

Original Research Article

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Association of Hepatitis Virus Infections with CD4 and CD8 Among Certain Group of Lymphoma Patients in Babylon Province

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 histopathologist'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 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 immunofluorescence 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 -29y) for HL, while adult age at (50-59y) 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-infection 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.

Introduction:-

33 | The lymphoma is used to describe proliferation-, malignant lymphomas are neoplasm of the
34 | lymphoid system , pathological, and epidemiological features showing heterogeneous clinical
35 | presentation and behavior (Swerdlow, 2008). Two major groups of malignancies can be distinguished,
36 | HL and NHL. The etiology of lymphomas is largely unknown. In particular, autoimmune diseases
37 | have repeatedly been shown to confer an increased risk for certain hematologic malignancies-. Linking
38 | infectious agents with neoplasia , however, is a more difficult task (-Anderson *et al.*, 2009). HL has a
39 | unique cellular composition, containing a minority of neoplastic cells (Reed-Sternberg cells and their
40 | variants) in an inflammatory background. Early epidemiologic data suggested that HL develops among
41 | persons with a delayed exposure to a ubiquitous infectious agent (-Alexander *et al.*, 2000). Nodular
42 | Sclerosing (NS)-, Mixed cellularity (MC) , and Lymphocyte Depletion (LD) are clinically similar to
43 | each other but different from the Lymphocyte predominant (LP) subtype. The possible etiological
44 | factors -include-, familial factors, some sex siblings of patient with HL, also associated with single
45 | family houses and early birth order. (Potlock and Yahole-, 2004).

46 | Lymphoma usually begins in a lymph node, but it can also begin in the stomach, intestines, and
47 | skin. The World Health Organization (WHO) recognizes three major categories of lymphoid
48 | neoplasms: B-cell neoplasms; T-cell, and natural killer (NK)-cell neoplasms-; and whether or not
49 | lymphoma are derived from primitive precursor cells or more mature peripheral cells (-Jaffe *et al.*,
50 | 2001).

51 | NHLs are broadly classified as B-cell or T-cell lymphomas, B-cell lymphomas represent
52 | approximately 90 % of NHL. , and other rare types T-cell lymphomas represent approximately 10 %
53 | (Kasamon and Swinnen-, 2004).

54 | Expression of cell surface antigens and immunoglobulin proteins is dependent on the type of
55 | lymphocyte and its stage of differentiation or maturation. Analysis of these proteins in the malignant
56 | cells is useful diagnostically as well as for determining tumor histogenesis (-Barbara *et al.*, 2006).

57 | The cluster of differentiation (designation) is a protocol used for the identification and
58 | investigation of cell surface molecules providing targets for immunophenotyping of cells (Zola *et al.*,
59 | 2007). Immunohistochemistry (IHC) refers to the process of detecting antigens (e.g., proteins) in cells
60 | of a tissue section by exploiting the principle of antibodies binding specifically to antigens in
61 | biological tissues. In the most common instance, an antibody is conjugated to an enzyme, such as
62 | peroxidase, that can catalyse a colour producing reaction-. Alternatively, the antibody can also be
63 | tagged to a fluorophore, such as flourescein or rhodamine (Ramos, 2005).

64 | Because of the method of fixation and tissue preservation, the sample may require additional
65 | steps to make the epitopes available for antibody binding, including deparaffinization and antigen
66 | retrieval (microwave method, enzyme method, hot incubation method) (Jorgensen *et al.*, 2007). The

67 samples are incubated with a buffer that blocks the reactive sites to which the primary or secondary
68 antibodies may otherwise bind. IHC is an excellent detection technique and has the tremendous
69 advantage of being able to show exactly where a given protein is located within the tissue examined. It
70 is also an effective way to examine the tissues (Jorgensen *et al.*, 2007). Physiologically, CD molecules
71 can act in numerous ways, often acting as [receptors](#) or [ligands](#) (the molecule that activates a receptor)
72 important to the cell-. Some CD proteins do not play a role in cell signaling, but have other functions,
73 such as [cell adhesion CD for humans](#) is numbered up to 350 most recently-. CD molecules are utilized
74 in cell sorting using various methods including [flowcytometry](#) and immunohistochemistry (-Ho *et al.*,
75 2009).

