Prevalence of Epstein - Barr virus in Malignant Lymphoid tumors in Basrah

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ABSTRACT

Background: **Different factors (genetic and environmental) appear to be involved in the pathogenesis of malignant lymphomas and one of these factors is Epstein Barr virus.**

Objective: **To evaluate the prevalence of Epstein Barr virus in Malignant Lymphoid tumors in Basrah**

Method: **Cross-sectional study of fifty-six cases of malignant lymphomas (Hodgkin and Non-Hodgkin) were collected. The initial diagnosis and subtyping of malignant lymphomas were performed by the microscopical examination of the hematoxylin and eosin stained sections. Then immunohistochemistry for Epstein Barr virus, Latent membrane protein was done.**

Results: **A total of 56 cases diagnosed as malignant lymphomas, 37 cases (66.1%) were Hodgkin lymphomas and 19 cases (33.9%) were Non-Hodgkin lymphomas. For Epstein Barr virus (Latent membrane protein) immunohistochemistry, 18 of 37 cases (48.6%) of Hodgkin Lymphomas and 6 of 16 cases (31.6%) of Non Hodgkin Lymphomas had positive viral expression. Mixed Cellularity Hodgkin Lymphomas and Diffuse Large B-Cell Lymphomas were the commonest subtypes that showed Epstein Barr virus expression (64% and 37.7% respectively). The immunohistochemical viral expression was more among those less than 50 years and male sex in both Hodgkin and Non-Hodgkin Lymphomas.**

Conclusion: **The high expression of Latent membrane protein -1 confirms the association of the virus with malignant lymphomas, especially in Hodgkin lymphomas.**

Key words: **Malignant lymphomas, Hodgkin lymphomas, Non-Hodgkin lymphoma, Epstein Barr virus, Epstein Barr virus latent membrane protein-1.**

انتشار فيروس ابشتاين – بار في أورام اللمفاوية الخبيثة في البصرة

الخلفية: **يبدو أن هناك عوامل مختلفة (وراثية وبيئية) متورطة في التسبب في الأورام اللمفاوية الخبيثة وأحد هذه العوامل هو فيروس إبشتاين بار.**

الهدف: **تقييم مدى انتشار فيروس ابشتاين بار في أورام اللمفاوية الخبيثة في البصرة**

الطريقة: **تم جمع دراسة مستعرضة من ستة وخمسين حالة من الأورام اللمفاوية الخبيثة (هودجكين وغير هودجكين). تم إجراء التشخيص الأولي والنماذج الفرعية للأورام اللمفاوية الخبيثة عن طريق الفحص المجهري لأقسام ملطخة الهيماتوكسيلين والأيوزين. ثم الكيمياء المناعية لفيروس ابشتاين بار، وقد تم البروتين الغشاء الكامنة.**

النتائج: **ما مجموعه ٥٦ حالة تم تشخيصها على أنها سرطان الغدد الليمفاوية الخبيثة، ٣٧ حالة (٦٦.١ ٪) كانت سرطان الغدد الليمفاوية هودجكين و ١٩ حالة (٣٣.٩ ٪) كانت ليمفوما اللاهودجكين. بالنسبة لفيروس Epstein Barr بروتين الأغشية الكامنة)، فإن ١٨ من ٣٧ حالة (٤٨.٦٪) من الأورام اللمفاوية هودجكين و ٦ من ١٦ حالة (٣١.٦٪) من الأورام اللمفاوية غير هودجكين كان لها تعبير فيروسي إيجابي. كانت الأورام اللمفاوية المكوّنة من نوع هودجكين الخلوية والأورام اللمفاوية الكبيرة من الخلايا المنتشرة هي النوع الفرعي الأكثر شيوعًا الذي أظهر تعبير فيروس إبستين بار (٦٤٪ و ٣٧.٧٪ على التوالي). كان التعبير الفيروسي المناعي أكثر بين أولئك الذين تقل أعمارهم عن ٥٠ عامًا والجنس الذكري في كل من الأورام اللمفاوية هودجكين وغير هودجكين.**

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الخلاصة: إن التعبير العالي عن بروتين الغشاء الكامن –١ يؤكد ارتباط الفيروس بالأورام اللمفاوية الخبيثة ، خاصة في الأورام اللمفاوية هودجكين.

