

Significant Expression of CD22 and CD 79b in the Classification of B- Cell non-Hodgkin's lymphoma in Khartoum State 2014-2015

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Abstract

The aim of this study was to study the CD22 and CD79b markers in the differentiation between different types of B-cell Non Hodgkin Lymphoma and precursor B-cell neoplasm (negativity).30 samples were collected from patients attended the lymphoma centre, 20 samples were FNA and 10 samples were BM. All samples were analyzed by the flowcytometre for the CD22 and CD79b. CD22 was negative in (47 %) of SLL cases, while (52.9%) of them were CD22 positive. Targeted marker came negative in (46.1%) NHL cases and positive in (53.8%) NHL cases. Also (76.4%) of SLL cases were CD79b negative, and (23.6%) of them were CD79b positive, CD79b was negative in (15.3%) in NHL cases and positive in (84.6%) of the cases.CD22 had an insignificant expression in the differentiation between B. cell NHLs subtypes as well as differentiation between SLL and other types of NHL other than cd79b which was considerable in the discrimination between SLL and other types of NHL. The study concluded that using CD79b is very efficient in the differentiation between SLL and other types of NHL, while CD22 was less effective in the differentiation between same groups.

Key words: CD22, CD 79b and B- Cell non-Hodgkin's lymphoma

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Introduction

The two main types of lymphocytes are B lymphocytes and T lymphocytes. Normal T cells and B cells do different jobs within the immune system. ⁽¹⁾ Different types of lymphoma can develop from these different types of lymphocytes. Treatment for each lymphoma depends on which type it is, so determining the exact type of lymphoma is important. ⁽¹⁾

Lymphoma is cancer of the lymph tissue; there are two main types of lymphomas Hodgkin lymphoma is named to Dr. Thomas Hodgkin, who first described it and Non Hodgkin's. Includes a group of more than 20 different malignant lymph proliferative diseases that originate from lymphocytes, types of non-Hodgkin lymphoma can be quite confusing because there are so many types and several different systems have been used, the most recent system is the WHO classification. ⁽¹⁾

Rates of NHL have increased dramatically over the past few decades, although the rate of increase has recently slowed. It is now the sixth most common cancer in Australia. Globally, it is somewhat more common in men than in women, and rates are highest in North America and Australia. The causes of the increase in NHL rates are largely unknown. The best described risk factor for NHL is immune deficiency; rates of NHL are greatly increased, with relative risks of 10-100, in people with immune deficiency associated with immune suppressive therapy ⁽⁴⁾. Non-Hodgkin lymphoma in the United States in 2014 the new cases are 70,800. ⁽⁵⁾

NHLs are common neoplasm in Middle East. The extra nodal forms are apparently far more frequent there than in the west. In addition, an unusual form of primary intestinal lymphoma, namely proliferative small intestinal disease, has been described in Middle Eastern and Mediterranean countries. Several reports indicate high incidence of NHLs in Turkey about 13% of all malignant neoplasm diagnosed in oncology clinic, the mean age for men was 45.5 years and 41 years for women ⁽⁶⁾.

The three most common cancer groups in children in North African countries were lymphomas (35%), leukemia (26%) and Wilms' tumors (13%). ⁽²⁾

In Sudan recent study analyze the cases during the period from 1979 to 1989 in Soba teaching hospital they found that there were 1205 patients with malignancy, 51 patients of them with NHLs (comprising 5.4% of all malignant tumors). The male: female ratio was 4.1:1, the age of patients ranged between few months to 90 years old, and the age group (40-70) years show higher frequency of NHLs ⁽⁷⁾.

CD79 is composed of CD79a and CD79b components expressed almost exclusively on B cells and B-cell neoplasm. CD79a and CD79b expression precedes immunoglobulin (Ig)⁽³⁾.

Lymphoma can be diagnosed using different methods such as biopsy (FNA, core needle , surgical) , routine histology , immunophenotyping or molecular or genomic feature. Immunophenotyping tells what kind of cell it is , classify whether its B. cell or T,cell according to CD and pattern of markers lead to diagnosis⁽⁸⁾. The flowcytometer performs this analysis by passing thousands of cells per second through a laser beam and capturing the light that emerges from each cell as it passes through. The data gathered can be analyzed statistically by flowcytometry software to report cellular characteristics such as size, complexity, phenotype, and health⁽⁹⁾.

Flow cytometric immunophenotyping remains an indispensable tool for the diagnosis, classification, staging, and monitoring of mature lymphoid cell neoplasm. flow cytometry improved ability to identify different normal cell populations and recognize phenotypic aberrancies, even when present in a small proportion of the cells analyzed.⁽¹¹⁾ Phenotypically abnormal populations have been documented in many neoplasms, including lymphoma, chronic lymphoid leukemia, plasma cell neoplasm, acute leukemia, mast cell disease, myelodysplastic syndromes, and myeloproliferative disorders.⁽¹¹⁾ The aim of this study was to study the CD22 and CD79b markers in the differentiation between different types of B-cell Non Hodgkin Lymphoma and precursor B-cell neoplasm (negativity).

