IJBC 2016; 8(3): 68-71

Iranian Journal of Blood & Cancer

Journal Home Page: www.ijbc.ir

ORIGINAL ARTICLE

The Evaluation of Immunophenotypes in Diffuse Large B Cell Lymphoma: A Single Center Study

Robab Sheikhpour[1]*, Fatemeh Pourhosseini[2]

[1]Hematology and Oncology Research Center, Shahid Sadoughi University of Medical Science, Yazd, Iran
[2]Kerman Medical University, Kerman, Iran

ARTICLE INFO

Article History:
Received: 20.06.2016
Accepted: 10.08.2016

Keywords:
Diffuse large B cell lymphoma
CD20
CD45
CD3
CD2

**Corresponding author:*
Robab Sheikhpour,
Hematology and Oncology Research Center, Shahid Sadoughi University of Medical Science, Yazd, Iran
Email: robab.sheikhpour@iauyazd.ac.ir

ABSTRACT

Background: Diffuse large B-cell lymphoma (DLBCL) is an aggressive malignancy of mature B lymphocytes. It is known as a heterogeneous disease with variable therapeutic responses and alternative therapies. Morphological and immunophenotypical evaluation of the biopsy specimens can help diagnose DLBCL.
Methods: In the current study, 44 patients were chosen from Shaheed Sadoghi Hospital (2010–2013), central Iran. Immunohistochemical method was used for detecting biomarkers such as CD2, CD3, CD20 and CD45.
Result: In this study, 54.5% of the patients were men and 45.5% were women. Most of the patients were 40-50 years old. Moreover, 10 (22.7%) patients had lymph node metastasis and 6 (13.6%) patients had stomach involvement. Positive expression of CD45 and CD20 biomarkers were expressed in 100% and 97.7% of the patients. Positive expression of CD3 and CD2 was expressed in 40.9% and 81.8% of the patients, respectively. C-expression of CD45 and CD20 biomarkers was seen in 43 patients. Moreover, there was no relation between biomarkers and sex and age (P>0.05).
Conclusion: The result of this study showed that high number of CD45 and CD20 have been seen in Iran's population. Moreover expression of CD20 and CD45 is different as compared with other populations. It seems that these differences can be due to ethnic groups and nature of malignant cells.

Please cite this article as: Sheikhpour R, Pourhosseini F. The Evaluation of Immunophenotypes in Diffuse Large B Cell Lymphoma: A Single Center Study. IJBC 2016; 8(3): 68-71.

Introduction

Diffuse large B-cell lymphoma (DLBCL) is an aggressive malignancy of mature B lymphocytes.[1,2] It is known as a heterogeneous disease[3] with variable therapeutic responses and alternative therapies.[4] The causes of diffuse large B cell lymphoma are not well understood yet.[5] Following nodular lymphocyte predominant HL, diffuse large B-cell lymphoma (DLBCL) can be observed as one of the most common lymphoid malignancy in adults diagnosed on basis of morphology and immunophenotype.[6] DLBCL accounts for 30–40% of adult non-Hodgkin lymphomas.[7] The peak occurrence of diffuse large B-cell lymphoma (DLBCL) arises in the seventh decade of life.[8] The first sign of this disease is rapidly growing mass, fever, weight loss, and night sweats.[9] Diffuse large beta cell lymphoma disorder manufactured of a clinically and pathologically heterogeneous group of lymphoproliferative malignancies, most of which are B-cell origin.[8] Morphological and immunophenotypical evaluation of the biopsy specimens can help to diagnosis of DLBCL.[10]

