Microbiology Research Journal International
18(1): 1-9, 2017; Article no.MRJI.25845
Previously known as British Microbiology Research Journal
ISSN: 2231-0886, NLM ID: 101608140

SCIENCEDOMAIN *international*
www.sciencedomain.org

Association of Hepatitis Virus Infections with CD4 and CD8 among Certain Group of Lymphoma Patients in Babylon Provence

Raheem T. Obyes Al-Mammori[1*], Azhar A. Lateef Al-Thahab[2] and Alaa Sadiq Al-Awad[3]

[1]Department of Clinical Immunology, Babylon Public Health Laboratory, Iraq.
[2]Department of Immunology, College of Science, Babylon University, Iraq.
[3]Medicine College, Babylon University, Iraq.

Authors' contributions

This work was carried out in collaboration between all authors. Author RTOAM designed the study, performed the statistical analysis, wrote the protocol, wrote the first draft of the manuscript and managed the literature searches. Authors AALAT and ASAA managed the analyses of the study and literature searches. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/MRJI/2017/25845
Editor(s):
(1) Alok K Upadhyay, Fox Chase Cancer Center, Philadelphia, Pennsylvania, USA.
Reviewers:
(1) Mark E. Mummert, University of North Texas Health Science Center, USA.
(2) Heshu Sulaiman Rahman, University Putra Malaysia, Malaysia.
(3) Juandy Jo, Singapore.
Complete Peer review History: http://www.sciencedomain.org/review-history/17187

Original Research Article

Received 22nd March 2016
Accepted 3rd December 2016
Published 10th December 2016

ABSTRACT

This study was planned to detect the immunological association impact of hepatitis infection in lymphoma patients, There are two branches of this study, firstly theoretical background and secondly is practical as diagnostically and immunological impact. Investigate the immunological association of Hepatitis viruses on lymphoma patients by estimation of certain, auto antibodies and specific CD markers among clinically diagnosed patients of Hodgkin's and Non Hodgkin lymphoma admitted to malignancies registry center of Babylon. At the time limited between February 2012 and February 2013. Specific diagnosis is clinically done by consultant physician and histopathology's. The sample selection was classified as the following; newly diagnosed patients (HL, n= 17), (NHL. n= 33) and (30) healthy samples were chosen as control group as well as (9) autopsy

**Corresponding author: E-mail: immunoraheem@yahoo.com;*

samples examined by histopathologist and confirm it as a normal section before used as healthy lymph node control for CD markers. The examination of (CD4 and CD8) used as a monitor to evaluating the lymph node environment during infection, as well as screening test for different types of hepatitis viruses for both, patients and control. To confirm the link between immune reactivity with such condition, the Antinuclear and Anti mitochondrial antibodies also done by using immunoflourescence technique, because there are a few studies dealing with such relation and to find out the accurate and early diagnostic protocol for detection of risk patients to infect with such diseases.

According to this application, the clinically significant results showed that; the age groups distributed among young age (20 -29 y) for HL, while adult age at (50-59 y) for NHL. There were no differences between males and females. The frequency of hepatitis viruses revealed that HCV is more distributed at all patient groups at a higher percent (35.64%) than HBV (11.76%) and no positive result of HAV as well as no co – infetion with viral hepatitis were seen at the time of study. Tumor infiltrating lymphocytes with positive CD4, CD8 markers could play a role in immunological microenvironment of both types of lymphoma .In conclusion mentioned that the increase of positive CD8 tumor infiltrating lymphocytes, and the ratio of CD4/ CD8 equal to (1:1) refers to that the tumor infiltrating lymphocytes are T lymphocytes, often CD8 CTLs, and there is progressive maturation of autoantibodies in association with viral activity among both lymphoma types.

Keywords: Hepatitis; CD4; CD8; lymphoma.

1. INTRODUCTION

The lymphoma is used to describe proliferation, malignant lymphomas are neoplasm of the lymphoid system, pathological, and epidemiological features showing heterogeneous clinical presentation and behavior [1]. Two major groups of malignancies can be distinguished, HL and NHL. The etiology of lymphomas is largely unknown. In particular, autoimmune diseases have repeatedly been shown to confer an increased risk for certain hematologic malignancies. Linking infectious agents with neoplasia, however, is a more difficult task [2]. HL has a unique cellular composition, containing a minority of neoplastic cells (Reed-Sternberg cells and their variants) in an inflammatory background. Early epidemiologic data suggested that HL develops among persons with a delayed exposure to a ubiquitous infectious agent [3]. Nodular Sclerosing (NS), Mixed cellularity (MC), and Lymphocyte Depletion (LD) are clinically similar to each other but different from the Lymphocyte predominant (LP) subtype. The possible etiological factors include, familial factors, some sex siblings of patient with HL, also associated with single family houses and early birth order [4].

