

Incidence of Epstein Barr Virus infection in newly diagnosed non-Hodgkin lymphoma in the national center of hematology-single center study

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Abstract:

Background: Non Hodgkin lymphoma (NHL) is a diverse group of disorders both in their original history and in response to therapy. The appearance of Epstein-Barr infection (EBV) in Non-Hodgkin's lymphoma (NHL) could be distinguished by enzyme link immune sorbent assay by detecting IgM and IgG. The role of EBV as causative agent for non-Hodgkin lymphoma has been supported by identification of large amounts of latent membrane protein 1 (LMP-1) outflow in tumors.

The aim: of this study was to determine the incidence of EBV infection in patient with newly diagnosed patients with non-Hodgkin lymphoma.

Materials and Methods: This study was done at the Department of clinical hematology, the national center of hematology (NCH), Baghdad, Iraq from August 2012 to November 2013. It is a prospective study. A total of 56 patients with age ranged from 18 – 56 years were included. They were diagnosed with NHL and then classified to subtypes by studying histopathology and immunohistochemistry. Assessment of EBV IgM and IgG were done by ELISA technique. A second group comprised of 22 patients with non-malignant condition regarded as control group with matched sex and age. Statistical analysis was performed utilizing SPSS version 17.0.

Result: Mean age of the patients was 51.44±15.93 years. Thirty-six (70.4%) were males and 20 (29.6%) were females. Seven (12.5%) out of 56 cases were positive for EBV-IgM, two cases (28.5%) were follicular lymphoma, 5 cases (71.5%) were diffuse large B cell lymphomas. For the EBV-IgG 12 patients (21.4%) were negative while majority 44 patients (78.6%) were positive, in comparison to control group the result was statistically not significant.

Conclusion: This study showed that the age, sex and clinical characteristics of Iraqi patients with NHL are different from those of surrounding countries and. The incidence of EBV is high in NHL. The most frequent subtype of NHL is diffuse large B cell lymphoma.

Keywords: incidence, Epstein-Barr virus, NHL.

Introduction:

Non Hodgkin lymphoma (NHL) is a diverse group of disorders both in their original history and in response to therapy. Accessible epidemiological information from different parts of world showed different geographical varieties in the frequency, histopathological and clinical outcome. Non Hodgkin lymphoma seems to be more prevalent in developing countries, where effect of different risk factors including environmental

and hereditary factors may influence the development of such diseases.(1)

The Non-Hodgkin lymphomas include B cell lymphomas which comprise 86% and T cell lymphomas around 14%. The most widely recognized histological subtypes of B cell NHL are Diffuse large B cell lymphoma (DLBCL), followed by follicular lymphoma, then Burritt lymphoma and Lymphoblastic lymphoma (2).

Epstein-Barr infection (EBV) is a well-known tumorigenic human herpes infection which characterized by long lasting asymptomatic infection of B cell in many people, without causing illness (3, 4). Epstein-Barr infection has different association with many types of lymphoproliferative disorders ranging from

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very common like Burkitt’s lymphoma and Hodgkin’s lymphoma (40%) to uncommon association like chronic lymphocytic leukemia (5). Epstein Barr virus (EBV) is considered to be an essential model of a changing virus involved in several NHL subtypes (6).

EBV is generally developed in early infancy; the principal infection of EBV in teens is associated with the clinical syndrome of infectious mononucleosis. After primary infection, EBV stays for long period in the host body in a dormant state in the memory B cell lymphocytes. Numerous EBV proteins can be shown in infected lymphocytes, among which latent membrane protein-1 is supposed to be most significant for transformation. In healthy infected persons, development of EBV-transformed B lymphocytes is stopped by the presence of integral T lymphocyte-mediated immunity (5).

