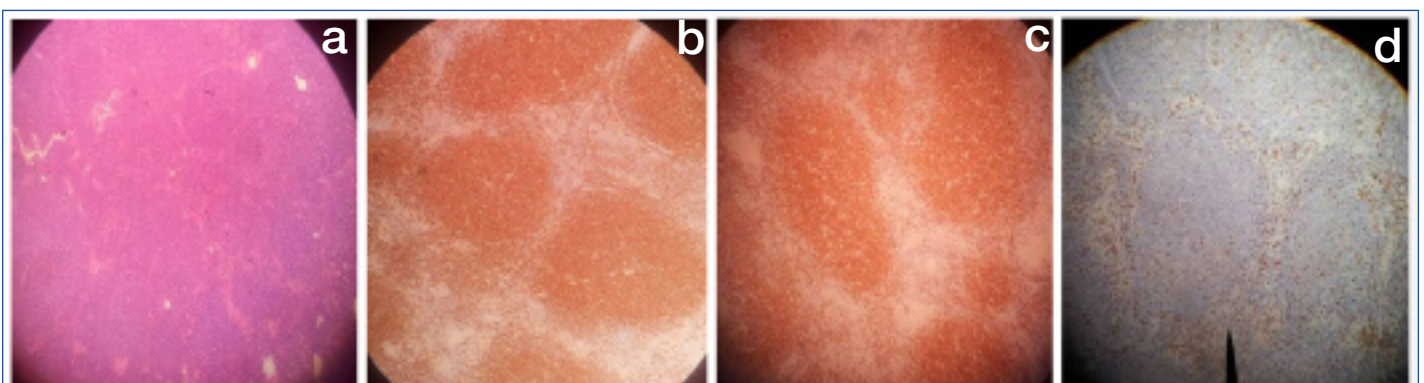
There was also only one case of B cell lymphoma which was seen in a 22-year-old man. It was a high grade lymphoma and the primary site was the cervical lymph node. The histomorphological and immunohistochemical features were indeterminate between Burkitt lymphoma and diffuse large B-cell lymphoma.



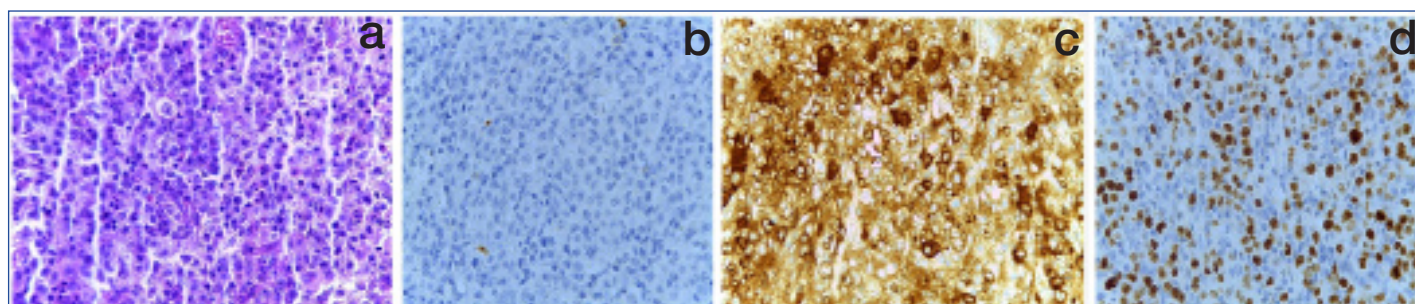
[Table/Fig-6a,b]: Photomicrographs of diffuse large B-cell lymphoma showing: (A) The histological features on routine haematoxylin and eosin (X100) (B) Diffuse CD 20 expression pattern (X60)



[Table/Fig-7a-d]: Photomicrograph of Burkitt Lymphoma showing: (A) The histological features on haematoxylin and eosin (X40), (B) The typical 'starry-sky appearance' on Giemsa (X40), (C) CD 20 expression pattern (X40), (D) Tumour cells with nuclear staining for EBV encoded RNA (X80)



[Table/Fig-8a-d]: Photomicrograph of follicular lymphoma showing: (A) The nodular growth pattern on haematoxylin and eosin (X40), (B) CD 20 expression pattern (X40), (C) BCL-2 expression (X40), (D) Ki-67 expression (X40)



[Table/Fig-9a-d]: Photomicrograph of anaplastic large cell lymphoma showing: (A) The histological features on routine haematoxylin and eosin (X100), (B) Lack of CD 20 expression (X100), (C) CD 30 expression (X100), (D) Ki-67 index (X100)

