EUROPEAN ACADEMIC RESEARCH
Vol. IV, Issue 9/ December 2016

Impact Factor: 3.4546 (UIF)
DRJI Value: 5.9 (B+)

ISSN 2286-4822
www.euacademic.org

The Important Role of HLA-DR in Differentiation between Different Types of Mature B-Cell Neoplasm in Sudanese Patients by Flowcytometry

AREEG ABDELAZIM HASSAN
Medical Laboratory Second Specialist
Department of Haematology, Faculty of Medical Laboratory Sciences
Al-Neelain University, Sudan
ENAAM A. ABDELGADER
Associate Professor of Hematology, Al Neelain University, Sudan
Consultant Clinical Hematologist
Radiation and Isotope Center - Khartoum (RICK)
OSAMA A. ALTAYEB
Flowcytometry Consultant
Flowcytometry Laboratory, Khartoum, Sudan
EMAN ABBASS F.
Flowcytometry Specialist
Flowcytometry Laboratory, Khartoum, Sudan
ASMA M. SHAMMET
Clinical Pathologist
Flowcytometry Laboratory, Khartoum, Sudan
ELDIRDIRI M. ABDELRHMAN
Associate Professor of Medicine and Oncology (KCMS)
Khartoum College of Medical Sciences

Abstract:

Background: *HLA-DR is an MHC class II cell surface receptor encoded by the human leukocyte antigen complex on chromosome 6 region 6p21.31. HLA-DR is expressed on B-Cell at different stage of maturation except of most mature plasma cell, hematopoietic progenitor cells, myloid, erythroid , and megakaryocytic*

precursor, monocyte\dendretic cells and immature T-Cells. HLA-DR is expressed on myeloblast but it lost during maturation to the promyelocytic stage, among malignant tumors, HLA-DR is positive on precursor and mature B-Cell lymphprolifrative disorder and the majority of AML (M0, M1,M2,M4,M5)- both hypergranular and hypogranular (M3) are HLA-DR negative. Plasma cell neoplasm and the majority of T-Cell lymphoma\leukaemia do not express HLA-DR.

***Aim of the study**: To evaluate the expression of HLA-DR (percentage and meanfluorescence intensity) in Sudanese patient wit mature B-cell neoplasm.*

***Methodology:** This is descriptive cross-sectional study, conducted in Flowcytometry centre in Khartoum state, 30 new cases of mature B-cell neoplasm analyzed by flowcytometry for HLA-DR expression. The flowcytometery lysing procedure for bone marrow aspiration and peripheral blood HLA-DR combination was done as the follows: All tubes were labeled then pipette into each tube 20 µL of HLA-DR marker and added 100 µL of sample. The tubes were incubated in dark room at temperature of 4°C, for 30 minutes or at room temperature (20-25) °C for 15 minutes. 1.0 ml of lysing solution added to each sample and mixed gently with a vortex for 2 seconds and then incubated for 10 minutes at room temperature in the dark, centerfugated at 1000xg for 5 minutes, the supernatant and discard gently aspirated leaving approximately 50 µl of fluid. 1ml of PBS 0.01 mol\L added and and resuspended the cells bu using a vortex mixture, all tubes were run on flowcytometer.*

***Result and discussion**: In this study we found that most case of B-LPD were positive for HLA-DR so we found that, (p.value= 0.00)is significant. Means that we can use HLA-DR in the identification of mature B-cell neoplasm and excluding of T & NK cases. There is no significant correlation between HLA-DR parameters (percentage & Mean intensity) and other general population distribution : Age, Gender, Sample type, Diagnosis and cases scores. (p.value= 2.8, 1.8, 3.4, 0.94 and 1.2 respectively). There is no significant correlation between HLA-DR parameters (percentage & Mean intensity) and complete blood count figures: TWBCs, RBCs, HB and PLTs. (p.value= 1.8, 2.4, 2.1 and 0.9 respectively).*

Key words: role of HLA-DR, mature B-Cell neoplasm, Sudanese patients, flowcytometry

INTRODUCTION:

The B-cell lymphomas are types of lymphoma affecting B-Cells. They are blood cancers in the lymph nodes . They develop more frequently in older adults and in immunocompromised individuals.

B-cell lymphomas include both Hodgkin's lymphomas and most Non-Hodgkin's lymphomas. They are typically divided into low and high grade, typically corresponding to indolent (slow-growing) lymphomas and aggressive lymphomas, respectively. As a generalisation, indolent lymphomas respond to treatment and are kept under control (in remission) with long-term survival of many years, but are not cured. Aggressive lymphomas usually require intensive treatments, with some having a good prospect for a permanent cure. (1)

They divide into common and rare, common are: (2)

Diffuse large B-Cell Lymphoma (DLBCL) (3), Follicular lymphoma, Marginal zone B-Cell lymphoma (MZL), Small lymphocytic lymphoma (chronic lymphocytic leukemia), Mantle cell lymphoma (MCL).