The detection of Epstein-Barr virus in Non-Hodgkin’s lymphoma can be done promptly by either immunohistochemistry for detection of Epstein-Barr virus latent membrane protein (LMP), or by detection of antibodies to viral capsid antigen. The role of Epstein-Barr virus as etiologic agent in the development of Non-Hodgkin lymphoma has been supported by expression of high levels of latent membrane protein 1 (LMP-1) in malignant lymphocytes. The incidence of EBV in NHL is has different widely according to geographical area, so it ranges from 10% in Japan to around 60% in Middle East(2,12,13,14). The aim of current study was to determine the incidence of Epstein-Barr virus in newly diagnosed patients with Non-Hodgkin lymphoma by using ELISA method for detection of IgM and IgG antibodies.

Material and Methods:

This study was conducted at Department of clinical hematology, the national center of hematology /Almustansiriya University (NCH) in Baghdad from August 2012 to November 2013. It was a prospective study. The study was approved by Institutional Review Board (IRB) of NCH. A total of 56 patients with new diagnosis as of NHL were included. The diagnosis was based on histological and immunohistochemical studies and according to this subtypes of NHL were assigned. All cases of nodal Non-Hodgkin lymphomas diagnosed according to WHO

Classification were included. Burkitt lymphoma, leukemic phase NHL, and extra nodal NHL were excluded from the study.

The control arm consisted of (22) patients who diagnosed with non-malignant disorders with sex and age matched. Five milliliters of venous blood samples were taken from patients and controls groups. The samples then centrifuged and serum separated. Serum was stored in aliquots at -20°C. Repeated freezing and thawing was avoided. EBV (viral capsid antigen) IgM and IgG were detected with commercially available enzyme linked immunosorbent assay (ELISA) (CUSABIO BIOTECH CO.LTD). The mentioned above tests was done according to the instructions of the kit provided by Cortez diagnostics

The statistical data were analyzed by using SPSS for windows version (17.0). P-value <0.05 was considered significant.

Results:

Mean age of the patients was 51.44±15.93 years. Thirty six patients (70.4%) were males and 20 patients (29.6%) were females. Seven (12.5%) out of 56 cases were positive for EBV-IgM, (cutoff value <1.1) with mean value of (0.35 ±0.54), 5 cases (71.5%) were diagnosed with diffuse large B cell lymphomas while the other 2 cases (28.5%) were diagnosed with follicular lymphoma. In Comparison to control group only 2 patients have IgM which statistically revealed non-significant relationship, which is shown in Table-2.

For the EBV-IgG antibody; 44 patients (78.6%) were positive while 12 patients (21.4%) were negative, (cutoff value >1.1) with mean value of (1.93 ±0.86) and in comparison to control group the result was statistically significant p value < 0.05, which is shown in Table-3.

A total of 53(94.6%) cases were B-cell Non Hodgkin lymphomas and only 3(5.4%) cases were T-cell Non Hodgkin lymphomas. This study included 26 (46.4%) cases of large B cell lymphoma, 19 (33.9%) cases of follicular lymphoma, 5(8.9%) cases of small lymphocytic lymphoma, 3 (5.4%) cases of T- cell lymphoma, 3 (5.4%) cases of mantle cell lymphoma, Table- 1 shows demographic characteristics of both patients and control group.

Table 1. demographic characteristics of both patients and control group.

Characteristic	Patients	Controls	P value
Number of patients	56	22	
Age - Mean (years)	51.44±15.93	47.23±11.43	NS
Range(year)	17-78	23-61	
Sex (male/Female)	36/20	13/9	NS
Types of NHL	Patient No. (%)		
Diffuse large B cell lymphoma	(46.4) 26		
Follicular lymphoma	(33.9) 19		
Small lymphocytic lymphoma	5 (8,9)		
T cell lymphoma	(5,4)3		
Mantle cell lymphoma	(5.4)3		

Table 1. demographic characteristics of both patients and control group.

IgM-VCA	Positive	Negative	Value-P
with Patients NHL	(12.5%)7	(87.5%)49	value-P 0.67 Significant Not
control	(6.6%)2	(93.4%)20	

The Chi-square statistic is 0.1798. The P value is 0.671509. This result is not significant at $p < 0.05$.