Lymphoma usually begins in a lymph node, but it can also begin in the stomach, intestines, and skin. The World Health Organization (WHO) recognizes three major categories of lymphoid neoplasms: B-cell neoplasms; T-cell, and natural killer (NK)-cell neoplasms; and whether or not lymphoma are derived from primitive precursor cells or more mature peripheral cells [5].

NHLs are broadly classified as B-cell or T-cell lymphomas, B-cell lymphomas represent approximately 90% of NHL., and other rare types T-cell lymphomas represent approximately 10% [6].

Expression of cell surface antigens and immunoglobulin proteins is dependent on the type of lymphocyte and its stage of differentiation or maturation. Analysis of these proteins in the malignant cells is useful diagnostically as well as for determining tumor histogenesis [7].

The cluster of differentiation (designation) is a protocol used for the identification and investigation of cell surface molecules providing targets for immunophenotyping of cells [8]. Immunohistochemistry (IHC) refers to the process of detecting antigens (e.g., proteins) in cells of a tissue section by exploiting the principle of antibodies binding specifically to antigens in biological tissues. In the most common instance, an antibody is conjugated to an enzyme, such as peroxidase, that can catalyse a colour producing reaction. Alternatively, the antibody can also be tagged to a fluorophore, such as flourescein or rhodamine [9].

Because of the method of fixation and tissue preservation, the sample may require additional steps to make the epitopes available for antibody binding, including deparaffinization and antigen retrieval (microwave method, enzyme method,

hot incubation method). The samples are incubated with a buffer that blocks the reactive sites to which the primary or secondary antibodies may otherwise bind. IHC is an excellent detection technique and has the tremendous advantage of being able to show exactly where a given protein is located within the tissue examined. It is also an effective way to examine the tissues [10]. Physiologically, CD molecules can act in numerous ways, often acting as receptors or ligands (the molecule that activates a receptor) important to the cell. Some CD proteins do not play a role in cell signaling, but have other functions, such as cell adhesionCD for humans is numbered up to 350 most recently. CD molecules are utilized in cell sorting using various methods including flowcytometry and immunohistochemstry [11].

In humans, the CD4 protein is encoded by the CD4 gene. CD4 is a co-receptor that assists the T- cell receptor (TCR) in communicating with an antigen-presenting cell. CD8 is a transmembrane glycoprotein that serves as a co receptor for the T cell receptor (TCR). Like the TCR, CD8 binds to a major histocompatibility complex (MHC) molecule, but is specific for the class I MHC protein [12] T cells are distinguished by the presence of either CD4 or CD8 surface markers, and the latter cells are involved in antitumor immunity. However, CD4 cells are also important in this regard, because such cells are able to recognize internalizing tumor antigens presented by APC in association with class 11 MHC molecules [13].

Chronic antigenic stimulation has long been implicated in the development of hematologic malignancies. Reasons are the observed prevalence and the increased risk of cancer particularly among patients with autoimmune diseases and the association of some infectious agents with certain malignancies. This has, in part, been ascribed to different rates of hepatitis infections in the general population of the respective countries [14]. In particular, autoimmune diseases have repeatedly been shown to confer an increased risk for certain hematologic malignancies or malignant lymphoma. Linking infectious agents with neoplasia, however, is a more difficult task [1].

2. MATERIALS AND METHODS

According to the clinical and theoritical backgraund of such patients (Lymphoma and Hepatitis). Clinically diagnosed patients of H. and NH lymphoma admitted to malignancies registry center of Babylon in the period between February 2012 and February 2013. Specific diagnosis is clinically done by consultant physician, histopathologist and hematologist. Certain group of lymphoma patients (50 patients) of newly diagnosed patients (17) of HL and (33) of NHL. With (9) healthy lymph node sections as control samples, all samples were examined by histopathologist. Apparently healthy person (30) as a control group, all the control samples are negative for viral hepatitis.

By using EnVesionTM Flex target can be detection of different CD markers on human cells or tissues (such as CD4 and CD8), this reagent provided by Dakocytomation company for laboratory investigation. The principle and methods for viral screening test of all patient samples and control are done according to procedural flow charts of ELISA protocol of Bioelisacompany.