الكلمات المفتاحية: الأورام اللمفاوية الخبيثة، الأورام اللمفاوية الهودجكينية، الأورام اللمفاوية غير الهودجكينية، فيروس إيبشتاين بار، فيروس إيبشتاين بار، بروتين الغشاء الكامن١

INTRODUCTION

Malignant lymphomas (MLs) are the commonest primary tumor of lymphatic system, particularly of lymphocytes and their precursor.[1] There are 2 main types of lymphomas: Hodgkin lymphoma (HL) and non-Hodgkin lymphoma (NHL).[2] Both types are subdivided into distinct subcategories.[1] Many factors associated with the increased risk of developing lymphomas such as impaired immune system and certain infections example: Epstein-Barr Virus (EBV), Human T- cell Lymphotropic Virus type 1 (HTLV-1), Human Immunodeficiency Virus (HIV),[3] and Helicobacter pylori infections.[4] According to Iraqi cancer registry in 2016, HLs account for 4.4% while NHLs were 8.2%. [5] Epstein-Barr virus (EBV), a member of the herpesvirus family, that have been implicated in the pathogenesis of several tumor types includes: Burkitt lymphoma, B-cell lymphomas in immunosuppressed individuals, Hodgkin lymphoma, nasopharyngeal carcinoma, some gastric carcinomas, and rare forms of T-cell and natural killer (NK) cell lymphoma.[6] Epstein - Barr virus infects B-lymphocytes and possibly epithelial cells of the oropharynx. The virus uses the complement receptor CD21 to attach to and infect B cells,[7] EBV-driven polyclonal B-cell proliferation in vivo is readily controlled. [8] A small percentage of B-cell can undergo a lytic infectious cycle while the majority remain latently infected for life.[9] EBV infection is controlled by an intact cellular immune response in which cytotoxic (CD8+) T-cells play a vital role. Latent infection is established in Memory B-cells.[10] Evasion of the immune system seems to be a key step in EBV-related oncogenesis, in which infected carrier B-cells can transform from their latent state into malignant cells.[11] Latent EBV infection express six" EBV-encoded nuclear antigens" (EBNAs 1, 2, 3A, 3B, 3C and- LP) and three "latent membrane proteins" (LMPs 1, 2A, and 2B), in addition to "EBV-encoded small RNA" (EBER) and "non-transcribed BART" (*Bam*HI-A region rightward transcript) RNAs.[7,12] Latency type I occur in Burkitt Lymphoma and expressed (EBER1/2 RNA, EBNA-1, BART RNA).[13] While latency type II is found in nasopharyngeal carcinoma and Hodgkin lymphoma that expressed ("EBER1/2 RNA, EBNA-1, LMP-2A/B, LMP-1 (type IIa) or EBNA-2 (type IIb), BART RNA").[13,14] Latency type III occur in lymphoproliferative disorder and in vitro infected B-lymphocyte and expressed ("EBER1/2 RNA, EBNA-leader protein (EBNA-LP), EBNA-2, EBNA-3ABC, EBNA-1, LMP-2A/B, LMP-1, BART RNA").[13]

METHODS

Cross-sectional study was conducted from October 2017 to the end of November 2018. Newly diagnosed cases of Malignant Lymphomas (HLs and NHLs) where collected from pathology department of Al-Sader teaching hospital and private laboratories in Basrah. Fifty-six patients were selected in this study. The clinico-pathological parameters were included: age, sex, histopathological diagnosis and EBV immunohistochemistry. The study protocol was approved by institutional ethical committee at Basrah College of Medicine. For each case two sections of 5 mm thickness were taken. One section was stained with the routine hematoxylin and eosin (H&E) stain. While other section was stained immunohistochemically for LMP-1 with horseradish peroxidase (HRP)-labelled-streptavidin-biotin method. This technique basically uses an primary antibody

from DaKo Denmark A/S, Anti-Epstein-Barr Virus, LMP. Mouse Monoclonal antibody, Code Number: M 0897. There will be an antibody-antigen interaction and an enzymatic reaction can be detected by the chromogen, diaminobenzidine (DAB), which can be visualized by light microscopy. Diffuse cytoplasmic and membranous brown staining pattern was considered as positive result.

Statistical analysis:

The software used was Microsoft excel 2010 and Statistical Package for Social Science (SPSS) version 23 to analyze the data. The means values and standard deviation were used for numerical data. Categorical data were presented as number and percentage. Chi-square (x^2) Test and Exact Fissure Test were used to assess the significance of differences between groups.

P-value less than 0.05 was considered as statistically significant and P-value less than 0.01 considered as highly significant.