Table 3: Result of EBV IgG in patients with NHL and controls group

IgG-VCA	Positive	Negative	Value-P
with Patients NHL	(78.6%)44	(21.4%)12	value-P 0.05> Significant
control	(31.8%)7	(68.2%)15	

The Chi-square statistic is 49.2858. The P value is 0. This result is significant at $p < 0.05$

Discussion:

Epstein–Barr virus, first labelled by Denis Burkitt in 1958, it is a member of herpes virus family. Like other herpes viruses, EBV is an enveloped virus that has a DNA core surrounded by nucleocapsid. Family members include herpes simplex I and II and varicella-zoster virus, cytomegalovirus and human herpes virus 6 and 7, and human herpes virus 8 and EBV (gamma herpes virus subfamily) (1,5).

Despite our growing understanding of the role of EBV in the pathogenesis of disease, the optimal management of EBV associated tumors remains unsatisfactory. Exploration of antiviral agents, immune-based therapies, and specific monoclonal antibodies is, however, proceeding with encouraging results (5, 6). Although NHL can be diagnosed in any age group, it is more common in 5th and 6th decade. The mean age in our study was around 50 years. This result was similar to the studies by Mushtaq et al., Jamal et al., and Veelken et al. In which mean ages were 58 years, 55 years, and 59 years respectively. (1, 7, 8). Worldwide, NHL is more common in males as compared to females. Comparable result was found in current study in which 64.2% of patients were males and 35.8% were females. The same results were stated in other studies, in which males consist of 68%, 70%, and 60%, respectively. (1, 7, 9)

In our study diffuse Large B-cell lymphoma was the most common type of NHL (46.4%), followed by follicular lymphoma (33.9%), small lymphocytic lymphoma (8.9%), these results were consistent with other international data (11, 13, 14), but This result differed from earlier study in Saudi Arabia which stated that low, intermediate and high grade of NHL comprise about 18%, 37% and 29%, respectively (12). These unpredicted

findings, however, should be taken carefully in that it difficult to clarify. In addition, these minor differences may be a coincident or need another study with larger sample size. The differences in the incidence of NHL subtypes in previous studies could be due to different study methodologies of classifications, difference in sample sizes.

A serological analysis used in this study to determine EBV antibodies in peripheral blood in patient with NHL was done with ELISA technique. According to the result concerning elevated EBV VCA-IgG antibodies in NHL patients in comparison to control group, incidence was determined. In this study, approximately 74.6% of NHL patient were positive for EBV VCA-IgG with significant difference when compared to control group which implicate a possible role for EBV in the pathogenesis of the disease. EBV could also play a direct role in the pathogenesis of NHL probably by activating the pathogenic mechanism(s) or it could reveal the presence of an inherited or acquired depression of immunoregulation that is run-up both malignant and to reactivation of EBV (16). Additionally, different studies from the Middle East have also reported a little bit lower incidence of EBV in NHL, namely Oman study with 65% NHL (13), Bahrain study with 66.7% NHL (14) and United Emirates with 59% NHL, (15). Those findings might be explained by the nature of our samples and by lower incidence of EBV in those countries. Traditionally the presence of EBV seems to occur more frequent in Hodgkin Lymphoma than NHL cases. On other hand, different studies reported higher association of the EBV with NHL as high as 80% in other developing countries (17), (18). It is also worth mentioning that Burkitt’s lymphoma is a high-grade malignant NHL that is most commonly associated with EBV infection (17,

18, and 19), in this study it was excluded because of its rarity in adult patient and its strong association with EBV infection.

From the mentioned results there is a significant association between EBV and NHL. Further studies using immunohistochemistry and PCR are highly indicated because its presence may have significant impact on prognosis and response to therapy. The current study also shows some evidence supporting the previous studies saying that people with recurrent EBV infection might develop lymphoma. Thus, there is an urgent need to educate our populations at different levels about the possible risk of EBV infection might cause serious harm to them. These results also provided a supplementary reason for doctors to take EBV

infection seriously in patients with lymphomas which should be treated promptly.