3. RESULTS AND DISCUSSION

The results were obtained from the clinically and laboratory application as illustrated in Fig. 1, lymphoma patients attending from different provinces of middle part of Iraq for malignancies registry center of Babylon at the time of study was that the majority of patients with HL and NHL were from Babylon when compared with patients from other middle Euphrates provinces, In the percentage (35.2%, 57.5 %) respectively, while the patients from Karbala (23.4%, 18.2%) for both HL and NHL in the second province, and in other provinces in Al-Najaf (11.7%) HL and (6.0%) NHL, AL-Diwania (11.7%) HL (15.1%) NHL and Al- Muthana (17.6%, 3.0%) for both HL and NHL. There are no related studies in such area dealing with the diseases distribution, and in the study done by Aladdin, [15] who mentioned that the childhood cancers are 8-10 times more common than in the west, with about 8% of the total cancers in Iraq compared to 0.5-1% in developed countries, the most common cancers in childhood are leukemia, followed by lymphomas. According to reports of World Health Organization (WHO) the annually incidence rate of lymphoma in the middle east until 2004 as examples in Iran about 15113 cases, Iraq 5681 cases, Jordan 1256 case, Kuwait 505 case, Lebanon 845 case, Saudi Arabia 5775 case, Syria 4033 case, United Arab Emirates 565 case, Yemen 4483 cases. The high mortality rate of such cases mostly in Iraq reach up to 19.8% from all cases [16].

According to the expermental, the age range of HL is great in young age than other age groups as mentioned in Fig. 2. The age range of HL refers to higher percent of patient groups at 20 - 29 y, 41.1% and 30 -39 y, 23.5%, than in other groups 10-19 y; 11.7%, 40-49 y; 11.7%, 50-59 y; 5.8%, 60-69; 0.0%, and >70; 5.8%. In the patients with NHL the age ranges differ from patients with HL and refer to the adult age higher than young, higher percent recorded at age groups 50-59 y; 24.2% and 40-49 y; 21.2%, while less percent in other groups as the following 10-19 y; 12.1%, 20-29 y; 12.1%, 30-39; 12.1%, 60 - 69 y; 12.1%, and >70; 6.0%. This result agreed with other studies such as Al –Barzinji [17] who stated that the age of Iraqi HL patients ranged between 10-73 years with a mean age of (29.31 ± 1.73) years, the age incidence below 45 years old formed (84%), versus (16%) incidence above 45 years. Nearly all NHL cases occur in adults, with the average age of diagnosis in the 60's. While scientists do not know the exact causes of NHL [18]. While Biermann, [19] who stated that incidence of lymphoma was higher in males

