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Immunohistochemical and Molecular Study of Epstein–Barr virus (EBV) Expression in Lymphoma and Its Correlation with Age , Gender of Patients and Type of Lymphoma in Thi-Qar

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Abstract:

Background: The lymphoid malignancies comprise one of the most diverse and heterogeneous sets of diseases that exist under a single type of malignancy. In 2012, the American Cancer Society estimated there were about 70,000 cases of non-Hodgkin lymphoma (NHL), and about 10,000 cases of Hodgkin lymphoma in the United States, collectively accounting for about 4% to 5% of all cancers. The estimated number of U.S.

cancer deaths attributed to lymphoma is about 3% for both males and females, ranking it about 8th among all causes of cancer death. Hodgkin lymphoma typically carries a better prognosis, accounting for approximately 1000 deaths per year. Whereas the lifetime risk of developing any cancer is about 1 in 2 for males and 1 in 3 for females, the lifetime risk of developing non-Hodgkin lymphoma is about 1 in 43 and 1 in 51 for males and females, respectively (for the years between 2006 and 2008).

Since the discovery in 1964 of the Epstein–Barr virus (EBV) in African Burkitt lymphoma, this virus has been associated with a remarkably diverse range of cancer types. Because EBV persists in the B cells of the asymptomatic host, it can easily be envisaged how it contributes to the development of B-cell lymphomas. However, EBV is also found in other cancers, including T-cell/natural killer cell lymphomas and several epithelial malignancies. Explaining the etiological role of EBV is challenging, partly because the virus probably contributes differently to each tumour and partly because the available disease models cannot adequately recapitulate the subtle variations in the virus–host balance that exist between the different EBV-associated cancers .

Aim of the study:- To assess EBV over expression in lymphoma and its correlation with age , gender of patients and type of lymphoma .

Materials and methods: this study was included 93 cases of archival lymphoid tissue, collected randomly from the period of February 2015- April 2017 from Al-imam Al-hussein Teaching Hospital and some private laboratories in Thi-Qar government . 41 patients of non Hodgkin's lymphoma (NHL) and 52 patients of Hodgkin's lymphoma (HL) ,their median age 50.04 years range from (14–70)years . A manual LSAB procedure was used in the imunohistochemical (IHC) analysis (DakoCytomation Copenhagen ,Denmark) and polymerase chain reaction (PCR) procedure was used in the molecular analysis (Bioneer company, Korea).

Results: IHC analysis of EBV over expression was positive in (17.1%) of NHL and (42.3%) of HL , while PCR analysis of EBV over expression was positive in (31.7%) of NHL and (61.5%) of HL were correlated with age of patients and type of tumor,

Conclusion: These finding support the role of EBVs in carcinogenesis of lymphoma regarding behavior and aggressiveness**.**

Keywords: PCR , a manual LSAB+ procedure , EBV , NHL , HL .

Introduction:

Lymphoma is a group of blood cell tumors that develop from lymphocytes (a type of white blood cell).[1] The name often refers to just the cancerous versions rather than all such tumors.[1] Signs and symptoms may include enlarged lymph nodes, fever, drenching sweats, unintended weight loss, itching, and constantly feeling tired. The enlarged lymph nodes are usually painless.[2] The sweats are most common at night.[2,3]

There are dozens of subtypes of lymphomas.[4] The two main categories of lymphomas are Hodgkin's lymphomas (HL) and the non-Hodgkin lymphomas (NHL).[5] The World Health Organization (WHO) includes two other categories as types of lymphoma: multiple myeloma and immunoproliferative diseases.[6] About 90% of lymphomas are non-Hodgkin lymphomas.[5,7] Lymphomas and leukemias are a part of the broader group of tumors of the hematopoietic and lymphoid tissues.[8]

Risk factors for Hodgkin lymphoma include infection with Epstein–Barr virus and a history of the disease in the family.[2] Risk factors for common types of non-Hodgkin lymphomas include autoimmune diseases, HIV/AIDS, infection with human T-lymphotropic virus, immunosuppressant medications, and some pesticides.[3,9] Eating large amounts of red meat and tobacco smoking may also increase the risk.[10-12] Diagnosis, if enlarged lymph nodes are present, is usually by lymph node biopsy.[2,3] Blood, urine, and bone marrow testing may also be useful in the diagnosis. [3] Medical imaging may then be done to determine if and where the cancer has spread[2,3] . Lymphoma most often spreads to the lungs, liver, and brain. [2,3]

