Clinicopathologic evaluation of different subtypes of Non Hodgkin's lymphoma according to WHO classification

Basem Hassan El-Esawy[1,2] and Ahmad Abd El Hakam Elaskary[2,3]

[1]Pathology Department, Faculty of Medicine, Mansoura University, Mansoura, Egypt.
[2]Department of Medical Laboratory Sciences, Taif University, College of Applied Medical Sciences, Taif, Saudi Arabia.
[3]Department of Medical Biochemistry, Faculty of Medicine, Al-Azhar University, Egypt
basemelesawy2@gmail.com

Abstract: Objectives: To assess the frequency of different subtypes of Non Hodgkin's Lymphomas (NHL) according to WHO classification & study of clinicopathologic correlation. **Methods:** Total 320 biopsy proven cases of NHL, from 2010 to 2013, were selected. The inclusion criteria in all newly diagnosed patients of NHL with appropriate clinical information regarding gender, anatomic location and occurrence of B symptoms. All the cases were evaluated on Haematoxylin and Eosin (H & E) and special stains. Cases were subjected to immunohistochemistry (IHC) using extensive panel of antibodies and classified according to WHO classification of lymphoid neoplasms 2008. **Results:** Clinical data showed that 215(67.2%) were males and 105(32.8%) females. The male to female ratio was 2:1. The B cell lymphoma comprised of 85 % as compared to T cell lymphoma consisting of 15%. The extra nodal involvement was seen in 130 (40.6 %) cases, while 190 (59.4%) cases showed nodal involvement. The B symptoms were found in overall 153 (47.8 %) cases. **Conclusion:** B cell NHL is more common as compared to T cell lymphoma. Diffuse large B cell lymphoma (DLBCL) was the most frequent B cell lymphoma. The major bulk of T cell lymphomas comprised of anaplastic large cell lymphoma (ALCL). Significant association was seen in the occurrence of B symptoms with extra nodal origin and male gender.
[Basem Hassan El-Esawy and Ahmad Abd El Hakam Elaskary. **Clinicopathologic evaluation of different subtypes of Non Hodgkin's lymphoma according to WHO classification.** *Life Sci J* 2015;12(1):170-174]. (ISSN:1097-8135). http://www.lifesciencesite.com. 23

Keywords: Non Hodgkin lymphoma, B & T cell lymphoma Immunohistochemistry , B symptoms

1. Introduction

Lymphoma is a solid tumors of lymphocyte those are important elements of the immune system). The incidence of lymphoma varies according to age, geographical location and exposure to various viral factors.(1) Lymphoma is a heterogeneous disease, has two main subtypes, which namely are Non-Hodgkin Lymphoma [NHL] and Hodgkin Lymphoma [HL].(2) NHL, which comprises 85% of all lymphomas & accounts for 3-4% of all cancers around the world.(3) The incidence of NHL and the patterns of occurrence of its various subtypes vary geographically. The rate of NHL is increasing worldwide and is higher in developed countries than in Africa and Asia according to the World Health Organization International Agency for Research on Cancer (IARC).(4) NHL incidence has shown to be on the rise. This increase is related to the increase in HIV incidence as well as the prevalent use of immune-suppressive drugs.(5) NHL has numerous histological subgroups, entails various biological behaviors, clinical properties and epidemiological differences.(6) Improved lymphoma reporting and changes in lymphoma classification have also contributed to the increased incidence of disease, and it is continuing to increase rapidly.(7) Accurate classification of these requires correlation of clinical features, morphology, immunohistochemistry and genetic testing.(8) Lymphomas cause B symptoms that include drenching night sweats, unexplained weight loss, fever and severe itching.(9) Patients having B symptoms show a more severe condition than asymptomatic patients with the same cancer stage, tumor location or size. Onset of B symptoms at the time of diagnosis suggests that lymphoma is progressing.(10) The lymphomas encompass an array of heterogeneous malignancies.(11) Deficient local data is available, hence clinical insight is needed to identify the exact picture of NHL for further prevention, control and disease etiology, especially in terms of efficacy of treatment protocols being followed(12) Hence The purpose of the present study is to characterize the distribution of malignant non Hodgkin lymphoma subtypes according to the World Health Organization (WHO) classification system 2008 and to demonstrate their clinicopathologic features with respect to histological subtypes.