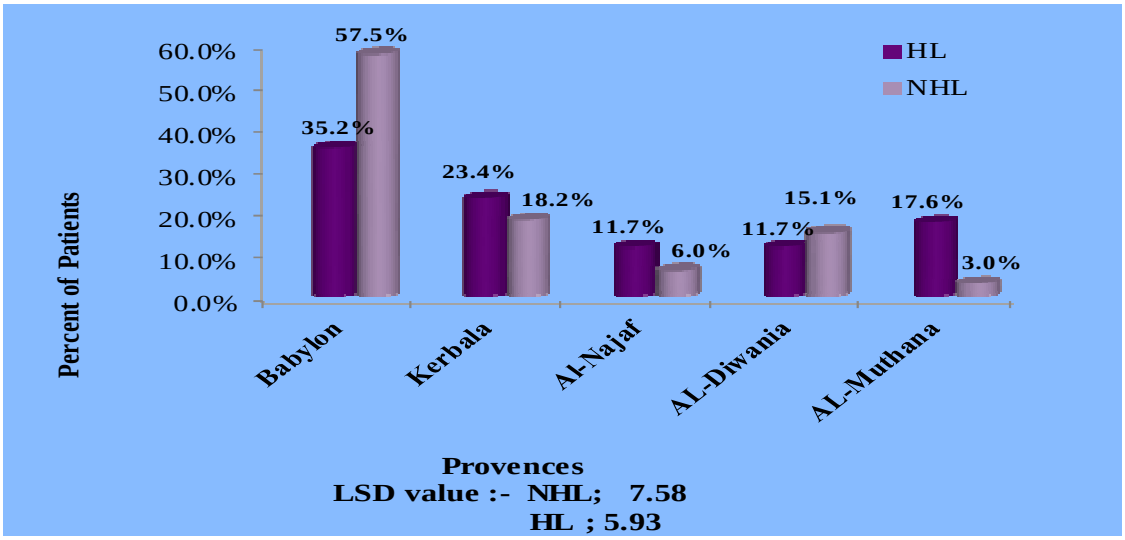


Fig. 1. The percentage of lymphoma in middle Euphrates area

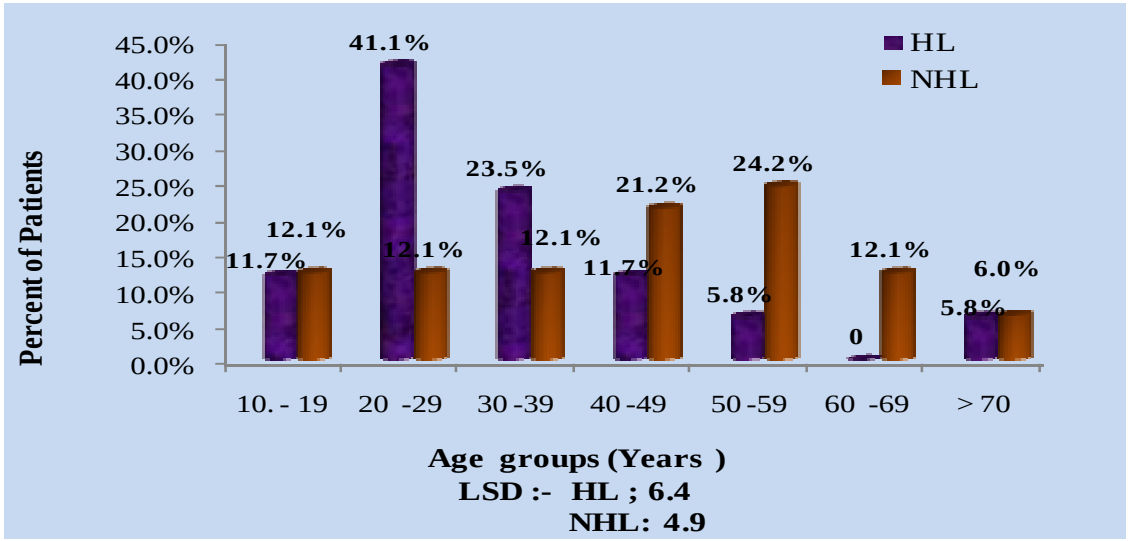


Fig. 2. Age groupsdistribution

than females. Increased with age, in older persons than young, low grade lymphoma accounts for only 16% of cases in those younger than age 35 year. According to this figure the result mentioned that the most recorded cases of NHL are in adult age less than 60 years below the universal age distribution of such disease.

In Fig. 3 the result show that no significant (P>0.05) differences between male and female in frequency of infected with both types of lymphoma according to the male and female distribution, the ratio of present study showed that mild increased in female more than male, 47.0% : 53.0% (at male : female ratio 1 : 1.25) of HL patients and in NHL; 42.3% : 57.7% (at male : female ratio 1 : 1.35). In comparison with other related study, Yahalom and David, [20] mentioned that the male to female ratio of HL is 1.3 : 1.0. The age specific incidence is bimodal with peak in third decade of life and second peak after age 50 years. It occurs more frequently in developed than the underdeveloped countries. The result of figure is inconsistent with James and Armitage, [21] who stated that the male is more than female. This might be due to the fact that age related disease or it might be due to disease types since HD is more common in female with nodular Sclerosing variants.

The results of hepatitis screening for (hepatitis A, B and C) among all patient groups, showed that Hepatitis C virus (HCV) gave an important virus associated with HL and NHL. The results as mentioned in Table 1 show a presence of antibody of hepatitis C in HL patients 17.64%, NHL patients 18.0%. While for antihepatitis B surface antigen (Anti HBs antigen) positive result in NHL patients 6.06%, but 5.7% for HL. The result of anti-hepatitis A antibody (HAV) was negative for both HL and NHL. HCV infection had higher percent (35.64%) for all studied patients than HAV and HBV.

The result of this figure might be consistent with different studies which demonstrated these relationship, such as Nermine et al. [22] who stated that a pathogenic role for HCV has been hypothesized to a subset of B cell NHL primary to the liver; therefore the immunophenotypic profile of NHL of the liver, biological properties and their association to HCV infection. Although the liver has its own lymphoid tissue mainly T-cells, NKT cells and few B cells, Bronowicki et al. [23] and Ramos et al. [24] have reported B cell NHL in patients who were infected with HCV and have suggested a role for HCV in its pathogenesis. Wang et al. [25] reported a decreased rate of HBV infections in patients with T-cell lymphoma compared to patients with B-cell malignancies. The HCV infections more distributedrather than other HBV and HAV as well as no co- infections were recording at the study time.

