The Role of CD5 and CD23 in Classification of B. Cell Non Hodgkin Lymphoma in patient of Khartoum State Using Flow Cytometer 2014-2015

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Abstract

The aim of this study was to determine the role of CD5 and CD23 in classification of B. cell non Hodgkin lymphoma and precursor B-cell neoplasm (negativity) in Khartoum state using flow cytometer. 30 samples were collected from patients attended the lymphoma center .19 samples were FNA and 11 samples were B.M. In this study sex distribution among study population found that 20% of patients were females and 80% were males. Age of patients was divided into two groups, below 16 year was 2 (6.6%) patients and above 16 year was 28 (93.4%) patients.

All samples were analyzed by the flowcytometre for the CD5 and CD23. CD5 was negative in (5.9%) of SLL cases, while (94.1%) of them were CD5 positive. Targeted marker came negative in (23.1%) NHL cases and positive in (76.9%) NHL cases. Also (11.8%) of SLL cases were CD23 negative, and (88.2%) of them were CD23 positive, CD23 was negative in all cases of NHL.

We concluded that using of co-expression of CD5/CD23 had remarkable efficiency in the differentiation between sub types of non hodgkin lymphoma in general as well as in identification of SLL. And using of this marker in scoring system is useful.

Key words: CD5, CD 23 and B- Cell non-Hodgkin's lymphoma

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Introduction

Lymphoma is a group of blood cell tumor that developed from lymphocyte [1].
Treatment for each lymphoma depends on which type it is, so determining the exact type of lymphoma is important. [1]Mature B-cell neoplasm (also known as non-Hodgkin's lymphoma, NHL) is a cancer that starts in lymphocytes [1] .B-cell lymphomas make up most (about 85%) of non-Hodgkin lymphomas in the United States. Non-Hodgkin lymphoma in the United States in 2014 the new cases are 70,800 [2]. Because lymphoid tissue is in many parts of the body, lymphomas can start almost anywhere. The major sites of lymphoid tissue are Lymph nodes are bean-sized organs throughout the body that are connected by a system of lymphatic vessels [1] . 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 [3].

Sudan is the largest country in Africa, in terms of geographical area, with a population of over 36 million and an average life expectancy of 57 years for men and 62 years for women. [4] In sudan among 10 years in soba hospital found 1205 patient malignancy .51 of them NHL5.4 of hole malignancy .male to female ratio 4:1 age of the patient range few months to 90 years old and the age group 40 /70 show higher frequency of NHL (SALWA HS 92) also resent study in sudan in 2014 agree with the previous study[5] The three most common cancer groups in children were lymphomas (35%), leukaemias (26%) and Wilms' tumors (13%). [6] In 2004 ,54370 new cases of NHL and approximately 19410 deaths from the disease are expected ·As the fifth most commonly occurring cancer in both men and women" there are approximately 19/ 100000 persons in the US each year ,the disease occurs more often in whites than in blacks ,and it is about 50% more common among men the women . There are 2 main types of lymphomas Hodgkin lymphoma (is named after Dr. Thomas Hodgkin, who first described it). Hodgkin lymphoma has characteristics that distinguish it from other diseases classified as lymphoma including the presence of Reed Sternberg cell. These are large, cancerous cell found in Hodgkin lymphoma tissues and non Hodgkin lymphoma. These 2 types of lymphomas differ in how they behave, spread, and respond to treatment [1] .Classifying of non hodgkin lymphoma can be quite because there are so many types and because several different systems have been used. The most recent system is the *WHO* classification. The WHO system groups lymphomas based on how they look under a microscope, the chromosome features of the lymphoma cells, and the presence of certain

chemicals on the surface of the cells [1]. CO-expression of CD5 and CD23 ,a finding frequently associated with neoplastic proliferations ,is also measured .CD10(CALLA),CD23,FM-7 and HLA-DR are included with the standard profile other markers may be added in assessing T-cell disorders (CD1a,CD30), hodgkin disease (CD15,CD30),or anaplastic (Ki-1)lymphoma (CD30)[7] .These lineage-specific cell surface markers are produced by the normal genetic program of the cells or by aberrant expression patterns that are pathologic. The cell markers are designated according to a standard nomenclature that defines Clusters of Differentiation (CD) by scientific consensus. They are detected by a process that combines fluorescently-labeled, monospecific immunological reagents and a flow cytometer to count and analyze the cell populations. [4]Flow cytometry is a powerful technique for the analysis of multiple parameters of individual cells within heterogeneous populations. Because it has many advantages which helping for quickly, accurate, specific and sensitive result of analysis [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]. The aim of this study was to study the expression of (CD5 and CD23) markers using in the differentiation between different types of B-cell Non Hodgkin Lymphoma and CLL using flowcytometer.

Materials and Methods

This study carried out in Khartoum flowcytometer center. 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). Fine needle aspiration was collected in sterile 5 ml plain container containing 3.0 ml fresh isotonic phosphate buffered saline solution PH. 7.2.

Method of sample collection and processing

Fine Needle Aspiration was collected in sterile 5 ml plain container containing 3.0 ml fresh Isotonic Phosphate Buffered Saline Solution PH. 7.2 (IPBSS).

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 Alneelain University.

Results

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

	AGE			SEX			Sample type		
	<16	>16	Total	Male	Female	Total	B.M	FNA	Total
Frequency	2	28	30	24	6	30	11	19	30
Percent	6.6%	93.3%	100%	80%	20%	100%	36.6%	63.3%	100%

Shows sex distribution among study population this study found that 20% of patient were female and 80% were male .sample provided in this study was B.M (36.6%) and FNA(63.3%) ,age less than 16 year was 2 (6.6%) and above 16 year was 28 (93.4%).