2. Patients and Methods

All the consecutive biopsy proven cases of NHL (n=320), from October 2010 to June 2013, referred from different laboratories. The inclusion criteria was,

all newly diagnosed patients of NHL having sufficient tumor material in paraffin embedded tissue blocks with appropriate clinical information regarding gender, anatomic location and occurrence of B symptoms. The clinical information of all the patients was noted for gender, site of the tumor and B symptoms, which were, fever (i.e., temperature >38°C for 3 consecutive days, weight loss exceeding 10% of body weight in 6 months, night sweats and severe itching. This was confirmed with the help of medical record, which was available with the patient\'s request at the time of registration of biopsy samples. The cases were classified according to WHO classification.(13)All the biopsied samples taken from nodal or extra-nodal sites were fixed in 10% neutral buffered formalin and processed for paraffin embedding, sectioning and staining by H and E and special stains. Immunohistochemistry (IHC) was done on 4µm thick sections of representative tumour areas, of all the cases. Histological slides were deparaffinized in xylene followed by target retrieval of histological sections with target retrieval solution (Cat # S1700, Dako, Denmark) in water bath at 95ºC for 40 minutes. Background quenching in all specimens was performed by 3% H2O2 for 10 minutes. The antibodies used were Kappa, Lambda, Ki-67, CD 15, CD 20, CD 79a, CD 3, CD 5, CD 45/LCA, CD 10, CD 56, CD 30, CD 23, CD 43, CD 138, Alk-1, EMA, bcl-6, bcl-2, TdT, CD 99 and cyclin D1 from (DAKO, Denmark). The panel of primary antibody was decided on the histomorphology. Primary antibody was incubated for 1 hour in optimized dilution at room temperature. IHC detection was performed using Envision + system (Cat # K4007, Dako, Denmark), previously used by others investigator\'s also.[11] Positive control slides were included with each batch. Slides were examined for the presence of nuclear/ cytoplasmic/ membranous staining (depending on the location of the positivity) within the tumour itself.(14) Each case for IHC was evaluated by an expert histopathologist. A disagreement was resolved by joint review on multi-head microscope and a final consensus was established in each case. Data was subjected to statistical soft ware, SPSS version 16. Gender, types of NHL, anatomic location and occurrence of B symptoms were computed in terms of frequency. Statistical significance of B symptoms with specific phenotype, anatomic location and gender was evaluated by applying chi square test. p value < 0.05 was considered statistically significant.

3. Results:

* Out of 320 patients, 215(67.2%) were males and 105(32.8%) females. The male to female ratio was 2:1. According to immunophenotypic profile, 272 (85%) cases belonged to B cell origin & 48 (15 %) cases were designated as T cell type. Out of total 320 cases, 190 (59.4 %) presented with nodal involvement and 130(40.6 %) had extra nodal site. However, out of 190 patients with nodal involvement, 56 (29.5%) showed the presence of B symptoms, whereas of 130 cases of extra nodal origin, the presence of B symptoms was seen in 92 (70.7%) patients. *Significant association (p = 0.001) was seen in the presence of B symptoms with extra-nodal involvement. Out of 215 males, 125 (58.1%) presented with B symptoms (p = 0.0181), while out of 105 females, B symptoms were present in 25 (23.8%) cases only. Overall B symptoms were found in 153 (47.8 %) cases, out of which 132 (86.3 %) cases were of B cell and 21 (13.7%) cases of T cell type. There was no significant association (p = 0.870) in this regard.