Rare: (4)

*DLBCL:

Primary mediastinal large B-cell lymphomas, T Cell histocyte rich large B-cell lymphoma, Primary cutaneous diffuse large B-cell lymphoma, EBV positive diffuse large B-cell lymphoma of the elderly, Diffuse large B-cell lymphoma associated with inflammation.

*Burkitt's lymphoma, Lymphomplasmacytic lymphoma, Nodal marginal zone B-cell lymphoma (NMZL), Splenic marginal zone lymphoma (SMZL), Intravascular large B-cell

lymphoma, Primary effusion lymphoma, Lymphomatoid granulomatosis, Primary central nervous system lymphoma, ALK positive large B-cell lymphoma, Plasmablastic lymphoma, Large B-cell lymphoma arising in HHV8 associated multicentric Castleman's disease, B-cell lymphoma unclassifiable with features intermediate between diffuse large B-cell lymphoma and Burkitt lymphoma, B-cell lymphoma unclassified with features intermediate between diffuse large B-cell lymphoma and classical Hodgkin lymphoma. (5)

CLL immunophynotypic score (Matutes score):

Flow cytometric analysis of peripheral blood or bone marrow if performed for expression of the cell surface markers.

A score _> 4 is individual of CLL. a score of <_ 3 should promote consideration of an alternative diagnosis. (6)

Quality control:

Depending up on pilot study in the quality control results (that saved in the Q.C system II software file) of EPICS XL Flowcytometer, which adjusted the cut off points between negative and positive scale for every marker, Positivity was considered when =30% of the population expressed the marker. The percentages, mean fluorescence intensity were also recorded for most of the markers.

RESULTS:

There is a highly significant relation between B-LPD and positivity of HLA-DR. (p.value= 0.00). Means that we can use HLA-DR in the identification of B-LPD and excluding of T & NK cases.

There is no significant correlation between HLA-DR parameters (percentage & Mean intensity) and other general population distribution : Age, Gender, Sample type, Diagnosis

and cases scores. (p.value= 2.8, 1.8, 3.4, 0.94 and 1.2 respectively).

There is no significant correlation between HLA-DR parameters (percentage & Mean intensity) and complete blood count figures: TWBCs, RBCs, HB and PLTs. (p.value= 1.8, 2.4, 2.1 and 0.9 respectively).

Male was 76.2% and female was 23.8%

Bone marrow samples were 16.7% and peripheral samples were 83.3%

Cases were 59.5% and NHL cases were40.5%

Scores of the cases were: Score 0 (14%), score 0.5 (5%), score 1.0(8%), score 1.5(7%), score 2.0(2%), score 2.5(2%), score 3.0(3%), score 3.5(2%), score 4.0(20%), score 4.5(6%) and score 5.0(30%)

Most cases showed high percentage of HLA with both diseases.

There was no significant correlation between the HLA percentage level and the diagnosis. (*p.value* = 1.75)

In high score (3 – 5), HLA percentage was correlate with high percentage more than low percentage while in low score (0 - 2.5) was different.

There were no low percentage with score 0.5, 1.0, 1.5 and 3.5 which mean that we can use the present of low percentage to exclude these scores.

There were no high percentage with score 2.5 which mean that we can use the present of high percentage to exclude the 2.5 score only.

Scores 2.0 and 2.5 were only two scores showed low percentage more than high percentage.

There was no significant correlation between the HLA percentage level and the score. (*p.value = 0.9*12)

In high score (3 – 5), HLA mean intensity was correlate with bright expression more than dim expression while in low score (0 - 2.5) was different.

There were no dim expressions with score 1.5 and 3.0 which mean that we can use the dim expression to exclude these scores.

There were no bright expression with score 2.5 which mean that we can use the present of bright expression to exclude the 2.5 score only.

Scores 2.0 and 2.5 were only two scores showed dim expression more than bright.