Diffuse large B cell lymphoma includes three variants

in term of morphology. Centroblastic, Immunoblastic[5,11] and anaplastic. Most DLBCL cases are centroblastic.[5] Each has a diverse clinical presentation and prognosis. However, the usual treatment for each of these is chemotherapy, often in combination with an antibody targeted at the tumor cells.[12] The disease is treatable in most patients, but fewer than half of them attain a durable remission.[1] CD3 is a marker for T cells and natural killer cells.[13] It is specific for T-cell derivation. CD2 is also expressed by T and natural killer (NK) cells and has been reported in T/NK cell lineage neoplasms as well as in immature B-lymphoblastic and myeloid leukemias.[14] CD45 (lymphocyte common antigen) is a receptor-linked protein tyrosine phosphatase[15] and expressed on all leucocytes. It plays a significant role in the action of these cells.[16] CD20 is an activated glycosylated phosphoprotein. It is expressed on the surface of B cells beginning at the pro-B phase (CD45R+, CD117+).[17] We aimed to evaluate CD markers such as CD45, CD20, CD3 and CD2 in patients with diffuse large B cell lymphoma in Yazd city, central Iran.

Materials and Methods

In this study, 44 patients were enrolled from Shaheed Sadoghi Hospital, Yazd, central Iran during 2010-2013. The specimens were conserved in formalin. Following fixation, the specimens were embedded on wax paraffin and sliced to 4 µm in thickness for staining. The hematoxylin and eosin staining method was used to stain the tissue sections. In immunohistochemical method, Endogenous peroxidase was blocked by 3% hydrogen peroxide in methanol for 10 minutes at room temperature. Heat-induced epitope retrieval was done by heating these sections in citrate buffer (pH 9.0) using the microwave technique. After cooling, the sections were exposed with primary antibody (table 1).

Then, the specimens were exposed to Horseradish peroxidase rabbit anti-mouse IgG for 30 min and incubated with 3,3-diamino-benzidine tetrahydrochloride. The sections were counterstained with hematoxylin and rinsed in tap water, followed by immersing in graded alcohol, xylene and finally mount. Negative control was done by displacement of the primary antibody with fetal bovine serum in each series.

Result

In our study, the mean±SD age of the patients was 55.8±9.5 years. 27 (61.2%) out of 44 patients were younger than 60 years old. 54.5% of the patients were men. 10 (22.7٪) patients had lymph node metastasis. Table 2 shows the primary tissue involvement.

In this study CD45 was expressed in 100٪, CD20 in 97.7٪, CD3 in 40.9٪, and CD2 in 81.8, respectively. Table 3 shows expression of these biomarkers in the patients.

Table 4 shows co-expression of Biomarkers. No relation between percentage of expression of biomarkers with sex was observed (P>0.05).

Discussion

In the present study, immunohistochemical staining of the specimens with DLBCL were positive for CD45 and CD20 in almost all of the patients and less than half expressed CD3 and CD2. Asano and colleagues reported negativity for CD20 and CD3 in their patients with DLBCL.[18] Kevin and co-workers reported that some cases of DLBCL with an anaplastic morphology may be rich in

Table 1: Antibodies used for immunohistochemical staining of the tumor markers in patients with DLBCL

Antibody	Isotype	Dilution	Source
CD2	Monoclonal mouse antibody AB75	Ready to use	Dako
CD20	Monoclonal mouse antibody L26	Ready to use	Dako
CD3	Polyclonol rabbit	Ready to use	Dako
CD45	Monoclonal mouse 2B11+PD7/26	Ready to use	Dako

Table 2: Primary tissue involvement in patients with DLBCL

Primary tissue involvement	Number/Percent
Stomach	6 (13.6٪)
Cervix	2 (4.5٪)
Thyroid	3 (6.8٪)
Breast	1 (2.2٪)
Bone marrow	2 (4.5٪)
Pelvis	2 (4.5٪)
Skin	2 (4.5٪)
Colon	2 (4.5٪)
Kidney	3 (6.8٪)
Testis	2 (4.5٪)
Neck	3 (6.8٪)
Lymph node	10 (22.7٪)
Tonsil	4 (9.09٪)
Mesentery	2 (4.5٪)
Total	44 (100٪)

Table 3: Positive expression of biomarkers in patients with DLBCL

Positive expression of biomarkers	Number/Percent
CD45	44 (100٪)
CD20	43 (97.7٪)
CD2	36 (81.8٪)
CD3	18 (40.9٪)