***Table (1): Frequency of different subtypes of NHL**

	B SYMPTOMS		P Value
	Present	Absent	
Cell Lineage B –Cell T-Cell	 132 21	 140 27	0.870
Site Nodal Extra-Nodal	 56 92	 134 38	0.001*
Gender Male Female	 125 25	 90 80	0.0181*

* NHL with nodal presentation showed cervical lymph node involvement in 110 (57.8%) cases followed by 45 (23.7%) inguinal lymph node cases. The site distribution of extra nodal origin was observed in 130(40.6%) of cases. On further analysis DLBCL (203 cases, 63.4 %) was the most common subtype, followed by follicular lymphoma (35 cases, 10.5 %) in B cell NHL. In contrast, anaplastic large cell lymphoma (36cases,11.3 %) dominated the picture in T cell NHL.

* Correlating the presence of B symptoms with types of NHL, it was commonly noted in DLBC, Burkitt lymphoma and lymphoma with precursor B &T cell phenotype, but no significant association (p=0.108) was found in relation to occurrence of B symptoms with specific NHL type.

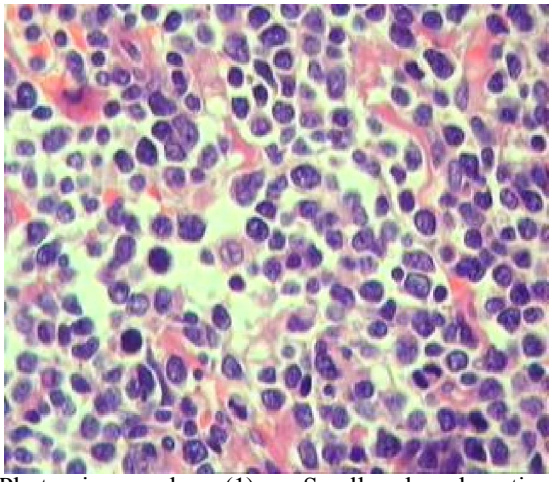
4. Discussion:

NHLs are lympho-proliferative diseases derived from B-lymphocytes, T-lymphocytes and Natural Killer [NK] cells. The incidence frequencies with regard to the cellular origins of NHLs varies across different parts of the world.(15) In the current study we assess the clinic-pathological characters and frequency of NHL. There is a male predominance in the present study in contrast to study performed by Ali

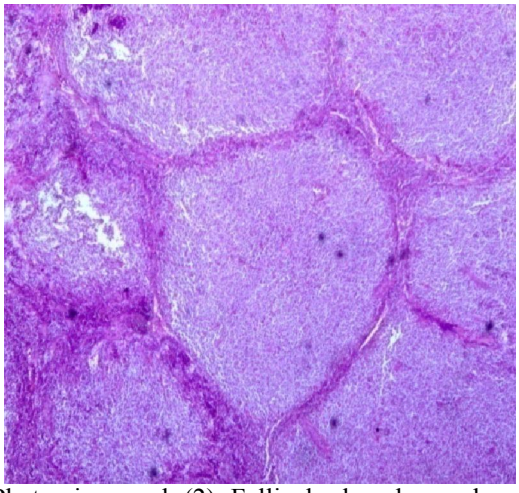
et al 1999.(16) that show female predominance. So there is still a need to generate more data regarding variation in the gender predominance. In the US, 80-85% of NHLs were derivated from B-cells, while 15-20% are from T-cells.(17) In a study performed by Erdal et al.(18) B –cell NHL represents 86.4% while T-cell NHL was in a percentage of 12% which is consistent with figures from the western literature. In the current study B-cell NHL represents 85% while T-cell NHL in a percentage of 15%. The histological subtypes of NHL is also influenced by geographical factors. For example, Diffuse large cell lymphoma is the most common B-cell lymphoma, and is identified at 63.4% among all NHLs in the present work in contrast in the study by Barışta et al. (19), DLBCL incidence of 30.1% was identified, which is consistent with the western literature. Burkitt Lymphoma incidence is only around 0.22 in Europe.(20,21) While in the current study its incidence is higher & represents 3.1% of NHL. Despite follicular lymphomas are more common in Europe and in the USA while it is rare in Africa, China and the Middle East.(22,23,24) In the current study, follicular lymphoma was the second most common subtype among B-cell lymphomas with a percentage of 10.9%. In a study by Işıkdoğan et al.(25), its incidence as 6.1%. Also, we found SLL/CLL with a higher ratio of 4.4%, which is above the ratios reported by Barışta et al.(19) Mantle-cell lymphoma has an incidence of 7% among all NHLs.(26) While the present study has found a lower incidence of 1.7%. Among T-cell lymphomas, ALCL is the most frequent in a percentage of 11.3% which is lower than 21.2% percentage in a study performed by Erdal et al 2013. Also angioimmunoblastic T-cell lymphoma was found in a percentage of 9.1%.(27) However it was not identified in the present work. Variation in nodal and extra nodal distribution was noted from a study to another. In year 2000 in US the percentage of primary extra nodal lymphoma was 27%, Also in western literature 34% of cases as primary extra nodal NHL. The ratio between nodal and extra nodal involvement has a wide range of dissimilarity.(28) In a study performed by Mushtak et al.(29) representing 62 % & 38 % of nodal & extra nodal involvement respectively the current study reveal 59.4% nodal & 40.6% extra nodal involvement. The current series shows 47.8% of patients have B symptoms while Hingorjo et al.(30) show the frequency of 37.1% Also in addition we found significant association of B symptoms with extra nodal involvement (P=0.001) & male gender (P=0.0181). Regarding clinicopathological parameters there is variation when compared to western literature which advocate that there is still a need to generate long term studies to further predict the prognosis of NHL with the aid of clinic-pathological parameters at the time of diagnosis as a strong supportive evidence.