76 In humans, the CD4 protein is encoded by the CD4 [gene](#). CD4 is a [co-receptor](#) that assists the
77 [T- cell receptor](#) —(TCR) in communicating with an [antigen-presenting cell](#). CD8 is a
78 [transmembraneglycoprotein](#) that serves as a [co receptor](#) for the [T cell receptor](#) (TCR). Like the TCR,
79 CD8 binds to a [major histocompatibility complex](#) (MHC) molecule, but is specific for the [class I MHC](#)
80 protein (Gao and Jakobsen,- 2000). T cells are distinguished by the presence of either CD4 or CD8
81 surface markers, and the latter cells are involved in antitumor immunity-. However , CD4 cells are also
82 important in this regard , because such cells are able to recognize internalizing tumor antigens
83 presented by APC in association with class II MHC molecules (Bendelac *et al.*, 2001).

84 Chronic antigenic stimulation has long been implicated in the development of hematologic
85 malignancies. Reasons are the observed prevalence and the increased risk of cancer particularly among
86 patients with autoimmune diseases and the association of some infectious agents with certain
87 malignancies. This has, in part, been ascribed to different rates of hepatitis infections in the general
88 population of the respective countries (Cooper *et al.*, 2009). In particular, autoimmune diseases have
89 repeatedly been shown to confer an increased risk for certain hematologic malignancies or malignant
90 lymphoma. Linking infectious agents with neoplasia, however, is a more difficult task (Swerdlow—,
91 2008).

92 **Materials and Methods**

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

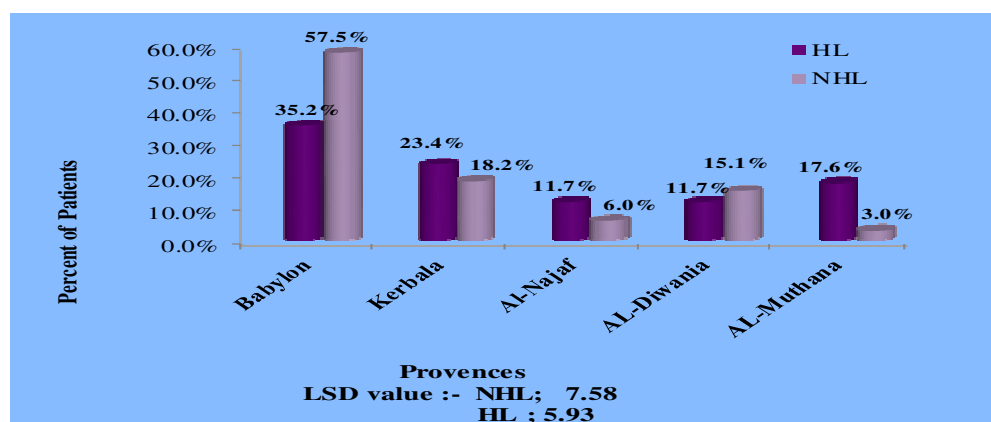
100 By using EnVesion™ Flex target can be detection of different CD markers on human cells or
101 tissues (such as CD4 and CD8), this reagent provided by Dakocytomation company for laboratory

102 investigation-. The principle and methods for viral screening test of all patient samples and control are
 103 done according to procedural flow charts of ELISA protocol of Bioelisa company.