HL is characterized by the presence of Reed-Sternberg cells and their variants in an appropriate background of inflammatory cells. Both components of this definition must be satisfied for a diagnosis of HL to be made. Although HL was originally defined on morphologic criteria alone, immunohistochemical confirmation is advisable.(13) HL includes two biologically and clinically distinct entities: nodular lymphocyte-predominant Hodgkin lymphoma (NLPHL) and classic Hodgkin lymphoma (CHL). NLPHL is a B-cell neoplasm, and CHL represents a neoplasm of "crippled" B cells.(14,15) In whites, HLs account for 25% to 40% of all lymphomas, with one age peak in the second to third decades and another in the sixth decade. In Asian and developing countries, HL accounts for only 5% to 10% of all lymphomas.(16) HL occurring in patients with acquired immunodeficiency syndrome (AIDS) exhibits several unusual features compared with sporadic cases.(17-20)

The Rye classification has been widely used for many years, and the "lymphocyte-rich CHL" category has been added in the recent classifications (Table A).(21-27) The most common type of HL is nodular sclerosis (NS) HL (NSHL), which accounts for more than 50% (up to 80%) of cases. Lymphocyte depleted (LD) HL (LDHL) is extremely rare, except in underdeveloped countries and immunocompromised hosts. Unclassifiable cases used to be placed in the mixed-cellularity (MC) category,(28) but the World Health Organization (WHO) classification recommends labeling such cases as "HL, not classifiable." (24) Most cases of "anaplastic large cell lymphoma, Hodgkin-like" have been shown to represent CHL, usually of the nodular sclerosis (NS) type.(25) In the past, the lymphocyte-predominant, NS, MC, and LD subtypes have been shown to exhibit very different clinical outcomes. These differences have been largely obliterated by modern therapy.(29)The Ann Arbor staging system has been used widely for staging of patients with HL and NHL (TableB).(30,31) The clinical stage is based on history, physical examination, radiologic studies, isotope scans, and laboratory tests and does not necessarily correlate with the pathologic stage.

TableA: Comparison of the Classification of Hodgkin Lymphoma .

WHO	Rye	Lukes and Butler
Lymphocyte predominant, nodular	Lymphocyte predominance	Lymphocytic and histiocytic Nodular and diffuse
Classic **lymphocyte rich,classic** **Mixed cellularity** **Nodular sclerosis**	Mixed cellularity Nodular sclerosis Lymphocyte depletion	Mixed cellularity Nodular sclerosis Reticular Diffuse fibrosis

WHO, World Health Organization .

Table B: Ann Arbor Staging of Hodgkin Lymphoma.

Stage I	**Involvement of a single lymph node region (I) or a single extralymphatic organ*/site (IE)**
Stage II	Involvement of two or more lymph node regions on the same side of the diaphragm (II) or Localized involvement of an extralymphatic organ and one or more lymph node region
Stage III	Involvement of lymph node regions on both sides of the diaphragm (III), which may also be accompanied by localized involvement of an extralymphatic organ (IIIE) or involvement of the spleen (IIIS) or both (IIISE)
Stage IV	Diffuse or disseminated involvement of one or more extralymphatic organs or tissues with or without associated lymph node enlargement
Sub-classification: **A. Without symptoms listed as follows.** **B. Systemic symptoms:** **• Unexplained fever 38° C** **• Unexplained weight loss 10% body weight in preceding 6 months** **• Night sweats** ***Extralymphatic organs are defined as those other than lymph node, spleen, thymus, Waldeyer ring, appendix, and Peyer patches.**	

As with the 2001 and 2008 classifications, an all-important Clinical Advisory Committee meeting was held in 2014 to obtain the advice and consent of clinical hematologists/oncologists and other physicians critical to the revision (supplemental Appendix, available on the Blood Web site). Additional editorial meetings and consultations followed leading to the updated classification (Table 1).[32]

Materials and Methods:

This study was carried out in the Department of Pathology in the College of Medicine – Thi-qar University ,it included 93 samples of archival lymphoid tissue that had been fixed with formalin and embedded in paraffin wax. Ages of patients had ranged from 14 to 70 years, with a mean of (50,04 ± 16,15 S.D. years). Forty one paraffin embedded samples (18 males and 23 females) cases with NHL and fifty two cases of HL (35 males and 17 females) cases were collected randomly from the period of February 2016- April 2017 from Al-imam Al-hussein Teaching Hospital in Thi-Qar government .