*** Table (2) Frequency of different subtypes of B & T -Cell NHL**

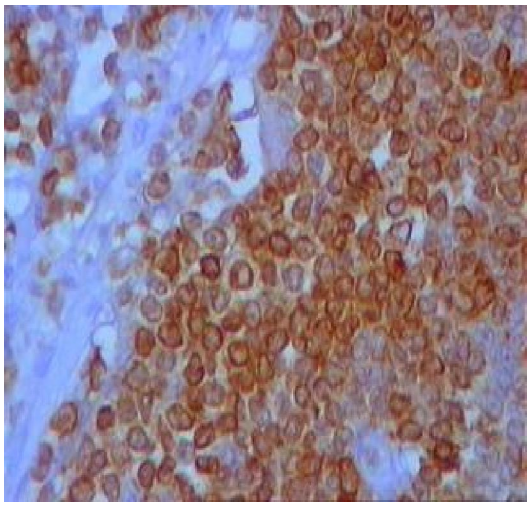
B-cell NHL (272)	Nodal (142)				Extra-Nodal (130)	B Symptoms	
	Cervical	Inguinal	Axillary	Supra-clavicular		Present (132)	Absent (140)
DLBCL 203 (63.4)	46	24	12	6	115	92	60
FL 35(10.9)	26	4	5	-	-	10	48
SLL/CLL 14(4.4)	7	-	2	-	5	8	20
MCL 5(1.7)	5	-	-	-	-	2	8
PCL 2(0.6)	1	-	-	-	1	0	3
BL 10(3.1)	4	-	-	-	6	16	0
B-ALL 3(.9)		-	-	-	3	4	1
T-cell NHL(48)	Nodal (48)				Extra-Nodal	B Symptoms	
	Cervical	Inguinal	Axillary	Supra-clavicular		Present (21)	Absent (27)
ALCL 36 (11.3)	16	10	10	-	-	16	23
T-ALL 7 (2.2)	-	7	-	-	-	5	0
PTCL 5 (1.5)	5	-	-	-	-	0	4