104

105 **Results -and discussion**

106 The results were obtained from the clinically and laboratory application as illustrated in Figure
 107 (1), lymphoma patients attending from different provinces of middle part of Iraq for malignancies
 108 registry center of Babylon at the time of study was that the majority of patients with HL and NHL were
 109 from Babylon when compared with patients from other middle Euphrates provinces, In the percentage
 110 (35.2%, 57.5 %) respectively, while the patients from Karbala (23.4%, 18.2%) for both HL and NHL
 111 in the second province, and in other provinces in Al-Najaf (11.7%) HL and (6.0%) NHL, AL-Diwania
 112 (11.7%) HL (15.1%-) NHL and AL- Muthana (17.6% , 3.0 %-) for both HL and NHL. There are no
 113 related studies in such area dealing with the diseases distribution, and in the study done by Aladdin-,
 114 (2004) who mentioned that the childhood cancers are 8-10 times more common than in the west, with
 115 about 8% of the total cancers in Iraq compared to 0.5-1% in developed countries, the most common
 116 cancers in childhood are leukemia, followed by lymphomas-. According to reports of World Health
 117 Organization (WHO) the annually incidence rate of lymphoma in the middle east until 2004 as
 118 examples in Iran about 15113 cases, Iraq 5681 cases-, Jordan 1256 case, Kuwait 505 case-, Lebanon
 119 845 case-, Saudi Arabia 5775 case-, Syria 4033 case-, United Arab Emirates 565 case-, Yemen 4483
 120 cases. The high mortality rate of such cases mostly in Iraq reaches up to 19.8 % from all cases (WHO,
 121 2011)-.



122 **Figure (1): The percentage of lymphoma in middle Euphrates area**

123

124 According to the experimental, the age range of HL is great in young age than other age groups
 125 as mentioned in Figure (2-). The age range of HL refers to higher percent of patient groups at 20 -29y ,
 126 41.1% and 30 -39y, 23.5%, than in other groups 10-19y ; 11.7%, 40-49 y; 11.7%, 50-59y ; 5.8%, 60-

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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 50-59y ; 24.2% and 40-49y ; 21.2% , while less percent in other groups as the following 10-19y; 12.1%, 20-29y ; 12.1%, 30-39; 12.1%, 60 -69y ; 12.1% , and >70 ; 6.0%. This result agreed with other studies such as Al –Barzinji (2006) 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- (Carol *et al.*, 2009). While, Biermann *et al.*, (2004) who stated that incidence of lymphoma was higher in males 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.

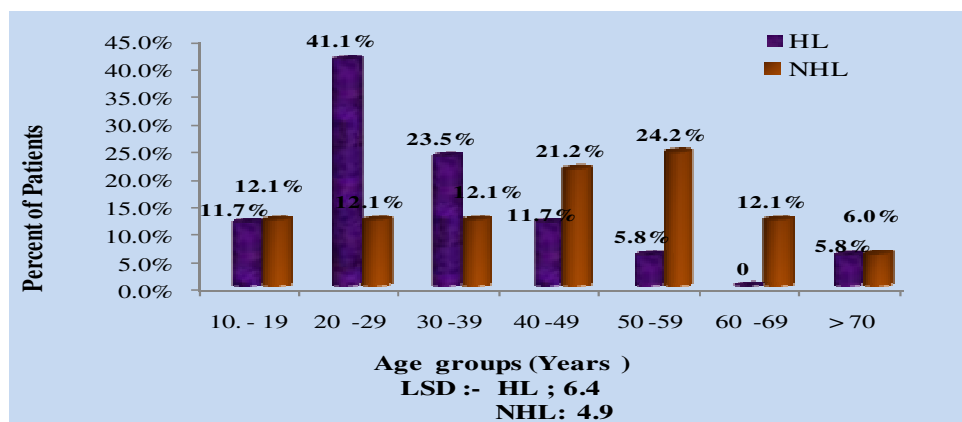


Figure (2-): Age groups distribution

In figure (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, (2006) 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, (2012) 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.