	Age (yrs)	Sex	Biopsy Site	Previous histologic diagnosis	Final diagnosis after immunohistochemistry
1	9	Female	Cervical lymph node	NHL	Alveolar rhabdomyosarcoma
2	52	Male	Inguinal lymph node	NHL	Melanoma
3	57	Female	Nasopharyngeal mass	NHL	Lymphoepithelioma
4	61	Male	Cervical lymph node	NHL	Undifferentiated carcinoma
5	70	Male	Cervical lymph node	HL	Anaplastic carcinoma

[Table/Fig-10]: Cases reclassified as non-lymphoid malignant disorders
p=0.218

Plasmablastic Lymphoma

The only case of plasmablastic lymphoma was seen in a 53-year-old HIV positive male who presented with a high grade tumour primary affecting the right inguinal node.

The Detection of Epstein-Barr Virus

The great majority (91.6%) of malignant lymphomas in this study were negative for EBV. Epstein Barr virus encoded RNA was detected in a total of seven cases (8.4%). This included 4 (23.5%) cases of classical Hodgkin lymphoma, 2 (28.6%) cases of Burkitt lymphoma and one case of plasmablastic lymphoma. All EBV positive cases of classical Hodgkin lymphoma were of the mixed cellularity type. The association between EBV positivity and lymphoma subtype was statistically significant ($p=0.002$).

Reclassified Cases

[Table/Fig-10] shows the list of the five cases reclassified as non-lymphoid malignancies. Four of these were carcinomas while the fifth was diagnosed as alveolar rhabdomyosarcoma. The carcinomas included one case each of lymphoepithelioma, melanoma, undifferentiated and anaplastic carcinoma. Four of these were primarily nodal in distribution. All the carcinomas were seen in middle-aged to elderly individuals while the sarcoma was diagnosed in a nine-year-old child.

DISCUSSION

Although lymphomas constitute an important cause of morbidity and mortality in Nigeria data on their immunohistochemical characteristics remain scanty [9,19]. This clinicopathologic review of lymphomas diagnosed in our hospital, thus, provides the first data on their immunohistochemical characteristics in our local Nigerian population.

The overall mean age of 41.7 years in this study compares with what has been reported in various parts of Nigeria and around the world [9,20]. Our study also showed that the disease is twice as common in males as it is in females. This contrasts sharply with those from Europe and North America which show nearly equal sex distribution [21-24]. This perhaps suggests that possible differences may exist between both sexes in the pathogenesis of these tumours in our local population.

Our study identified only nine subtypes of lymphomas, eight of which were non-Hodgkin lymphomas. Overall, intermediate and high grade subtypes predominated unlike in Western countries

where low grade lymphomas are more common [1,6,21,23-26].

The most frequent low grade lymphomas from this study were marginal zone lymphoma, small lymphocytic lymphomas and follicular lymphomas. The marginal zone lymphomas were predominantly extranodal unlike the majority of SLL which presented as cervical lymph node masses. The rarity of follicular lymphoma in Africans was demonstrated in this study [22]. Similarly, our study also confirms the rarity of anaplastic large cell lymphoma and plasmablastic lymphoma as there was only one case of each lymphoma subtype.

Diffuse large B-cell lymphomas formed the vast majority of our cases. These were either intermediate or high grade lesions in nearly equal distribution. Although, they were widely distributed among all age groups, more than two-thirds occurred in individuals older than 20 years old. This is similar to various reports from around the world and Nigeria [9,23,26,27]. It is well known that DLBCL's are a heterogeneous group of tumours with variable morphology, immunophenotype and molecular cytogenetics. They may also represent the endpoint of several indolent lymphomas and therefore could vary widely in incidence and clinical course even in the same region. A large proportion of our cases were sub-classified as belonging to the germinal centre subtype of DLBCL which is typically associated with a favourable prognosis [27,28]. There were, however, about 16 cases which could not be classified into any of the prognostic subgroups based solely on their immunohistochemical profile. Molecular studies will be valuable in unravelling these.

Burkitt lymphoma was the most common childhood lymphoma we encountered in this study. Its frequency was, however, much lower than what has been reported in other series [1,6,15]. This may be due to the fact that majority of cases are diagnosed by the haematologists and do not get to our histopathology department [15]. In addition, there is a reported decline in the incidence of this tumour in our country associated with improvements in our general living conditions [29].