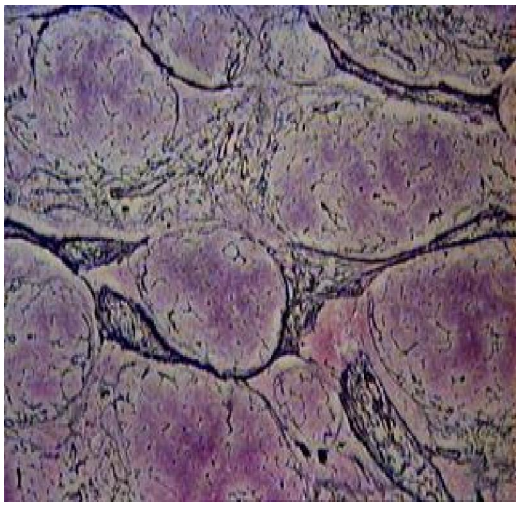
Photomicrograph (1): Small lymphocytic lymphoma with proliferation centers formed of prolymphocytes & paraimmunoblasts (H & E X 400)



Photomicrograph (2): Follicular lymphoma shows tightly packed, fairly uniform follicles distributed throughout the nodal parenchyma (H & E X 100)



Photomicrograph (3): Mantle cell lymphoma shows high expression & diffuse nuclear staining of cyclinD 1 (immunoperoxidase staining, X 400)



Photomicrograph (4): Follicular Lymphoma shows back to back arrangement of neoplastic follicles (reticulin stain X 40)

5. Conclusion:

B cell NHL is more common as compared to T cell NHL, with the most common subtype of B cell NHL is DLBCL, whereas ALCL is the most frequent subtype of T cell NHL. Male gender & extra-nodal involvement show more aggressive behavior as supported by significant association of B symptoms with male gender & extra-nodal origin.

References:

1. Clarke CA, Glaser SL, Dorfman RF, Bracci PM, Eberle E, Holly EA. Expert review of non-Hodgkin's lymphomas in a population-based cancer registry: reliability of diagnosis and subtype classifications. Cancer Epidemiol Biomarkers Prev 2004; 13: 138-43.
2. Eser S, Yakut C, ozdemir R, et al. Cancer incidence rates in Turkey in 2006: a detailed registry based estimation. Asian Pac J Cancer Prev 2010; 11: 1731-9.
3. Cancer incidence, mortality and prevalence worldwide, version 1.0 IARC Cancer Base No.5 [Internet]. IARC. Cited 2001.
4. Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer 2010; 27: 2893-7.
5. Naresh KN, Srinivas V, Soman CS. Distribution of various subtypes of non-Hodgkin's lymphoma in India: a study of 2773 lymphomas using R.E.A.L.