The clinical information were collected including age, gender and histological type of the lymphoid tissue from the clinical reports of the hospital, the median age 50.04 years range from (14–70) years.

Tissue sections of 5-Mm thickness from formalin –fixed , paraffin –embedded blocks were taken for the LSAB+ and PCR procedures were used for imunohistochemical and molecular detection of EBV.

Results :

In the study group, The results revealed that immunostaining of EBV protein was exclusively accumulated in the cytoplasm (cytoplasmic stain) of malignant cells.. In the studied group, positive immunoexpression for EBV was reported in 7(17.1%) out of 41 cases of NHL and 22(42.3%) out of 52 cases of HL, while 34 (82.9 %) cases of NHL and 30 (57.7 %) cases of HL were negative, with significant difference among them (P value=0.009 , R=0.271) (Table 1and figure 1&2) .

Table 1:- EBV immunoexpression in Hodgkin and non Hodgkin lymphoma .

			EBV-IHC		Total
			positive	negative	
TYPE	NHL	Count	7	34	41
		% within TYPE	17.1%	82.9%	100.0%
	HL	Count	22	30	52
		% within TYPE	42.3%	57.7%	100.0%
Total		Count	29	64	93
		% within TYPE	31.2%	68.8%	100.0%

(P value=0.009 , R=0.271)

In our study according to the age distribution, EBV immunohistochemical analysis in relation to age of the patients revealed that 6 out of 42 of age group >50 years were positive represented (14.3%) within age of the patients . 23 out of 51 cases of age group ≤ 50 years were positive represented (45.1%) within age of the patients.. There was significant difference among these groups (P value=0.001 , R=0.331) (Table 2) .

Table 2 :- the relation between EBV immunoexpression and age of malignant lymphoma .

			EBV-IHC		Total
			positive	negative	
Age	>50	Count	6	36	42
		% within Age	14.3%	85.7%	100.0%
	≤50	Count	23	28	51
		% within Age	45.1%	54.9%	100.0%
Total		Count	29	64	93
		% within Age	31.2%	68.8%	100.0%

(P value=0.001 , R=0.331

According to gender of the patients , EBV immunoexpression was reported in 17 (32.1%) out of 53 cases of male group. 12 (30%) out of 40 cases of female group. There was not a significant difference among them (P value =0.833, r=0.022) (Table 3) .

Table 3 :- the relation between EBV immunoexpression and gender of malignant lymphoma .

			EBVIHC		Total
			positive	negative	
Gender	Male	Count	17	36	53
		% within Gender	32.1%	67.9%	100.0%
	Female	Count	12	28	40
		% within Gender	30.0%	70.0%	100.0%
Total		Count	29	64	93
		% within Gender	31.2%	68.8%	100.0%

(P value =0.833, r=0.022)

In the study group, The results revealed that PCR detection of EBV was reported in 13(31.7%) out of 41 cases of NHL and 32(61.5%) out of 52 cases of HL, while 28 (68.3 %) cases of NHL and 20 (38.5%) cases of HL were negative, with significant difference among them (P value=0.004 , R=0.296) (Table 4) .

Table 4 :- PCR detection of EBV in Hodgkin and non Hodgkin lymphoma .

			EBV-PCR		Total
			POSITIVE	NEGATIVE	
TYPE	NHL	Count	13	28	41
		% within TYPE	31.7%	68.3%	100.0%
	HL	Count	32	20	52
		% within TYPE	61.5%	38.5%	100.0%
Total		Count	45	48	93
		% within TYPE	48.4%	51.6%	100.0%

(P value=0.004 , R=0.296)

In our study according to the age distribution, PCR analysis of EBV in relation to age of the patients revealed that 10 out of 42 of age group > 50 years were positive represented (23.8%) within age of the patients . 35 out of 51 cases of age group ≤50 years were positive represented (68.6%) within age of the patients.. There was significant difference among these groups (P value=0.0001 , R=0.446) (Table 5) .