Table 2: Relation between diagnosis and CD5, CD23 result

RESULT		DIAGNOSIS SLL NHL		Total
CD5	+	16	3	19
	-	1	10	11
CD23	+	15	0	15
	-	2	13	15

P. value 0.00

Table 2 Shows the results of CD5,which were positive in (94.1%) and negative in (5.9%) of SLL. While it is positive in (23.1%) and negative in (76.9%) of NHL. The results of CD23, which were positive in (88.2%) and negative in (11.8%) of SLL. While it was negative in all cases of NHL.

Table: 3. Relation between CD5 and CD23 remark with B. cell NHL subtypes

Remark		**Sub diagnosis**						
		DLBCL	MCL	HCL	SPVL	BL	FL	TOTAL
CD5	negative	5	0	2	1	1	1	10
	positive	0	2	0	1	0	0	3
CD23	Negative	5	1	2	2	1	1	13

B. cell NHL subtypes result of CD5 was negative DLBCL,HCL,SPVL,BL,FL and positive in MCL,SPVL. CD23 was negative in DLBCL, MCL, HCL, SPVL, BL, FL (Table 3).

Discussion

CD23 is one of the B cell markers that appears in MBCN cells and do not appear in the stages of B cells maturation .show insignificant importance in the differentiation between B-cell NHL with P.V (.027) while it was significant in the differentiation between SLL and other type of B-cell non hodgkin lymphoma with P.V (.000) due to highly positive of CD23 in SLL cases (88%) this finding match with Gujral et al,(2009)[12]. Who reported that Frequency of CD23 negativity in SLL is very low while most cases of NHL showed negative expression except few cases of MCL..so we can use the co-expression of CD5/CD23 for the proper diagnosis and differentiation between SLL and other type of non hodgkin lymphoma ,this finding was agree with Kaleem et al (2000)[13] who have shown phenotype of CD5+/CD23- for diagnosis of SLL was highly specific and had a high PPV(93%).

CD5 is one of the T cell markers and it has aberrant expression in some B cell of MBCN. It is considered as one of the most important markers in the diagnosis and classification of mature B cell neoplasms, CD5 showed significant importance in the differentiation between B-cell non hodgkin lymphoma with P,V (.028) .our finding showed that the positive expression of sCD5 were SLL/MCL. While negative expression with HCL/BL FL. Also CD5 showed highly importance in the differentiation between (SLL/MLL) and other type of B-cell NHL with P,V(.000). the percent of CLL positive cases (94.1%) and (100%) for MCL while the positive cases of NHL were only (23%). This finding match with Salomon et al.,(1995)[14]. Who mention that jest 8% of CLL cases were negative for CD5.therefore the using of CD5 had less importance in differentiation between SLL/MCL due to high positivity showed in both of them using of CD23be more beneficial in differentiation between CLL/MCL lymphoma ,this finding compatible with W.Gorczyca [15] who mention that SLL differ from MCL by CD23positivity.

Conclusion

We concluded that using of co-expression of CD5/CD23 had remarkable efficiency in the differentiation between sub types of non Hodgkin lymphoma in General as well as in Identification of SLL. And using of this marker in scoring system is useful.

References:

1. American Cancer Society,(2009), what is non Hodgkin lymphoma, page: 1-9.

2. American cancer society (2014). Surveillance and health services program Cancer facts and figures book .15.

3.Andrew E. Grulich . Claire M. Vajdic (2005). The epidemiology of NHL. Taylor and fancies .Australia. pp409-419.

4/ Dafalla O. Aduidris, Mohamed E. Ahmed, Elgaili M Elgaili (2007). Childhood cancer in Sudan, 38 (4): 208 – 212.

5.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

6. Yousif M Y A (2007). Presentation Current Status of Radiation Therapy in Sudan, Radiotherapy and Nuclear Medicine Department, College of Medical Radiological Science, SUST.

7. Borowitz M, Guenther KL, Shults KE, Stelzer GT (1991). Immunopheno- heterogeneity at diagnosis. Leukemia ; 5: 757–767. typing of acute leukemia by flow cytometry analysis. Use of CD45 14 Abrahamsen JF, Lund-Johansen F, Laerum OD, Schem BC, Sletand right-angle light scatter to gate on leukemic blasts in three- vold O, Smaaland R. Flow cytometric assessment of peripheral colour analysis. Am J Clin Pathol 1993; 100: 534–540.

8. Bikoue, A., G. Janossy, and D. Barnett.(2002). Stabilised cellular immmunofluorescence assay (SCIFA): CD45 expression as a calibration standard for human leukocytes. J. Immunol. Methods 266:19–32.

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. 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.

12. 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

13. Kaleem, Z., B. A. Zehnbauer, G. White, and M. M. Zutter (2000). "Lack of Expression of Surface Immunoglobulin Light Chains in B-Cell Non-Hodgkin Lymphomas." *Am J Clin Pathol* 113, no. 3: 399-405

14. Salomon-Nguyen, F., F. Valensi, H. Merle-Beral, and G. Flandrin (1995). "A Scoring System for the Classification of Cd5-B Cll Versus Cd5+ B Cll and B Pll." *Leuk Lymphoma* 16, no. 5-6: 445-50..

15. W.ojciech .Gorczyca (2010). (flow cytometer in Neoplastic Hematology.pag 96.informa,UK.

16. R M Rowan, B J Bain, et al, (1994). Immunophenotyping in the diagnosis of acute leukaemias, General Haematology Task Force of BCSH,; 47:777-781.