Table 1. Distribution of viral hepatitis infections among all patient groups

Diseases	Total	HAV %	HBV %	HCV %
HL	17	0.0	5.70	17.64
NHL	33	0.0	6.06	18.0
Control	30	0.0	0.0	0.0
Total	80	0.0	11.76%	35.64%

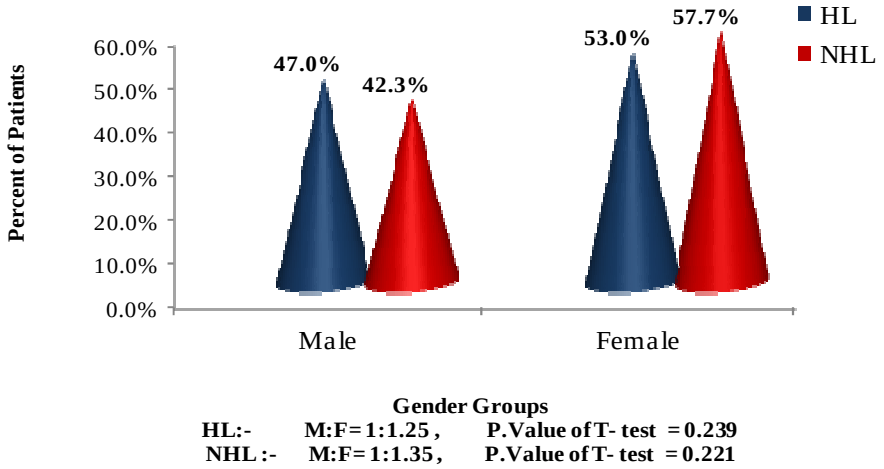


Fig. 3. Gender distribution of HL and NHL patients

The specific marker for T-cell CD 4 (speciallyTh cells) positive result refers to the immunity state of lymphoid tissue or local lymph node immunity against the disease or tumor. The result shows that positive CD4 in 10 : 20 (50.0%) patients at different score in comparison with 22.2% of healthy lymph nodes sections. The major distribution of positive result mainly among mixed cell lymphoma, small B-cell as well as T-cell types of NHL. While positive CD4 staining in patients with HL gave low percent. There are 4 : 12 (33.34%) positive results at different scores, in similar distribution for both nodular and mixed cellular type of HL. Table 2 shows this results and Fig. 4A shows the picture of positive staining of CD4 on tissue paraffin section. The T-cell NHLs generally correspond to normal activated CD4+ T cells. The lymphoblastic lymphomas, which are generally of T-cell lineage often co expression of CD4 and CD8 [26]. CD4 T cells are activated and secretes various cytokines, Thus the CD4 T cells play a central role in both humoral and cell mediated immunity [27].

Antitumor activity have been shown to destroy tumors, in order for this to take place CD4 T h1 should be able to infiltrate to the tumor sites. The lymphocyte should recognize tumor specific antigen in association with MHC class II molecules on the surface of professional antigen presenting cells (APC) and receive specific signal and intiate immune response [28].

Similar to CD4 the CD 8 marker is specific for T cell specially Tc cells and the positive result reflex the immunity state of lymphoid tissue or local lymph node immunity against infections or tumors. Table 3 shows that positive CD8 in 10 out of 20 (50.0%) patients at different scores in comparison with 11.1% of healthy lymph nodes sections. The major positive results at score III and score IV. The main positive results are distributed among large B-cell, small B- cell, mixed B–cell lymphoma, but there is no positive result at follicular type of NHL. While the results of CD8 in HL show that there are 4: 12 (33.34%) gave positive result at different scores. The major

Table 2. CD 4 with tumor types of lymphoma

Disease	Types	CD 4 positive score. (%)				
		Score II	Score III	Score IV	Total	H.L.N. sections (No. 9)
HL	M. C.	-	16.67	-	16.67	
(No=17)	N. S.	-	8.33	8.33	16.66	
Total		-	25.0	8.33	33.33	22.2%
NHL	L. B-cell	-	-	3.03	5.0	
(No= 33)	S. B- cell	5.0	10.0	-	15.0	
	Follicular	-	-	-	-	
	L. and S. B-cells	-	5.0	20.0	25.0	
	T-cell	-	-	5.0	5.0	
Total		5.0	15.0	30.0	50.0	
P. value (Test and control)			HL = 0.014 (S.)		NHL= 0.031 (S.)	