Table 5 :- the relation between PCR detection of EBV and age of malignant lymphoma patients .

			EBV- PCR		Total
			POSITIVE	NEGATIVE	
Age	> 50	Count	10	32	42
		% within Age	23.8%	76.2%	100.0%
	≤ 50	Count	35	16	51
		% within Age	68.6%	31.4%	100.0%
Total		Count	45	48	93
		% within Age	48.4%	51.6%	100.0%

(P value=0.0001 , R=0.446)

According to gender of the patients ,EBV-PCR detection was reported in 26 (49.1%) out of 53 cases of male group. 19 (47.5%) out of 40 cases of female group . There was not a significant difference among them (P value =0.883, r=0.015) (Table 6) .

Table 6 :- the relation between PCR detection of EBV and gender of malignant lymphoma patients .

			EBVPCR		Total
			POSITIVE	NEGATIVE	
Gender	Male	Count	26	27	53
		% within Gender	49.1%	50.9%	100.0%
	Female	Count	19	21	40
		% within Gender	47.5%	52.5%	100.0%
Total		Count	45	48	93
		% within Gender	48.4%	51.6%	100.0%

(P value =0.883, r=0.015)

Discussion:

In the current study the results of IHC study have clarified that 42.3% (22 out of 52) of HL and 17.1% (7 out of 41) of NHL were expressing immunohistochemical cytoplasmic staining of EBV protein in their histological sections, with a significant difference in comparison with control group (P value=0.009).While The results of polymerase chain reaction (PCR) technique revealed that the prevalence of EBV was 61.5% (32 out of 52) in HL and 31.1% (13 out of 41) in NHL , with a significant difference in comparison with control group (P value=0.001).

It looks that EBV is reported in both HL and NHL lymphoid tissue with a variable significant percentage, this means that EBV expression has a significant role in early event of neoplastic process of lymphoid lesion. This event may undergo more amplification and augmentation of mutant genes due to subsequent exposure to co carcinogens that explain the evolution into malignancy later on (multistep theory). This necessitates to introduce a successful screening program for early detection and early treatment of these suspicious lesions.

This proposed explanation is supported by some researchers investigated the molecular basis of EBV in malignant lymphoma .

According to the age distribution of malignant lymphoma patients , EBV immunohistochemical analysis revealed that (14.3%) (6 out of 42) in age group >50 years were positive and (45.1%) (23 out of 51) in age group ≤ 50 years were positive. There was significant difference among these groups (P value=0.001 , R=0.331). While the analysis of PCR results of EBV in relation to age distribution of malignant lymphoma patients was detected in 68.6% (35 out of 51) in age group ≤ 50 years and 23.8% (10 out of 42) in age groups > 50 years , with significant differences between the advanced age and those ≤ 50 years (P value=0.0001) . Furthermore, it looks well correlated to the age of patients (r= 0.446).

The low prevelance of EBV expression in IHC study inspite of the presence of EBV DNA in PCR study , suggests that EBV don't reach a level to be expreessed immunohitochemically, or its protein has been lost due to excessive utilization by replicative tumor cells .

It can be proposed that the EBV affect occurs mainly before 50 years of age with highly tropism to lymphoid cells in young age groups . This notice is so important to plan further for proper management of malignant lymphoma .

According to gender of the patients , EBV immunoexpression was reported in 32.1% of male group and in 30% of female group. There was not a significant difference among them (P value =0.833) . While EBV-PCR detection was reported in 49.1% of male group and 47.5% of female group . There was not a significant difference among them (P value =0.883) .

This means that the biological behavior of tumors in both male and female patients is the same regarding grade, stage and age, i.e EBV expression is not affected by hormonal status of patients.

Conclusion:

EBV expression in HL and NHL is well correlated with types of tumor , indicating biologically aggressive tumor. This notice is so important to plan further for proper management of malignant lymphoma .