RESULTS

The mean age of the 56 patients with ML was 37.1 years ± 16.1 SD with a range of 13 to 71 years. 44 cases (78.6%) were < 50 years, while 12 cases (21.4%) were ≥ 50 years. Thirty- two patients (57.1%) were males and 24(42.9%) were females with a male to female ratio (M:F) of 1.3:1. (Table-1) Regarding the histopatological type distribution of MLs, 37 cases (66.1%) were HLs and 19 cases (33.9%) were NHLs. Among HLs: 25 cases (67.6%) were mixed cellularity (MC) and 12 cases (32.4%) were nodular sclerosis (NS) subtype. 19 cases (76%) of MC subtype were males while 6 cases (24%) females (M:F 3.1:1) , and of 12 cases of NS subtype 4 cases (10.8%) were males and 8 cases (21.6%) females with (M:F 0.5:1). While for NHLs: 16 cases (84.2%) were Diffuse large B-cell lymphoma (DLBCL), 2 cases (10.5%) were small lymphocytic lymphoma (SLL), and 1case (5.3%) natural killer T- cell lymphoma (NKTCL) -nasal type. Equal numbers of DLBCL had been occurred in both males and females (8 cases for each). Two cases of SLL was expressed in females, while the 1 cases of NKTCL (nasal type) presented in male**.**

Table 1. Age and sex distribution of 56 cases of Malignant lymphoma

Age in years	Hodgkin lymphoma				Non-Hodgkin lymphoma			
	Sex				*Sex*			
	Male		Female		male		Female	
	No.	%	No.	%	No.	%	No.	%
50 <	21	91.3	12	85.7	5	55.6	6	60
≥ 50	2	8.7	2	14.3	4	44.4	4	40
Total	23	100	14	100	9	100	10	100

X^2 test, P-value >0.05

The immunostaining for anti-EBV antibodies was positive in 24 cases (42.9%) of ML and negative in 32 cases (57.1%). In HLs, 18 cases (48.6%) had positive expression for EBV and 19 cases (51.4%) were negative . For NHLs, 6 cases (31.6%) were positive for viral expression while 13 cases (68.4%) were negative. Among histopathological subtypes for HLs, MC showed the commonest immunohistochemical expression of EBV antibody, in which 16 cases (64%) were positive while in NS, 2 cases (16.7%) were positive (Table:2). There is a highly significant correlation between EBV immunohistochemistry and MC subtypes.

Table 2. Anti-EBV (LMP) expression among histological subtypes of Hodgkin Lymphoma

EBV expression	Histological subtypes					P- value
	Mixed Cellularity		Nodular Sclerosis			
	No.	%	No.	%	No.	
Positive	16	64*	2	16.7	18	0.012
Negative	9	36	10	83.3	19	
Total	25	100	12	100	37	

X^2 test, EBV= Epstein - Barr virus, LMP= latent membrane protein

For NHLs subtypes only DLBCL showed positive expression in 6 cases (37.7%) while other subtypes had negative expression. (Table-3)

Table 3. Anti-EBV (LMP) expression among histological subtypes of Non-Hodgkin lymphoma

EBV expression	Histological subtypes							
	Diffuse large B-cell lymphoma		Small lymphocytic lymphoma		Natural Killer T-cell lymphoma		Total	
	No.	*%*	*No.*	*%*	*No.*	*%*	*No.*	
Positive	6	37.5	0	0	0	0	6	0.67
Negative	10	62.5	2	100	1	100	13	
Total	16	100	2	100	1	100	19	

X^2 test, EBV= Epstein - Barr virus, LMP=latent membrane protein

Fourteen cases (77.8%) of EBV positive HLs were males and 4 cases (22.2%) were females (M:F 3.5:1), but in NHLs all positive cases were males (Table-4). Statistically, there is a highly significant association between EBV expression in NHL and male sex (P-value = 0.003).

Table 4. Sex distribution of Epstein - Barr virus (LMP)

Histological type	Epstein-Barr virus positive				Epstein-Barr virus negative				P- value
	Male		Female		Male		Female		
	No.	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*	
Hodgkin Lymphoma	14	77.8	4	22.2	9	47.4	10	52.6	
Non-Hodgkin Lymphoma	6	100*	0	0	3	23.1	10	76.9	0.003

X^2 test, LMP= latent membrane protein

The distribution of anti- EBV (LMP) in cases of ML in relation to age, 18 cases (54.5%) of those less than 50 years were anti-EBV positive while no viral expression in older age group (≥50 years). While For NHL, four (36.4%) out of 6 positive cases were less than 50 years and 2 (25%) cases ≥ 50 years. (Table-5)

Table 5. Age distribution of Epstein - Barr virus (LMP)