and WHO Classifications. Ann Oncol 2000; 11: 63-7.
6. Anderson JR, Armitage JO, Weisenburger DD. Epidemiology of the non-Hodgkin's lymphomas: distributions of the major subtypes differ by geographic locations. Non-Hodgkin's Lymphoma Classification Project. Ann Oncol 1998; 9: 717-20.
7. Eltom MA, Jemal A, Mbulaiteye SM, Devesa SS, Biggar RJ. Trends in Kaposi\'s sarcoma and non-Hodgkin\'s lymphoma incidence in the United States from 1973 through 1988. J Natl Cancer Inst 2002; 94: 1204-10.
8. Teruya Feldstein J. Getting the diagnosis right in NHL: role of immunohistochemistry and molecular diagnostic testing. J Natl Compr Canc Netw 2008; 6: 422-7.
9. National Comprehensive Cancer Network. NCCN Clinical Practice Guidelines in Oncology: Non-Hodgkin\'s Lymphoma. Cancer Epidemiol Biomarkers Prev 2007; 16: 369-72.
10. Ekstram Smedby K. Epidemiology and etiology of non-Hodgkin lymphoma - a review. Acta oncologica 2006; 45: 258-71.
11. Altekruse SF KC, Krapcho M, Neyman N, eds. Cancer Statistics Review 2007; 1975-2007.
12. Fisher SG, Fisher RI. The epidemiology of non-Hodgkin\'s lymphoma. Oncogene 2004; 23: 6524-34.
13. Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, eds. WHO classification of tumours of Haematopoietic and Lymphoid Tissues. Lyon, France: IARC Press; 2008.
14. Rudiger T, Hofler H, Kreipe HH, Nizze H, Pfeifer U, Stein H, Dallenbach FE, Fischer HP, Mengel M, von WR, Muller-Hermelink HK. Quality assurance in immunohistochemistry: results of an interlaboratory trial involving 172 pathologists. Am J Surg Pathol. 2002;26:873–882.
15. Anderson JR, Armitage JO, Weisenburger DD. Epidemiology of the non-Hodgkin's lymphomas: distributions of the major subtypes differ by geographic locations. Non-Hodgkin's Lymphoma Classification Project. Ann Oncol 1998; 9: 717-20.
16. Ali A, Dilek D, Hakan A, Handan O, Ahmet D, Ahmet DC, et al. Clinicopathologic features and prognostic factors of primary extra nodal non-Hodgkin\'s Lymphomas in Turkey. Am J Clin Oncol 1999; 22: 587.
17. Jemal A, Siegel R, Xu J, Ward E (2010). Cancer statistics, 2010. CA Cancer J Clin 2010; 60: 277-300.
18. Erdal K, Mustafa Y, Hülya K, Mustafa Y, Sevil G, Dinç S, Vildan K. Clinicopathological properties of Non-Hodgkin Lymphomas in the south- west of Turkey. Cancer Research Journal 2013; 1(2): 15-19.
19. Barista I, Tekuzman G, Firat D, et al. Non-Hodgkin's lymphomas in Turkey:eighteen years' experience at the Hacettepe University. Jpn J Cancer Res 1994; 85: 1200-7.
20. Orem J, Mbidde EK, Lambert B, de Sanjose S, Weiderpass E. Burkitt's lymphoma in Africa, a review of the epidemiology and etiology. Afr Health Sci 2007; 7: 166-75.
21. Sant M, Allemani C, Tereanu C, et al. HAEMACARE Working Group. Incidence of hematologic malignancies in Europe by morphologic subtype: results of the HAEMACARE project. Blood 2010; 116: 3724-34.
22. Shih LY, Liang DC. Non-Hodgkin's lymphomas in Asia. Hematol Oncol Clin North Am 1991;5:983–01.
23. Ho FC, Todd D, Loke SL, Ng RP, Khoo RK. Clinicopathologic features of malignant lymphomas in 294 Hong Kong Chinese patients, retrospective study covering an eight-year period. Int J Cancer 1984; 34: 143–8.
24. Piccaluga PP, Sapienza MR, Agostinelli C, et al. Biology and treatment of follicular lymphoma. Expert Rev Hematol 2009; 2: 533-47.
25. Sikdogan A, Ayyildiz O, Buyukcelik A, et al. Non-Hodgkin's lymphoma in southeast Turkey: clinicopathologic features of 490 cases. Ann Hematol 2004; 83: 265-9.
26. Armitage JO, Weisenburger DD. New approach to classifying non-Hodgkin's lymphomas: clinical features of the major histologic subtypes. Non-Hodgkin's Lymphoma Classification Project. J Clin Oncol 1998; 16: 2780-95.
27. Dorfman DM, Brown JA, Shahsafaei A, Freeman GJ. Programmed death-1 (PD-1) is a marker of germinal centerassociated T cells and angioimmunoblastic T-cell lymphoma. Am J Surg Pathol 2006; 30: 802-10.
28. Zucca E, Cavalli F.Extranodal lymphomas.Ann Oncol 2000;11:219-22.
29. Mushtaq S, Akhtar N, Jamal S, Mamoon N, Khadim T, Sarfaraz T, Waqar A. Malignant lymphomas in Pakistan according to WHO classification of lymphoid neoplasms. Asia Pacif J Canc Prevention 2008; 9: 229-32.
30. Hingorjo MR, Syed S. Presentation, staging and diagnosis of lymphoma: A clinical perspective. J Ayub Med Coll 2008; 20: 100-3.

1/16/2015