This study confirms the rarity of nodular lymphocyte predominance Hodgkin lymphoma in Nigerians [6,7,15,16,21]. The predominance of the mixed cellularity variant in this study may not be unconnected with its well known association with the EBV [16]. Just like a previous study from our centre has shown, only the mixed cellularity variant was associated with the EBV, an endemic DNA virus which has been incriminated in the aetiopathogenesis of several malignant B cell lymphomas especially in immunocompromised individuals even in Nigeria [16,17]. Although EBV infection is

known to be endemic in Nigeria, its role in the pathogenesis of malignant lymphomas has not been clearly defined. Many reports from Nigeria only provide circumstantial evidence that suggests that such a link does exist. As demonstrated in this study as well as previous such studies from other parts of the country, the implicated lymphoma subtypes include endemic BL, CHL and B cell lymphomas associated with immunosuppression such as HIV-associated lymphomas, plasmablastic lymphomas and many post-transplant lymphoproliferative disorders [16,17,29]. Obviously there is need for a larger multi-centre study to confirm and clearly define the association of EBV with the various subtypes of lymphomas in Nigerians.

The importance of the routine use of immunohistochemistry in the diagnosis of malignant lymphomas even in resource poor countries like Nigeria is underscored by the reclassification of five cases in this study as non-lymphoid malignancies. It therefore becomes imperative that concerted efforts be made to ensure that immunohistochemical techniques are incorporated into the routine diagnostic workup for lymphomas.

CONCLUSION

Immunohistochemistry is vital to the proper classification of lymphomas even in a resource poor environment like Nigeria. Although nine subtypes of lymphomas were identified, B-cell and indeed diffuse large B-cell lymphomas formed the majority of cases. Males were more commonly affected than females. Epstein-Barr virus probably plays an important role in the pathogenesis of malignant lymphomas in our environment although a larger multicentre study is required to prove this.

ACKNOWLEDGEMENT

We acknowledge Professor KN Naresh and all staff of the Histopathology laboratory of the Hammersmith Hospital London NHS trust for the technical assistance received from them. Our appreciation also goes out to the staff of the department of Morbid Anatomy and Histopathology of the Obafemi Awolowo University Teaching Hospitals Complex, Ile-Ife Nigeria for their contributions towards this study.