Materials and Methods

30 samples were collected from known lymphoma patients attended flow cytometre center, of whom 20 samples were FNA and 10 samples were bone marrow. Tow ml of bone marrow aspiration was collected in Lithium Heparin vacontainer (5ml).⁽¹⁰⁾ Fine needle aspiration was collected in sterile 5 ml plain container containing 3.0 ml fresh isotonic phosphate buffered saline solution PH. 7.2.

Monoclonal antibody combination procedure for fine needle aspiration and B.M aspiration

20 uL of monoclonal antibody was added into tubes, tubes were incubated at room temperature for 15 minutes. Then, 1 ml of the "fix-and-lyses" mixture was added to the tubes and vortexes immediately for three seconds, this step was done only for FNA samples.

Each tube was incubated at room temperature for at least 10 minutes and was protected from light. Then centrifuged at 150 x g for 5 minutes and discards the supernatant by aspiration. 3 mL of PBS was added. All tubes were centrifuged in 150 x g for 5 minutes and the supernatant were discarded by aspiration. The pellets were Re-suspended by addition of 0.5 to 1 mL of 0.1% formaldehyde. All tubes were vortexes for 5 seconds. All tubes were analyzed by the flow cytometer.

Study was approved by the ethical committee, College of Medical Laboratory Science Research Board, EL Neelin University. Also ethical clearance from ministry of health was obtained.

Results

Table 1 shows sex frequency among study population, males were (80 %) females were (20 %) also shows age distribution. NHL and SLL diagnosis showed 2 people of the age group (0-16) years and 28 people of the age group (16-85) years (13.3% - 93.3%) respectively. and sample type distribution. (33.3%) samples were collected from bone marrow. (66.7%) samples were collected from lymph node.

Table 2 shows the results of CD22 in SLL was negative in 47 % and positive in 52.9% of cases. While CD22 in NHL was negative in 46.1% and positive in 53.9 % of cases. The results of CD79b in SLL were negative in 76.4% of cases, and positive in 23.6% of cases. CD79b in NHL negative in 15.3% of cases and positive in 84.6% of the cases.

Table 3 in B. cell NHL subtypes result of CD22 was negative MCL/SPV1/BL/FL and positive in DLBCL/HCL. CD79b was negative in DLBCL/MCL and was positive in all of the subtypes.

Table: 1. Frequency of sex, age and sample types.

	Sex			Age			Sample type		
	F	M	total	<=16 years	>16 years	total	BM	FNA	Total
Frequency	6	24	30	2	28	30	10	20	30
Percent	20	80	100 %	6.6	93.3	100%	33.3	66.7	100 %

Table: 2. Relation between diagnosis and cd22 and cd79b results

	Results	diagnosis		p. value
		SLL	NHL	
CD22	Negative	8 (47%)	6 (46.1%)	0.962
	Positive	9 (52.9%)	7(53.9%)	
CD79b	Negative	13 (76.4%)	2 (15.3%)	0.000
	Positive	4 (23.6%)	11 (84.6%)	

Table: 3. Relation between CD22 and CD79b remark with sub types

Remark		Sub diagnosis						
		DLBCL	MCL	HCL	SPVL	BL	FL	TOTAL
Cd22	negative	0	2	0	2	1	1	6
	positive	5	0	2	0	0	0	7
Cd79b	Negative	1	1	0	0	0	0	2
	Positive	4	1	2	2	1	1	11

Discussion

In this study men were more affected than females 80%, 20% respectively, this finding is similar to that of Ansell, *et al.* Who reported that most non-Hodgkin lymphomas (NHLs) are common in men.¹² and Gujral, *et al* ⁽¹³⁾ also reported that the ratio between men to women was 4:1.

In the present study the diagnosis of NHL and SLL revealed the age group (0-16) years and (16-85) years were 13.3% and 93.3% respectively. Therefore NHL majority of patients were among the adulthood, which means that the MBCN is generally spread in the age groups of relatively higher ages, To some extent, this finding is similar to that of Ansell *et al*, ⁽¹²⁾ who reported that the median age at diagnosis of most MBCNs exceeds 70 years and Gujral *et al* ⁽¹³⁾ findings who found that the median age was 57 years.

In the present study CD22 expression was insignificant in the differentiation between B. cell NHLs subtypes showed same percent of positive expression in most of these types. However some of (SLL/MCL/SPVI/BL/FL) showed negative expression. This finding

disagreed with W. Gorczyca⁽¹⁴⁾, who approved that spvl cases were CD22 bright. While in DLBCL/HCL cases CD22 had positive expression which compatible with Gorczyca⁽¹⁴⁾ who showed CD22 was bright in HCL.

CD22 found to have an insignificant role in differentiation between SLL and other types of NHL as delgado, *et al*⁽¹⁵⁾ showed that CD22 is of little diagnostic value in differentiation between SLL and other type of mature B. cell NHL.