Score II, 10 -20 cells/field, Score III, 30 -50 /field, Score 1V, more than 50 cells/field, M.C. (Mixed cellularity), NS (Nodular Sclerosing), L. large cells, S. Small cells. S. Significant . H.L.N (Healthy Lymph Node)

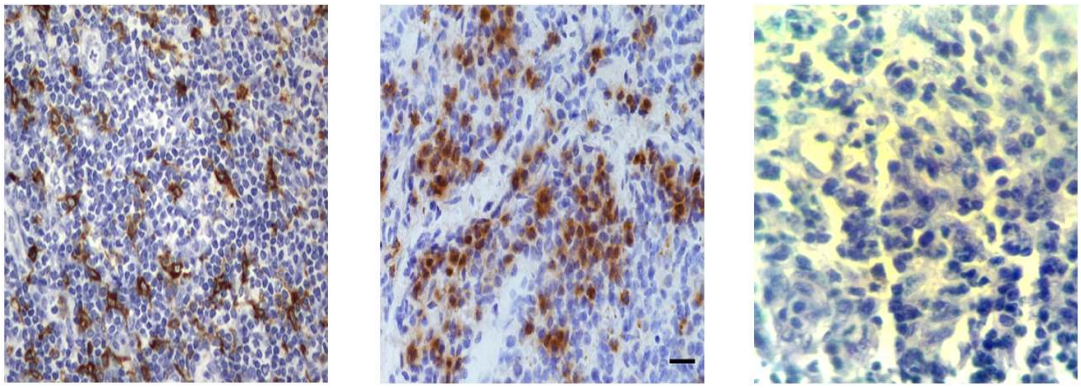
Table 3. CD 8 with tumor types of lymphoma

Disease	Types	CD 8 positive score. (%)			
		Score III	Score IV	Total	H. L.N sections (No. 9)
HL	M. C.	16.67	-	16.67	
(No= 17	N. S.	8.33	8.33	16.66	
Total		25.0	8.33	33.33	
NHL (No= 33)	L. B-cell	-	5.0	5.0	11.1%
	S. B- cell	10.0	5.0	15.0	
	L. and S. B-cells		25.0	25.0	
	T-cell		5.0	5.0	
Total		10.0	40.0	50.0	
P value (Test and control)		HL = 0.017 (S.)		NHL = 0.006 (H.S.)	

Score III, 30 -50 /field, Score 1V, more than 50 cells/field, M.C. (Mixed cellularity), NS (Nodular Sclerosing), L. large cells, S. Small cells. H.L.N (Healthy Lymph Node)

percent at score III, but there is similar distribution in the results for both nodular and mixed cellular type of HL. Fig. 4B shows the positive staining of CD 8 on tissue paraffin section. Tumor infiltrating lymphocytes are T lymphocytes, often CD8 CTLs. They also include some CD4 T cells and NKT cells. Certain tumors are known to lack or to be poor expressers of MHC antigens. In the early development of tumor, the amount of antigen may be too small to stimulate the immune system and due to rapid proliferation of malignant cells [29]. CD8 expression on the pathologic B cells of bone marrow samples from patients with various types of B-NHL [30].

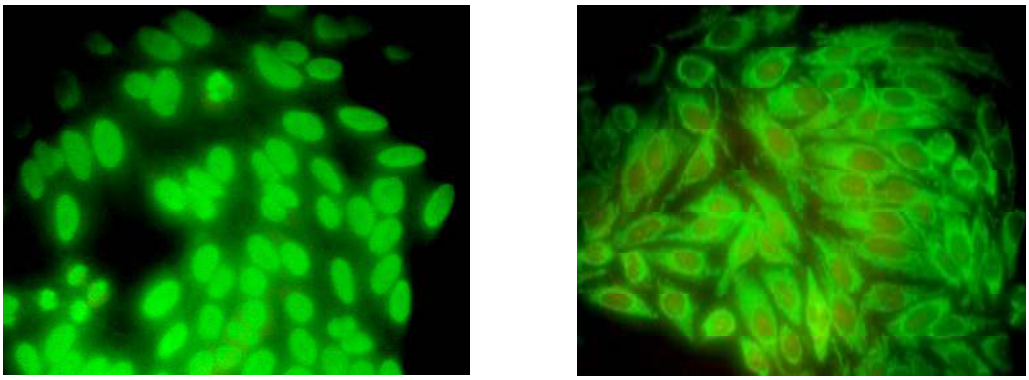
Table 4 shows that certain autoantibodies detected in number of patients have lymphoma for both types H. and NHL some of them have positive CD+4/CD+8, this specific and uncommon result shows autoimmune activity in lymphoma patients. The result explained as : 7 patients of HL having different autoantibodies, while in NHL 9 patients show ANA and AMA, 4 out of 9 (44.4%) and 5 out of 9 (55.6%) respectively. There are 2 HL patients (28.6%) and 3 NHL (33.3%) have co-occurrence of autoantibodies and CD+4/CD+8 markers gave a role of predisposing factor for infected with malignant autoimmune T cell lymphoma rather than other types of lymphoma. Fig. 5 show the different fluorescence pictures of both pattern Antinuclear (ANA) and Anti mitochondrial antibodies on Hep-20 substrate. Autoimmunity often exists in patients with non-Hodgkin lymphoma (NHL) and that autoimmune disorders carry an increased risk of NHL. Autoimmune phenomena occurs frequently in the course of lymphoproliferative malignancies, and sometimes be the first sign of malignancy.