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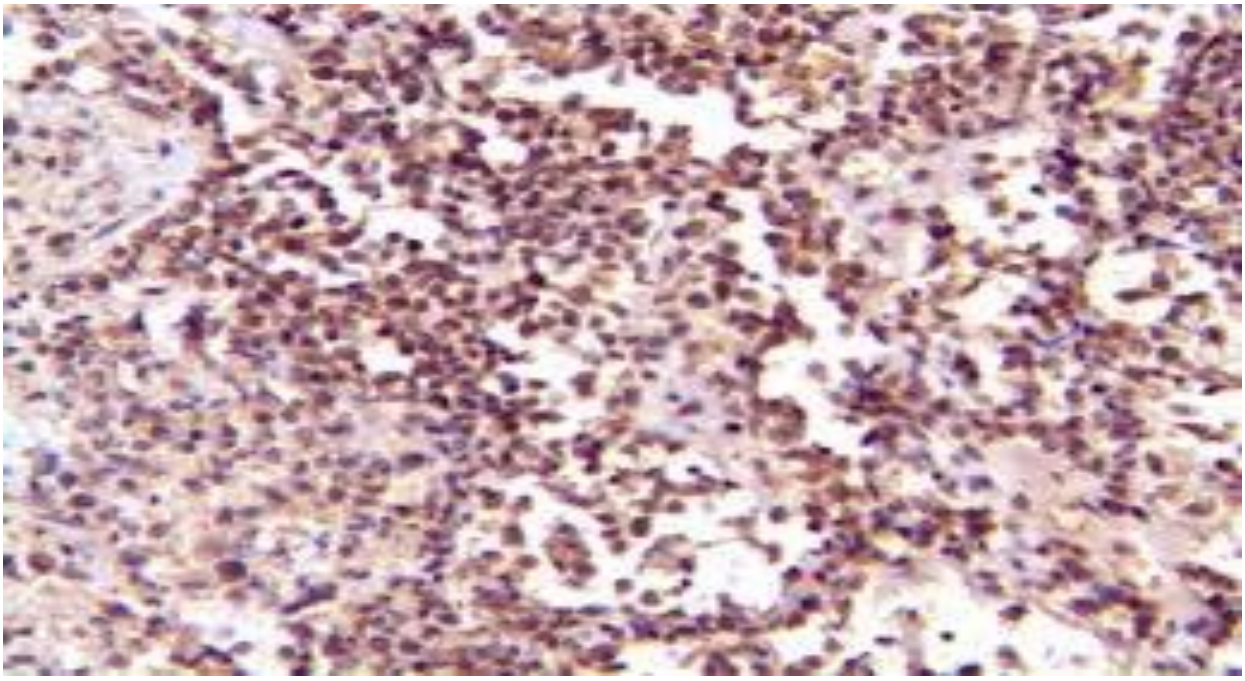

Figure 1 :- EBV immunoexpression in non Hodgkin lymphoma .