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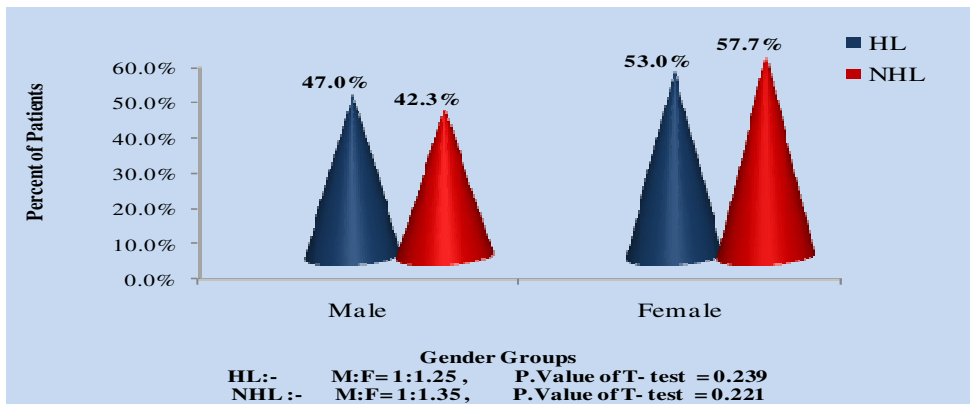


Figure (-3): Gender distribution of HL and NHL patients .

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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 antihepatitis 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.*, (2010) 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.*, (2003) and Ramos *et al.*,(2005) 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.*, (2007) reported a decreased rate of HBV infections in patients with T-cell lymphoma compared to patients with B-cell malignancies. The HCV infections more distributed rather 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 %

The specific marker for T-cell CD 4 (especially Th 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 Figure (4-A) 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 (Ansari – Iari *et al.*, 1996). 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 (Doan *et al.*, 2008).

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 initiate immune response (Warren and Earnst-, 2000).

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 (No=17)	M. C.	-	16.67	-	16.67	22.2 %
	N. S.	-	8.33	8.33	16.66	
Total		-	25.0	8.33	33.33	
NHL (No= 33)	L. B-cell	-	-	3.03	5.0	
	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 IV, 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).

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 percent at score

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III, but there is similar distribution in the results for both nodular and mixed cellular type of HL. Figure (4-B) shows the positive staining of CD 8 on tissue paraffin section. Tumor infiltrating lymphocytes are T_h 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 (Harvey and Champe, 2008). CD8 expression on the pathologic B cells of bone marrow samples from patients with various types of B-NHL (Carulli *et al.*, 2009).

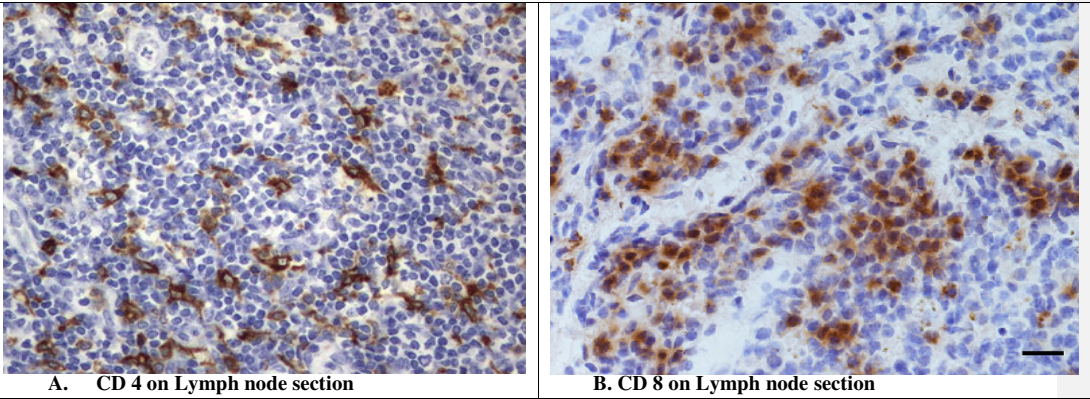
Table (-3): CD 8 with tumor types of Lymphoma.

Disease	Types	CD 8 positive Score. (%)			
		Score III	Score IV	Total	H. L.N sections (No. 9)
HL (No= 17)	M. C.	16.67	-	16.67	11.1 %
	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	
	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.)	