Conclusion and recommendation

Data from this study showed that the age, sex and clinical characteristics of Iraqi patients with NHL are somewhat different from those of surrounding countries and incidence is higher in NHL. The most frequent subtype of NHL is diffuse large B cell lymphoma. Furthermore, we suggest that a high sensitive technique must be carried out to verifying our observations like use of PCR in detection of EBV.

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نسبة حدوث عدوى فيروس ابشتاين بار (EBV) في المرضى المشخصين حديثاً بمرض ورم الغدد اللمفاوية اللاهوجكي في المركز الوطني لأمراض الدم في بغداد

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الخلاصة:

خلفية: ورم الغدد اللمفاوية اللاهوجكي هو سرطان الغدد اللمفاوية و هو مجموعة متنوعة من الاعراض التي تصيب الاشخاص و تتنوع سواء في تاريخ المرض الأصلي والاستجابة للعلاج. ان ظهور و اكتشاف الإصابة بابشتاين بار (EBV) في ورم الغدد اللمفاوية اللاهوجكي يمكن تمييزها عن طريق فحص بتقنية الاليزا حيث يتم اكتشاف الغلوبولين المناعي.. وقد تم دعم دور EBV كمسبب للمرض أو في نشوء ورم الغدد اللمفاوية اللاهوجكي عن طريق تحديد كميات كبيرة من البروتين الغشاء الكامنة 1 (LMP-1)..

الهدف: كان الهدف من هذه الدراسة تحديد معدل عدوى انتشار فيروس ابشتاين بار EBV في المرضى المشخصين حديثاً بمرض ورم الغدد اللمفاوية اللاهوجكي في المركز الوطني لأمراض الدم في بغداد.

المواد و الطرق: أجريت هذه الدراسة في قسم أمراض الدم السريرية، في المركز الوطني لأمراض الدم (NCH) ، بغداد، العراق من أغسطس 2012 إلى نوفمبر 2013. وكانت دراسة استطلاعية . ما مجموعه 56 من المرضى الذين يعانون من ورم الغدد اللمفاوية اللاهوجكي تراوحت اعمارهم ما بين 18-56 عاماً. تم التشخيص والتصنيف إلى أنواع فرعية من خلال دراسة التشريح المرضي و المناعية. وقد أجريت تقييم EBV الغلوبولين المناعي بواسطة تقنية ELISA. المجموعة الثانية تتكون من 22 مريض مشخصين بحالة غير خبيثة وتعتبر المجموعة الضابطة المتطابقة مع الجنس والعمر لمرضى ورم الغدد اللمفاوية. تم إجراء التحليل الإحصائي باستخدام التكييف SPSS

النتائج: كان متوسط عمر المرضى 51.44 ± 15.93 عاماً. 36 مريضاً (70.4 %) كانوا من الذكور و 20 مريضاً (29.6 %) من الإناث . سبعة (12.5 %) من أصل 56 حالة كانت إيجابية لل EBV - الغلوبولين المناعي ، و 2 من الحالات (28.5 %) كان تشخيصها لمفوما جريبية ، 5 حالات (71.5 %) تم تشخيصها بالأورام اللمفاوية ذات خلية كبيرة . كانت نتيجة EBV IgG - لاثنا عشر مريضاً (21.4 %) سلبية في حين كانت الغالبية 44 مريضاً (78.6 %) إيجابية ، و بالمقارنة مع المجموعة الضابطة كانت النتيجة إحصائياً غير ذات دلالة .

الاستنتاج : ان عدوى انتشار فيروس ابشتاين بار EBV في مرض ورم الغدد اللمفاوية اللاهوجكي هو شائع مع نسبة انتشار تساوي 12.5 % و بصورة عامة فهي شائعة في النوع المعروف ب ورم اللمفاوي ذات الخلية الكبيرة (ب). بينما غالبية المرضى مع NHL تحمل EBV IgG .

كلمات البحث: الإصابة ، وفيروس ابشتاين بار ، ورم الغدد اللمفاوية اللاهوجكي