A. CD 4 on lymph node section **B. CD 8 on lymph node section** **C. Negative control section**

Fig. 4. Immunohistochemical staining of CD4 and CD8 by Dakocytomation staining of tissue paraffin sections

A (CD4), B. (CD8) and C. (-ve Negative control)



ANA **AMA**

Fig. 5. Fluorescent staining of different antibodies on Hep-20 substrate

Table 4. Autoantibodies among lymphoma patients (Autoimmune lymphoma)

Autoantibodies	Autoimmune lymphoma				
	HL			NHL	
	No. (%)		$CD4^+/CD8^+$	No. (%)	$CD4^+/CD8^+$
ANA	2 (28.6)	1 (14.3)	4 (44.4)	1 (11.1%)	
AMA	5 (71.4)	1 (14.3)	5 (55.6)	2 (22.2%)	
Total	7 (100)	2 (28.6)	9 (100)	3 (33.3%)	

4. CONCLUSION

In conclusions of the present study the clinically significant results revealed that; the age groups distributed among young and adult ages for HL and NHL, respectively with no differences between males and females. The Anti-HCV antibody is more distributed at all patient groups than HBV. Tumor infiltrating lymphocytes with positive CD4, CD8 markers could play a role in immunological microenvironment of both types of lymphoma .Increase of positive CD8 tumor infiltrating lymphocytes, and the ratio of CD4/ CD8 equal to (1:1) refers that the tumor infiltrating lymphocytes are T - lymphocytes, often CD8 CTLs, autoimmune T-cell lymphoma is newly form of lymphoma in which recored in this result.

DISCLAIMER

This manuscript was presented in the conference "16th International and Iranian Congress of Microbiology" available links are: "http://16.ismcongress.ir/MckUpload/file/Poster_ID.pdf & http://16.ismcongress.ir/list_Abs_accepted.aspx" Date 25-27/2015, Place "Iran Tehran".

ACKNOWLEDGEMENTS

I hope to thank the lord Allah who honored and made me of the slaves, and peace and blessings be up on the best creation of our prophet Muhammad and his good pure family. I would like to express my deepest appreciation and very honest gratitude to my friends and supervisors, Prof. Dr. Azhar EmranLateif Al- Thahab, and Assist. Prof. Dr. AlaaSadeq Al- Awad. University of Babylon for their guidance, support, interest and their encouragement. My sincere thanks and gratitude are to all staff, members and friends of Malignancies Registry Center in Babylon / Merjan Medical City who helped me, with special thanks for all old and new friends and members of laboratory department.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Swerdlow SH. WHO classification of tumors of haematopoitic and lymphoid tissues. 2008;1-439.
2. Anderson LA, Gadalla S, Morton LM. Population based study of autoimmune conditions and the risk of specific lymphoid malignancies. Int J Cancer. 2009;125(2): 398-405.
3. Alexander FE, Jarrett RF, Lawrence D. Risk factor for Hodgkin`s diseases by Epstein Barr Virus (EBV) status: Prior infection by EBV and other agents. British. J. Cancer. 2000;82:1117.
4. Potlock CS, Yahalom J. Hodgkin`s disease. In Cecil textbook of medicine; by Lee Goldman and Dennis Ausie. 22nd ed. USA. 2004;1:1166-1183.
5. Jaffe E, Harris N, Stein H. World Health Organization classification of tumours: Pathology and genetics of tumors of hematopoietic and lymphoid tissues. WHO, Geneva, Switzerland; 2001.
6. Kasamon YL, Swinnen LJ. Treatment advances in adult Burkitt's lymphoma and leukemia. Curr. Opin. Oncol. 2004;16:429-435.
7. Barbara BR. Overview of non-Hodgkin's lymphoma. Seminars in Oncology Nursing. 2006;22(2):67-72.
8. Zola H, Swart B, Nicholson I, Voss E. Leukocyte and stromal cell molecules: The CD Markers. Hoboken, New Jersey: John Wiley and Sons; 2007.
9. Ramos-Vara JA. Technical aspects of immunohistochemstry. Vet Pathol. 2005; 42(4):405–426.
10. Jorgensen JT, Kirsten VN, Bent E. Pharmacodiagnostics and targeted therapies: A rational approach for