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



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Figure (-4): Immunohistochemical staining of CD4 and CD8 using by Dakocytomation staining of tissue paraffin sections. A (CD4) , B. (CD8). (please mention which one is A and which one is B, label them correctly)

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

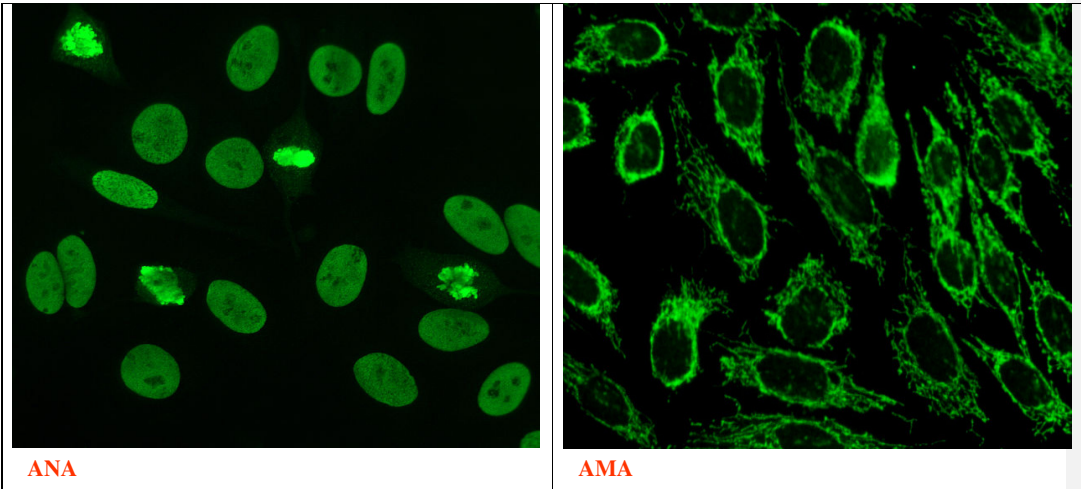
215 shows autoimmune activity in lymphoma patients. The result explained as : 7 patients of HL having
216 different autoantibodies, while in NHL 9 patients show ANA and AMA, 4 out of 9 (44.4%) and 5 out
217 of 9 (55.6%) respectively. There are 2 HL patients (28.6%) and 3 NHL (33.3%) have co-occurrence of
218 autoantibodies and CD⁴/CD⁸ markers gave a role of predisposing factor for infected with malignant
219 autoimmune T cell lymphoma rather than other types of lymphoma. Figure (-5-) show the different
220 fluorescence pictures of both pattern Antinuclear (ANA) and Anti mitochondrial antibodies on Hep-20
221 substrate-. Autoimmunity often exists in patients with non-Hodgkin lymphoma (NHL) and that
222 autoimmune disorders carry an increased risk of NHL. [Autoimmune phenomena occurs](#)
223 [phenomena occur](#) frequently in the course of lymphoproliferative malignancies, and sometimes be the
224 first sign of malignancy.

225 **Table (4-): Autoantibodies among lymphoma patients (Autoimmune**
226 **lymphoma).**

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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 %)

227



228 **Figure (-5): Fluorescent staining of different antibodies on Hep-20 substrate.**

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229 In conclusions of the present study the clinically significant results revealed that-; the age
230 groups distributed among young and adult ages for HL and NHL , respectively with no differences
231 between males and females. The Anti-HCV antibody is more distributed at all patient groups than
232 HBV. Tumor infiltrating lymphocytes with positive CD4, CD8 markers could play a role in
233 immunological microenvironment of both types of lymphoma .Increase of positive CD8 tumor

234 infiltrating lymphocytes , and the ratio of CD4/ CD8 equal to (1:1) refers that the tumor infiltrating
235 lymphocytes are T - lymphocytes, often CD8 CTLs , autoimmune T-cell lymphoma is newly form of
236 lymphoma in which recorded in this result.

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