CD79b had an insignificant importance in separation between B.cell NHL subtypes with however some cases of HCL/SPVL/BL/FL came with a positive expression. While SLL/DLBCL/MCL were with negative expression.

CD79b was highly significant in the discrimination between SLL and other types of NHL with. Due to low positivity of CD79b in 23.6% of SLL cases while high positivity of CD79b in 84.6% of NHL cases. CD79b seems to be a useful addition to a standard flow cytometry panel for the evaluation of BCLPDs⁽¹⁶⁾. The pattern of expression and expression level of CD79b used for distinguishing SLL from CLL patients⁽¹⁷⁾. Our finding compatible with Tijana Dragović Ivančević *et al* whose reported that these characteristics make this antigen CD79b a good marker for differential diagnosis of SLL⁽¹⁸⁾.

Conclusion

The study concluded CD79b is very efficient in the differentiation between SLL and other types of NHL. While CD22 was less effective in the separation between same groups.

References

1. American cancer society (2009). What is non- Hodgkin's lymphoma. pp 1-9.
2. Yousif M Y A (2007). Presentation Current Status of Radiation Therapy in Sudan, Radiotherapy and Nuclear Medicine Department, College of Medical Radiological Science, SUST.
3. Chan J K and Hui P K (1988). A simple guide to the terminology and application of leukocyte monoclonal antibodies. *Histopathology*, 12: pp 461–480.
4. Andrew E. Grulich . Claire M. Vajdic (2005). The epidemiology of NHL. Taylor and Fancis .Australia. pp409-419.
5. American cancer society (2014). Surveillance and health services program Cancer facts and figures book .15.
6. Sarpel SC, Paydas S, Tuncer I, Varinli S, Koksall M, Akoglu T (1988). Non-Hodgkin's lymphomas in turkey. *Cancer Oct*. 62(8):1653-7.
7. Khalid H H, Adel H E, Badereldin M Y and Magdi M A (2014). Immunophenotyping of Non-Hodgkin's lymphomas in Sudan statistics by Salwa H.S NHL soba university (1992) in soba university hospital (1979-1989). *Pan Afr Med J*. 2014; 18: 82.
8. Shannon M Campbell Sara B Roberts Mathewo J, and Henry K (2010). Immunophenotypic diagnosis of primary cutaneous lymphoma. *J Clin Aesthet Dermatol*. 3(10): 21–25.
9. American cancer society (2014). NHL diagnosis report. How NHL is diagnosed. 24.
10. Falini B, Tiacci A, Liso K, Basso E, Sabattini R, Pacini R, Foa, *et al* (2004). Simple Diagnostic Assay for Hairy Cell Leukaemia by Immunocytochemical Detection of Annexin A1 (Anxa1). *Lancet* 363, 9424. 1869-70.
11. Craig F E and Foon K A (2008). Flow Cytometric Immunophenotyping for Hematologic Neoplasm. *Blood* 111, 8. 3941-67.

12. Ansell P J, Simpson T, Lightfoot A, Smith E, Kane D, Howell R, Newton, *et al* (2011). Non-Hodgkin Lymphoma and Autoimmunity: Does Gender Matter. *Int J Cancer* 129, 2: 460-6.
13. Gujral S S, Polampalli Y, Badrinath A, Kumar P G, Subramanian R, Nair S, Gupta M, Sengar and Nair C (2009). Immunophenotyping of Mature B-Cell Non Hodgkin Lymphoma Involving Bone Marrow and Peripheral Blood: Critical Analysis and Insights Gained at a Tertiary Care Cancer Hospital. *Leuk Lymphoma* 50, 8. :1290-300.
14. Gorczyca W.ojciech (2010). Flow cytometry in neoplastic hematology. Informa, UK. pp 96.
15. Delgado J E, Matutes A M, Morilla R M, Morilla K A, Owusu-Ankomah F, Rafiq-Mohammed I, del Giudice and Catovsky D (2003). Diagnostic Significance of Cd20 and Fmc7 Expression in B-Cell Disorders. *Am J Clin Pathol* 120, 5 : 754-9.
16. McCarron K F, Hammel J P, and His E D (2000). Usefulness of Cd79b Expression in the Diagnosis of B-Cell Chronic Lymphoproliferative Disorders. *Is J Clin Pathol* 113, 6: 805-13.
17. Jovanovic D, Djurdjevic P, Andjelkovic N, Zivic L(2014). Possible role of CD22, CD79b and CD20 expression in distinguishing small lymphocytic lymphoma from chronic lymphocytic leukemia. *Contemp Oncol (Pozn)* .; 18(1):29-33.
18. Tijana Dragović Ivančević¹, Nada Kraguljac Kurtović¹, Vesna Knežević¹ *et al*, (2014). The Role of Immunophenotyping in Differential Diagnosis of Chronic Lymphocytic Leukemia, *Srp Arh Celok Lek.* ; 142(3-4):197-203.