There was no significant correlation between the HLA mean intensity level and the score. (*p.value = 0.858*)

Gender distribution

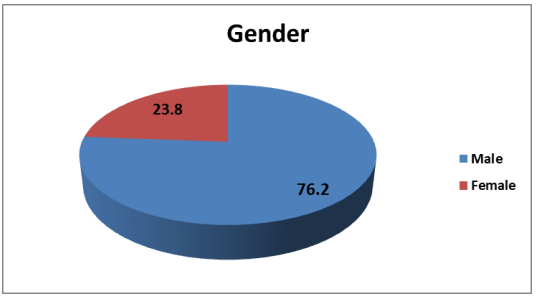

Figure 1: showed that male was 76.2% and female was 23.8%

Sample type distribution

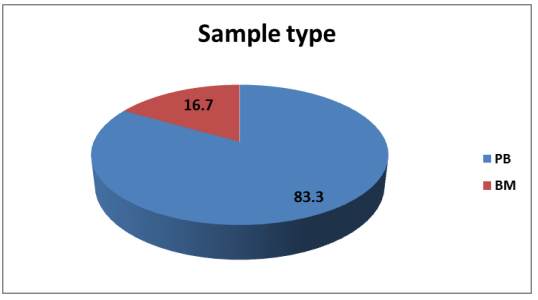

Figure 2: showed that bone marrow samples were 16.7% and peripheral samples were 83.3%

Cases diagnosis distribution

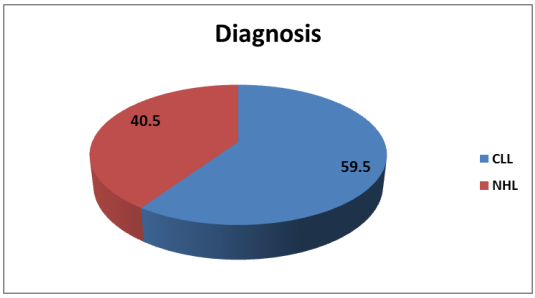

Figure 3: showed that CLL cases were 59.5% and NHL cases were 40.5%

Cases score distribution

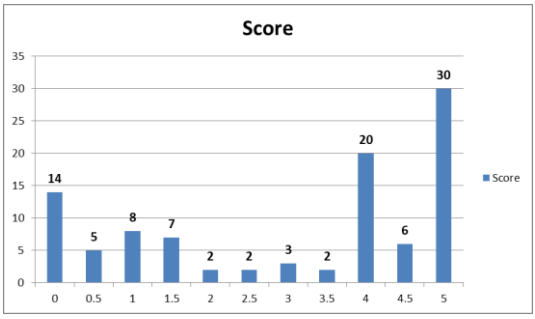

Figure 4: showed that scores of the cases were: Score 0 (14%), score 0.5 (5%), score 1.0(8%), score 1.5(7%), score 2.0(2%), score 2.5(2%), score 3.0(3%), score 3.5(2%), score 4.0(20%), score 4.5(6%) and score 5.0(30%).

HLA Percentage level X diagnosis

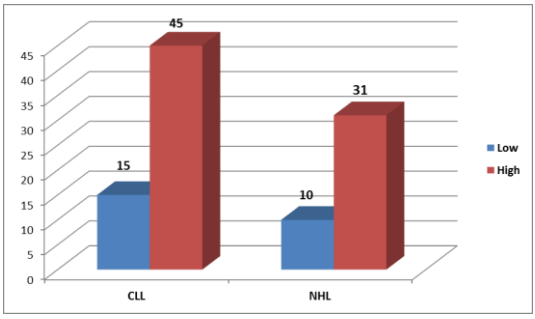

Figure 5: Most cases showed high percentage of HLA with both diseases. There was no significant correlation between the HLA percentage level and the diagnosis. (*p.value* = 1.75).

HLA mean intensity level X diagnosis

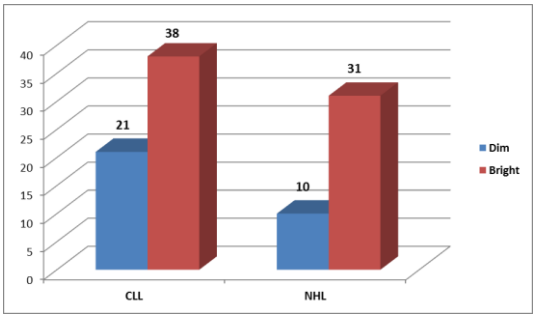

Figure 6: Most cases showed bright expression of HLA with both diseases. There was no significant correlation between the HLA mean intensity level and the diagnosis. (*p.value* = 2.04).