individualizing medical anticancer therapy in breast cancer. The Oncologist. 2007; 12(4):397–405.
11. Ho IC, Tai TS, Pai SY. (GATA3 and the T-cell lineage: Essential functions before and after T-helper-2-cell differentiation. Nat. Rev. Immunol. 2009;9(2):125–35.
12. Gao G, Jakobsen B. Molecular interactions of co receptor CD8 and MHC class I: The molecular basis for functional coordination with the T-cell receptor. Immunol Today. 2000;21(12):630–6.
13. Bendelac A, Bonneville M, Kearney JF. Autoreactivity by design: Innate B and T lymphocytes. Nat. Rev Immunol. 2001;1: 177-186.
14. Cooper GS, Bynum ML, Somers EC. Recent insights in the epidemiology of autoimmune diseases: Improved prevalence estimates and understanding of clustering of diseases. J. Autoimmun. 2009;33(3-4):197-207.
15. Aladdin A. A comparative study of the distribution of Leukemia's in Iraq: 1989-1998, 2000-2002. Ministry of health. Iraqi Cancer RegistryUnpublished Data; 2004.
16. World Health Organization Incidence (annual) of Lymphoma: Statistics by Country for Lymphoma. Health Grades Inc; 2011.
17. Al-Barzinji RM. Hodgkin's lymphoma: An epidemiological study in the Iraqi patients. The Iraqi Postgraduate Medical J. 2006; 5:3.
18. Carol P, Julie M, Vose MD, Bruce D, Cheson F. Diffuse large B-cell lymphoma. American Cancer Society.Cancer Facts and Figures. Lymphoma Research Foundation; 2009.
19. Beirmann PJ, Harris NL, Armitage JO. Non Hodgkin`s lymphoma. In Cecil text book of medicine; by: Lee Goldmann and Dennis Ausiello (Editors) 22nd ed. W.B. Saunders Com. USA. 2004;1:1174-1184.
20. Yahalom, David S. Hodgkin`s lymphoma in cancer mangment handbook, chap 29, 2005 -2006 from Cancer network.com; 2006. (Abstract)
21. James O, Armitage J. Who benefits from surveillance imaging. Journal of clinical oncology: Official journal of the American Society of Clinical Oncology. 2012;30(21): 2579-80.
22. Nermine E, Mohamed TB, Amany ER, Inas El A. Diffuse large B cell lymphoma of the liver: Immunophenotypic profile, biological properties and association with hepatitis C virus infection. Nile Liver J. 2010;1(1):43-48.
23. Bronowicki JP, Bineau C, Feugier P. Primary lymphoma of the liver: Clinical pathological features and relationship with HCV infection in French patients. Hepatology. 2003;37(4):781-787.
24. Ramos-Vara JA. Technical aspects of immunohistochemstry. Vet Pathol. 2005; 42(4):405–426.
25. Wang F, Xu RH, Han B. High incidence of hepatitis B virus infection in B-cell subtype non-Hodgkin lymphoma compared with other cancers. Cancer. 2007;109(7):1360-64.
26. Ansari-Lari MA, Muzny DM, Lu J, Lu F, Lilley CE, Spanos S, Malley T, Gibbs RA. A gene rich cluster between the CD4 and trios phosphate isomerase genes at human chromosome 12p13. Genome Res. 1996;6(4):314–26.
27. Doan T, Mevold R, Viselli S, Waltenbaugh C. Blood groups in: Lippincott`s illustrated review: Immunology Lippincott Williams and Wilkins. 2008;297-310. USA.
28. Warren L, Ernest J. Medical microbiology and immunology. 6th ed. McGraw Hill; 2000.
29. Harvy RA, Champe PC. Immunology. Lippincott`s illustrated review. Williams and Wilkins. 2008;302-304.
30. Carulli G, Stacchini A, Marini A, Ciriello MM, Zucca A, Cannizzo E, Aliberti S, Demurtas A, Novero D, Calcagno L, Callegari T, Petrini M. Aberrant expression of CD8 in B-cell non-Hodgkin lymphoma: A multicenter study of 951 bone marrow samples with lymphomatous infiltration. Am J Clin Pathol. 2009;132(2):186-90.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/17187