Table 4: Co-expression of biomarkers in patients with DLBCL

Co-expression of biomarkers	Number	Percent
CD20, CD45	43	97.7
CD20, CD2	36	81.8
CD20, CD3	18	40.9

Reed-Sternberg like cells and thus simulate lymphocyte-depleted classical Hodgkin lymphoma. However, in contrast to Hodgkin lymphoma, these neoplastic cells were uniformly positive for both CD20 and CD79a antigens and were negative for CD15. Therefore, they reported that the majority (65-85%) of the cases of DLBCL in their study were of B-cell type.[11]

Stein et. al reported that CD30 which was constantly expressed in Reed-Sternberg cells, also is expressed by a subset of DLBCL patients.[19] In another study, aberrant expression of a single T-cell-associated antigen (CD5) on specimens of DLBCLs was reported.[20] The researchers also reported aberrant co-expression of 2 T-cell-associated antigens; CD2 and CD7 in patients with diffuse DLBCL. In a study by Toyama et. al, flowcytometric immunophenotyping of the DLBCL cases were negative for CD2, CD3 and positive for CD20.[21] Asano et. al in another study reported that CD30 and CD45 were positive in DLBCL (anaplastic variant), but negative for CD3, CD10, CD20, CD15.[18]

Conclusion

The result of this study showed high percentage of CD45 and CD20 seen in Iran's positivity in Iranian patients with lymphoma. It seems that these differences could be due to ethnic factors.

Conflict of Interest: None declared.

References

1. Alizadeh AA, Eisen MB, Davis RE, Ma C, Lossos IS, Rosenwald A, et al. Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. Nature. 2000; 403(6769):503-11. doi: 10.1038/35000501. PubMed PMID: 10676951.
2. Damm JK, Gordon S, Ehinger M, Jerkeman M, Gullberg U, Hultquist A, et al. Pharmacologically relevant doses of valproate upregulate CD20 expression in three diffuse large B-cell lymphoma patients in vivo. Exp Hematol Oncol. 2015; 4:4. doi: 10.1186/2162-3619-4-4. PubMed PMID: 25973343. PubMed Central PMCID: PMC4429466.
3. Monti S, Savage KJ, Kutok JL, Feuerhake F, Kurtin P, Mihm M, et al. Molecular profiling of diffuse large B-cell lymphoma identifies robust subtypes including one characterized by host inflammatory response. Blood. 2005; 105(5):1851-61. doi: 10.1182/blood-2004-07-2947. PubMed PMID: 15550490.
4. Slack GW, Steidl C, Sehn LH, Gascoyne RD. CD30 expression in de novo diffuse large B-cell lymphoma: a population-based study from British Columbia. Br J Haematol. 2014; 167(5):608-17. doi: 10.1111/bjh.13085. PubMed PMID: 25135752.
5. Swerdlow SH, Campo E, Jaffe ES, Pileri SA, Stein H, Thiele J, et al: WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. 4th ed. World Health Organization, 2008.
6. Javier G, Ferres R. Large B cell diffuse lumphoma. Orphanet Encyclopedia. 2004; 1-5
7. Shipp MA, Ross KN, Tamayo P, Weng AP, Kutok JL, Aguiar RC, et al. Diffuse large B-cell lymphoma outcome prediction by geneexpression profiling and supervised machine learning. Nat Med. 2002; 8(1): 68-75. doi: 10.1038/nm0102-68. PubMed PMID: 11786909.
8. Cultrera JL, Dalia SM. Diffuse large B-Cell lymphoma: Current strategies and future directions. Cancer Control. 2012; 19(3): 204- 13. PubMed PMID: 22710896.
9. Freeman AS, Aster JC: Epidemiology, clinical manifestations, pathologic features, and diagnosis of diffuse large B cell lymphoma. In Basow, Denise S. UpToDate. Waltham, MA: UpToDate.
10. Hu Y, Yang K, Krause JR. Diffuse large B-cell lymphoma, differential, diagnosis and molecular stratification. N Am J Med Sci. 2011; 4(2): 67-7. doi: 10.7156/v4i2p067.
11. Gatter K, Pezzella. Diffuse large B-cell lymphoma. Diagn Histopathol. 2010; 16(2): 69-8. doi: 10.1016/j.mpdhp.2009.12.002.
12. Akyurek N, Uner A, Benekli M, Barista I. Prognostic significance of MYC, BCL2, and BCL6 rearrangements in patients with diffuse large B-cell lymphoma treated with cyclophosphamide, doxorubicin, vincristine, and prednisone plus rituximab. Cancer. 2012; 118(17):4173-83. doi: 10.1002/cncr.27396. PubMed PMID: 22213394.
13. Sharma S, Juffer AH. An atomistic model for assembly of transmembrane domain of T cell receptor complex. J Am Chem Soc. 2013; 135(6): 2188-97. doi: 10.1021/ja308413e. PubMed PMID: 23320396.
14. Kingma DW, Imus P, Xie XY, Jasper G, Sorbara L, Stewart C, et al. CD2 is expressed by a subpopulation of normal B cells and is frequently present in mature B-cell neoplasms. Cytometry. 2002; 50(5):243-8. doi: 10.1002/cyto.10131. PubMed PMID: 12360573.
15. Clark MC, Pang M, Hsu DK, Liu FT, de Vos S, Gascoyne RD, et al. Galectin-3 binds to CD45 on diffuse large B-cell lymphoma cells to regulate susceptibility to cell death. Blood. 2012; 120(23): 4635–44. doi: 10.1182/blood-2012-06-438234. PubMed PMID: 23065155. PubMed Central PMCID: PMC3512238.