Score X HLA Percentage level

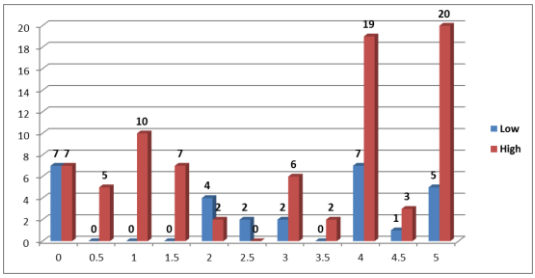

Figure 7:

- In high score (3 – 5), HLA percentage was correlate with high percentage more than low percentage while in low score (0 - 2.5) was different.
- There were no low percentage with score 0.5, 1.0, 1.5 and 3.5 which mean that we can use the present of low percentage to exclude these scores.
- There were no high percentage with score 2.5 which mean that we can use the present of high percentage to exclude the 2.5 score only.
- Scores 2.0 and 2.5 were only two scores showed low percentage more than high percentage.
- The was no significant correlation between the HLA percentage level and the score. (*p.value* = *0.9*12)

Score X Min intensity level

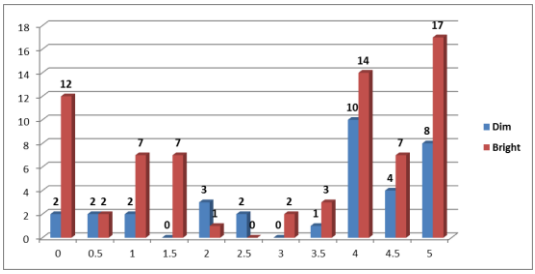

Figure 8:

- In high score (3 – 5), HLA mean intensity was correlate with bright expression more than dim expression while in low score (0 - 2.5) was different.
- There were no dim expressions with score 1.5 and 3.0 which mean that we can use the dim expression to exclude these scores.
- There were no bright expression with score 2.5 which mean that we can use the present of bright expression to exclude the 2.5 score only.
- Scores 2.0 and 2.5 were only two scores showed dim expression more than bright.
- There was no significant correlation between the HLA mean intensity level and the score. (*p.value = 0.858*)

DISCUSSION:

In this study we found that there is a highly significant relation between B-LPD and positivity of HLA-DR. (p.value= 0.00). So that we can use HLA-DR in the identification of B-LPD and excluding of T & NK cases.

There is no significant correlation between HLA-DR parameters (percentage & Mean intensity) and other general population distribution : Age, Gender, Sample type, Diagnosis and cases scores. (p.value= 2.8, 1.8, 3.4, 0.94 and 1.2 respectively).

There is no significant correlation between HLA-DR parameters (percentage & Mean intensity) and complete blood count figures: TWBCs, RBCs, HB and PLTs. (p.value= 1.8, 2.4, 2.1 and 0.9 respectively).

This result agreed with HLA-DR expression in B-cell non-Hodgkin's malignant lymphomas'a multiparameter flow cytometry study. Ratech H, which found HLA-DR has a role in differentiation between the sub types of B-Cell lymphomas according to the distribution of HLA-DR. (7)

CONCLUSION:

The present study demonstrated that HLA-DR has an important role in differentiation and classification of mature B-neoplasm and subtypes.

ACKNOWLEDGMENT:

We are indebted to the staff of Hematology Department (Alneelain University). We are grateful to the staff of Flowcytometry Laboratory for their collaboration. Finally special thanks to the patients for being cooperative.

REFERENCES:

(1) Merck Manual home edition, Non-Hodgkin Lymphomas, Garnock-Jones, K.P. (2010). Bendamustine. Drugs, 70(13), 1703-1718.

(2) Bope, Edward T. Kellerman, Rick D. (2015). *Conn's Current Therapy 2016. Elsevier Health Sciences. p.878. ISBN 9780323355353.*

(3) Mazen Sanoufa; Mohammad Sami Walid; Talat Parveen (2010). "B-Cell Lymphoma of the Thoracic Spine Presenting with Spinal Cord Pressure Syndrome". JOCMR. **2** (1): 53–54. doi:10.4021/jocmr2010.02.258w.

(4) Rowan, R. M., BAIN, B., England, J. M., Hyde, K., Matutes, E., Reilly, J. T., ... & Wood, J. K. (1994). Immunophenotyping in the diagnosis of acute leukemias.

(5) Dr. Rachel Dommett, Academic Clinical Lecture, University of Bristol, School of Clinical Science, Bristol, UK, Ch.21: Malignant disease

(6) Matutes E, Owusu-Ankomah K, Morilla R, Gracia Marco J, Houlihan A, Que TH et al. The immunological profile of B-cell disorders and proposal of a scoring system for the diagnosis of CLL. Leukemia 1994; 8:1640-5.

(7) Ratech, H. (1990). HLA-DR expression in B-cell non-Hodgkin's malignant lymphomas: a multiparameter flow cytometry study. *Human pathology*, *21*(12), 1275-1282.