REFERENCES

- Jaffe ES, Harris NL, Stein H, Isaacson PG. Classification of lymphoid neoplasms: the microscope as a tool for disease discovery. *Blood*. 2008;112(12):4384-99.
- Kluin P, Schuurding E. Molecular cytogenetics of lymphoma: where do we stand in 2010? *Histopathology*. 2011;58(1):128-44.
- Higgins RA, Blankenship JE, Kinney MC. The application of immunohistochemistry in the diagnosis of non-Hodgkin and Hodgkin lymphoma. *Arch Pathol Lab Med*. 2008;132(3):441-61.
- Parsonnet J, Hansen S, Rodriguez L, Gelb AB, Warnke RA, Jellum E, et al. Helicobacter pylori infection and gastric lymphoma. *N Engl J Med*. 1994;330(18):1267-71.
- Lazzi S, Ferrari F, Nyang 'O A, Palumbo N, de Milito A, Zazzi M, et al. HIV associated malignant lymphoma in Kenya (Equatorial Africa). *Hum Pathol*. 1998;29(11):1285-89.
- Adelusola KA, Adeniji KA, Somotun G.O. Lymphoma in adult Nigerians. *West Afr J Med*. 2001;20(2):123-26.
- Oluwasola, O, Olaniyi JA, Otegbayo, JA, Ogun GO, Akingbola, TS, Ukah, CO, et al. A fifteen-year review of lymphomas in a Nigerian tertiary healthcare centre. *J Health Popul Nutr* 2011;29(4):310-16.
- Ferlay J, Shin H, Bray F, Forman D, Mathers C, Parkin MD. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *Int J Cancer*. 2010;127(12):2893-917.
- Omoti CE, Halim NK. Adult lymphomas in Edo state, Niger delta region of Nigeria- Clinicopathologic profile of 205 cases. *Clin Lab Haematol*. 2005;27(5):302-06.
- Baris D, Zahm SH. Epidemiology of lymphomas. *Curr Opin oncol*. 2000;12(5):383-94.
- Gopal S, Wood WA, Lee SJ, Shea TC, Naresh KN, Kazembe PN, et al. Meeting the challenge of hematologic malignancies in sub-Saharan Africa. *Blood*. 2012;119(2):5078-87.
- Akang EEU. Recent methods and techniques in diagnostic histopathology: Impact on tropical pathology practice. *Annals of Tropical Pathology*. 2010;1(1):7-16.
- Campo E, Swerdlow SH, Harris NL, Pileri S, Stein H, Jaffe ES. The 2008 WHO classification of lymphoid neoplasms and beyond: evolving concepts and practical applications. *Blood*. 2011;117(19):5019-32.
- Dunphy CH. Applications of flow cytometry and immunohistochemistry to diagnostic Haematopathology. *Arch Pathol Lab Med*. 2004;128:1004-22.
- Adelusola KA, Sabageh DO, Ukah CO. Lymphoreticular diseases in Nigerians. *Afr Health Sci*. 2008;8(1):20-24.
- Adelusola KA, Titiloye NA, Durosini MA, Rotimi O. Epstein Barr Virus Latent Membrane Protein-1 in Hodgkin's Lymphoma in Nigerians. *Afr Health Sci*. 2009;9(3):174-78.
- Ilyasu Y, Ayers LW, Liman AA, Waziri GD, Shehu SM. Epstein-Barr virus association with malignant lymphoma subgroups in Zaria, Nigeria. *Niger J Surg Sci*. 2013;23(1):6-8.
- Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, et al. WHO classification of tumours of the haematopoietic and lymphoid tissues. 4th ed. Lyon: IARC Press; 2008.
- Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. *CA Cancer J Clin*. 2005;55(2):74-108.
- Hartge P, Devesa SS, Fraumeni JF Jr. Hodgkin and non-Hodgkin lymphomas. *Cancer Surv*. 1994;19(20):423-53.
- Roman E, Smith AG. Epidemiology of Lymphomas. *Histopathology*. 2011;58:4-14.
- Cool CD, Bitter MA. The malignant lymphomas of Kenya: morphology, immunophenotype, and frequency of Epstein-Barr virus in 73 cases. *Hum Pathol*. 1997;28(9):1026-33.
- Peh SC. Host ethnicity influences non-Hodgkin's lymphoma subtype frequency and Epstein-Barr virus association rate: the experience of a multi-ethnic patient population in Malaysia. *Histopathology*. 2001;38(5):458-65.
- Anunobi CC, Banjo AA, Abdulkareem FB, Daramola AO, Akinde RO, Abudu EK. Adult lymphomas in Lagos Nigeria: a fourteen year study. *Nig Q J Hosp Med*. 2007;17(2):63-66.
- Arcan A, Dincol D, Akbulut H, Onur H, Demirkazk A, Cay F, et al. Clinicopathologic features and prognostic factors of primary extranodal non-Hodgkin's lymphomas in Turkey. *Am J Clin Oncol*. 1999;22(6):587.
- Almasri NM, Habashneh MA, Khalidi HS. Lymphoma in Jordan - types and patterns of 111 cases classified according to the WHO classification of haematological malignancies. *Saudi Med J*. 2004;25(5):609-14.
- de Leval L, Harris NL. Variability in immunophenotype in diffuse large B-cell lymphoma and its clinical relevance. *Histopathology*. 2003;43(6):509-28.
- Hans CP, Weisenburger DD, Greiner TC, Gascoyne RD, Delabie J, Ott G. Confirmation of the molecular classification of diffuse large B-cell lymphoma by immunohistochemistry using a tissue microarray. *Blood*. 2004;103(1):275-82.
- Ojesina AI, Akang EE, Ojemakinde KO. Decline in the frequency of Burkitt lymphoma relative to other childhood malignancies in Ibadan, Nigeria. *Ann Trop Paediatr*. 2002;22:159-63.

PARTICULARS OF CONTRIBUTORS:

- Senior Registrar, Department of Morbid Anatomy and Forensic Medicine, Obafemi Awolowo University Teaching Hospitals Complex, Ile-Ife, Osun State, Nigeria.
- Consultant Pathologist, Department of Morbid Anatomy and Forensic Medicine, Obafemi Awolowo University Teaching Hospitals Complex, Ile-Ife, Osun State, Nigeria.
- Consultant Haematologist, Department of Haematology and Blood Transfusion, Obafemi Awolowo University Teaching Hospitals Complex, Ile-Ife, Osun State, Nigeria.
- Consultant Pathologist, Department of Morbid Anatomy and Histopathology, Ladoke Akintola University of Technology Teaching Hospital, Ogbomoso, Oyo State, Nigeria.
- Consultant Pathologist, Department of Anatomic Pathology, Asokoro District Hospital, Abuja, Nigeria.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:

Dr. Donatus Sabageh,
Consultant Pathologist, Department of Morbid Anatomy & Histopathology,
Ladoke Akintola University of Technology Teaching Hospital, Ogbomoso, Nigeria.
E-mail: dsabageh@yahoo.com

FINANCIAL OR OTHER COMPETING INTERESTS: None.

Date of Submission: **Nov 12, 2014**
Date of Peer Review: **Feb 19, 2015**
Date of Acceptance: **May 11, 2015**
Date of Publishing: **Jun 01, 2015**