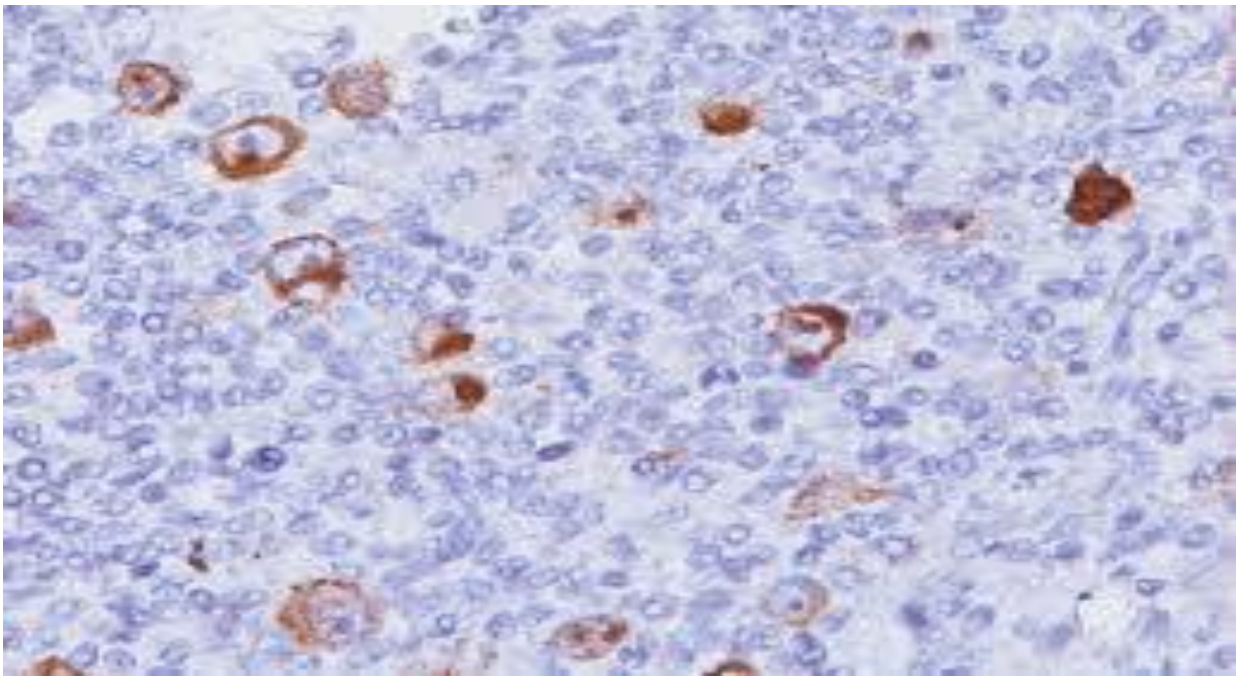

Figure 2 :- EBV immunoexpression in Hodgkin lymphoma .

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الخلاصة
الخلفية: الأورام الخبيثة التي تصيب الانسجة اللمفاوية واحده من أكثر المجموعات المتنوعة وغير المتجانسة من الامراض . في 2012 ، والجمعية الأمريكية للسرطان قدرت هناك حوالي 70,000 حاله من الأورام اللمفاوية نوع (NHL) ، وحوالي 10,000 حاله من اورام الغدد اللمفاوية نوع (HL) في الولايات المتحدة ، والتي تمثل مجتمعه نحو 4 ٪ إلى 5 ٪ من جميع أنواع السرطان. العدد المقدر للولايات المتحدة من وفيات السرطان المعزوة إلى الغدد اللمفاوية حوالي 3 ٪ لكل من الذكور والإناث ، وترتيب ذلك حوالي الثامن بين جميع أسباب وفيات السرطان. وعاده ما يحمل الورم اللمفاوي هودجكين تشخيصا أفضل ، وهو ما يمثل حوالي 1000 حاله وفاه في السنه. في حين ان خطر الإصابة بالسرطان علي مدي الحياة هو حوالي 1 في 2 للذكور و 1 في 3 للإناث ، والمخاطر الحياتية لتطور الغدد اللمفاوية غير هودجكين هو حوالي 1 في 43 و 1 في 51 للذكور والإناث علي التوالي (للسنوات بين 2006 و 2008)
منذ اكتشاف فيروس EBV في 1964 في الغدد اللمفاوية ، وقد ارتبط هذا الفيروس مع مجموعه متنوعة بشكل ملحوظ من أنواع السرطان. هو يستطيع بسهوله كنت يتصور كيف هو يساهم إلى التطوير من [ب-خليه] أورام لمفاوية. ومع ذلك ، يوجد أيضا في الأورام السرطانية الأخرى ، بما في ذلك الخلايا اللمفاوية/الخلايا القاتلة الطبيعية ، والعديد من الأورام الخبيثة للبشرة. شرح الدور المسبب لل EBV و قابليتة المرضية على انشاء السرطان ، من جانب لان الفيروس ربما يساهم بشكل مختلف لكل الورم وجانب اخر لان النماذج المتاحة للمرض لا يمكن تلخيصها بشكل كاف للاختلافات الخفية في الفيروس—و الوسط المضيف الذي توجد بين أنواع السرطان المرتبطة بالأمراض التي تصيب الانسان .
كلمات مفتاحيه: سرطان الغدد اللمفاوية ، طريقة (+LSAB) و PCR للكشف عن فيروس EBV.