EBV expression	Hodgkin lymphoma				Non-Hodgkin lymphoma			
	< 50 years		≥ 50 years		< 50 years		≥ 50 years	
	No.	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*
EBV- positive	18	100	0	0	4	66.7	2	33.3
EBV- negative	15	78.9	4	21.1	7	53.8	6	46.2

X^2 test, p-value >0.05, EBV= Epstein - Barr virus, LMP=latent membrane protein

DISCUSSION

The mean age of the patients in the present study was 37.1 years with highest frequency (78.6%) reported in those less than 50 years. This finding is in agreement with other studies such as in Eastern India (2014),[15] and Cairo (2010).[16] The male predominance observed in this study was in agreement with eastern India study in 2014 (M:F 3.1:1).[15] Regarding the histological type the proportion of HLs and NHLs was 66.1% and 33.9% respectively. This is in agreement to what was reported in Jordan 2010 (54.5% for HL and 45.5% for NHL),[17] but in contrast with Northern Iraqi studies in 2011,[18] and Lebanon 2014.[19] This disagreements were mostly due to small sample size. The mixed cellularity is the commonest subtype among HLs. This result was in concordance with other studies such as AL-Mudallal study (Baghdad 2012),[20] Eastern India[15] and Punjab, Pakistan.[21] The higher incidence of MC subtypes was reported among male, Similar finding was documented in other studies such as Punjab, Pakistan (M:F 3.8:1),[21] and Korea (M:F 2.4:1).[22] In NS subtypes, the higher incidence was reported among female (M:F 0.5:1), these differs from Cairo[16] Iraq,[18] and Punjab.[21] Regarding NHL subtypes, DLBCL was the most commonly encountered which is consist with other Iraq study,[18] and Lebanon (44%).[19] DLBCL had equal sex distribution (M:F 1:1), comparing with other studies, in Iraq.[18] and Lebanon,[19] and Punjab.[21] This difference in histological subtypes of HLs and NHLs may be attributed to geographical variation, which had been suggest different molecular pathway in development MLs in different geographical areas. In current study, EBV was positive in 24 cases (42.9%) of MLs. Among them 48.6% of HLs and 31.6% of NHLs express positivity. This result is in agreement with what was reported in others such as Jordan in which 60% of HLs were positive compare to 32% of NHLs,[17] India 2017 (51% of HLs and 5% of NHLs).[23] But in

contrast to that reported in Lusaka, Zambia 2018 (40.9% of HLs and 54.5% of NHLs were positive).[24] Sixty-four percent of MC and 16.7% of NS subtypes were positive viral expression. Comparing with other research that were done in Cairo (100% for MC and 60% for NS), [16] and United Arab Emirates 2008 (53% of MC and 27% of NS).[25] For NHLs subtypes, 37.5% (6 cases) of DLBCLs were positive. Which is similar to ALwan study (19.2% from 26 cases),[26] and in India 2% were positive. [23] None of other subtypes of NHL express positivity while in India 2 of 3 cases of NK/TCL were viral positive.[23] In present study, 77.8% of EBV positive HLs were male and 22.2% were female (M:F 3.5:1). This difference in sex was not statistically significant (P-value = 1). These in agreement with other studies in Lusaka, Zambia (77.8% male and 22.2% female),[24] and United Arab Emirates (70.6% male and 29.4% female).[25] In NHLs, all viral positive cases were present in male, which is highly statistically significant (P-value = 0.003). Comparable with other studies, in Lusaka, Zambia (60.4% male vs 39.6% female),[24] and Sudanese patients 2016 (84.6% male VS 15.4% female).[27] All cases of EBV positive HLs in current study were less than 50 years of age. This finding showed agreement with Kuwaiti study (2003) (82.7%) [28], this may attributed to same environmental exposure. For NHLs, 66.7% of EBV positive cases were those < 50 years and 33.3% of those ≥ 50. In correlation with Ismail study (2016) who mentioned that high incidence of EBV positive NHLs among Sudanese patients between 40-60 years (30.7%),[27] while in Korea the peak age at 60 years.[22] These significant variations in EBV positivity and lymphoma may be related to ethnic background and geographic location (different in socioeconomic level, genetic susceptibility, and early life exposure to EBV and other chronic infections associated with depressed immunity).

CONCLUSION

- In both HLs and NHLs higher percentage of EBV expression was among males and those less than 50 years of age
- Epstein-Barr Virus (LMP) expression was more in HLs than NHLs.
- Epstein-Barr Virus immunohistochemistry is highly express among MCHL and DLBCL.

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