16. Altin JG, Sloan EK. The role of CD45 and CD45-associated molecules in T cell activation. Immunol Cell Biol. 1997; 75(5):430-45. doi: 10.1038/icb.1997.68. PubMed PMID: 9429890.
17. Richard H. "Chapter 7: B lymphocyte development and biology". In William P, editor: Fundamental Immunology. Philadelphia; Lippincott Williams & Wilkins; 2009. P.237–269.
18. Asano H, Imai Y, Ota S, Yamamoto G, Takahashi T, Fukayama M, et al. CD30-positive anaplastic variant diffuse large B cell lymphoma: a rare case presented with cutaneous involvement. Int J Hematol. 2010; 92(3):550-2. doi: 10.1007/s12185-010-0675-9. PubMed PMID: 20838960.
19. Stein H, Foss HD, Dürkop H, Marafioti T, Delsol G, Pulford K, et al. CD30 anaplastic large cell lymphoma: a review of its histopathologic, genetic, and clinical features. Blood. 2000; 96(12): 3681-95. PubMed PMID: 11090048.
20. Sangle NA, Agarwal AM, Smock KJ, Leavitt MO, Warnke R, Bahler D, et al. Diffuse large B-cell lymphoma with aberrant expression of the T-cell antigens CD2 and CD7. Appl Immunohistochem Mol Morphol. 2011; 19(6):579-83. doi: 10.1097/PAI.0b013e318221c672. PubMed PMID: 21836500.
21. Toyama T, Kubuki Y, Sasaki H, Hidaka T, Okamoto M, Suzuki M, et al. [Primary splenic CD8-positive diffuse large B-cell lymphoma]. Rinsho Ketsueki. 2001; 42(12):1187-91. PubMed PMID: 11828722.
22. Johnson NA, Boyle M, Bashashati A, Leach S, Brooks-Wilson A, Sehn LH, et al. Diffuse large B-cell lymphoma: reduced CD20 expression is associated with an inferior survival. Blood. 2009; 113(16):3773-80. doi: 10.1182/blood-2008-09-177469. PubMed PMID: 19029441. PubMed Central PMCID: PMC2943836.