Association of Epstein- Barr virus (EBV) with development of Hodgkin lymphoma (HL) in western Region of Iraq: Unmatched Molecular case-control study

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Abstract

Background: Hodgkin's disease is a malignant disease in the blood cells, especially the lymphatic ones, and accounts for 30% of the lymphomas. The Epstein-Barr virus (EBV) is associated with a proportion of cases of Hodgkin lymphoma (HL), and this association is believed to be causal. In Iraq, there are very few studies on this subject.

Objectives: The purpose of the current study was to determine the association between EBV infection and Hodgkin lymphoma of Iraqi patients.

Patients and Methods: Unmatched case-control study was conducted 55 paraffin-embedded tissues of HL of Iraqi patient’s in addition to 110 normal noncancerous nasopharyngeal biopsy samples as control group. DNA of EBV was extracted from both controls and neoplastic tissues and analyzed by PCR technique using primers specific to EBV Latent Membrane Protein-1 Oncogene (LAMP-1) for the presence of EBV.

Results: This study has shown that EBV were found in 51/55 Hodgkin lymphoma cases (92.73%) and in 85/110 controls (77.27%). This study has identified Gender of HL cases was not risky and not significantly associated with illness ((OR 1.00, CI 0.5220–1.9157, P. Value = 1.000). The results of this investigation show that exposure to EBV was risky and independently associated with HL (OR 3.7500, CI 1.2345–11.3916, P. Value = 0.016).

Conclusions: The results do support an association between EBV infection and HL.

Keywords: Epstein - Barr virus, PCR, EBV-LMP-1, Hodgkin lymphoma (HL).

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Introduction

Hodgkin's lymphoma (HL) has been alluded to as an unordinary neoplasm, it is a sort of lymphoma wherein disease begins from a particular kind of white blood cells called lymphocytes(1). Classical HL (cHL) is characterized by the presence of clonal, malignant multinucleated Reed Sternberg giant cellsin a background of reactive inflammatory cells that includes lymphocytes, plasma cells, granulocytes, and histiocytes(2)(3).

Age of identification of HL differs from one center to another and yearly occurrence of HL is 2.7 per 100,000 for every people for each year, and in 2017, approximately 990 Canadians will be determined to have Hodgkin lymphoma, and 140 will die of the disease (4).

The EBV is associated with a proportion of cases of HL, and this association is believed to be causal (2)(5),(6). The tumors cells in HL, and EBV latent gene products are expressed. The latter include the EBER RNAs and EBNA1, LMP1 and LMP2 proteins; both LMP1 and 2 are postulated to play a role in disease pathogenesis(6)(5).

EBV-positive HL is more frequent in childhood, in older adults (>45 years) and in mixed cellularity cases. The survival of EBV-positive HL in the elderly and the immunosuppressed is particularly poor (7). For the young adults, it has been further suggested that delayed exposure to a common childhood pathogen may play a role in disease.
Increased knowledge of the virus’ role in the basic biology of HL may generate novel therapeutic strategies for EBV-positive HL and the presence of EBV-latent antigens in the malignant HL cells may represent a target for cellular immunotherapy. In Iraq, there are few studies on the relationship EBV with Hodgkin’s lymphoma.

**METHODS**

**Study design**

A matched case-control study was conducted in the Anbar province and its neighbor area to match two controls with each case as the most appropriate strategy in order to maximize the study power. Subjects were matched by age, sex and general geographical location of the case’s residence to determine the Risk factors that affect the development Hodgkin lymphoma (HL). To detect an association with a matched odds ratio of 2.0 at the 5% significance level with 80% power (assuming 20% exposure level among controls), a sample size of 55 cases and 110 controls was required. The molecular analyses were carried out at private laboratories in Baghdad city. The study was retrospective, so informed consent not taken from the patients. This study was approved by the Microbiology Department, Anbar medical College, University of Anbar, Ramadi city, Iraq. A standard questionnaire was design from the information obtained from the previous medical case history and the previous laboratory report of each patient and healthy control for potential risk factors like age and gender of each patient and healthy control.

**Detection EBV Nasopharyngeal carcinoma and healthy control cases**

A total of 55 archival paraffin wax-embedded tissue blocks diagnosed with Hodgkin lymphoma (HL) were selected from the archive of pathology laboratory of dr. Arkan-Al -Essawi, college of medicine Anbar, Iraq. All cases diagnosed histopathologically between January 1, 2010 to February 31, 2019, a period of 9 years according to the WHO classification. Archived slides were reviewed by two pathologists for confirmation of diagnosis. EBV DNA was extracted from paraffin blocks of 55 HL cases in addition to 110 normal noncancerous nasopharyngeal biopsy samples and tonsillectomy specimens from patients with chronic hypertrophic tonsillitis as controls were enrolled in the study to investigate the presence of LMP-1 gene. Controls were matched on Gender, Residence and year of birth.

**Tissue Processing, PCR Amplification and Gel Electrophoresis**

The tissue samples were obtained from archival paraffin embedded tissue blocks as described previously. DNA of Epstein-Barr virus was extracted from both controls and neoplastic tissues as described previously. The amount and purity of DNA were determined by spectrophotometer. The concentration of EBV DNA for cases and control was estimated (1.6-1.8) at O.D260. DNA was stored at – 80º C. PCR amplification were done using oligonucleotide primers for detecting LMP1 gene (sense BN1, antisense BN2). The sequence regions in the EBV-LMP1 gene were chosen for amplification as follow (sense BN1: 58-AGC GAC TCT GCT GGA AAT GAT-38 or antisense BN2: 58-TGA TTA GCT AAG GCA TTC CCA-38) for identification of EBV DNA as described previously. The products were then examined on 1.5% agarose gel electrophoresis in 1× Tris-boric acid–EDTA (TBE) solution and stained with red safe as -stained to verify the presence of PCR product for wt-LMP1 under ultraviolet-illuminated agarose gels under UV transilluminator.

**Statistical Analysis**

A P. value Descriptive data were generated for all variables using SPSS, version 24 in addition to Epi Info Version 7±02 was used to calculate crude matched odds ratios (OR) with 95% confidence intervals (CI) and two-tailed P-values to estimate the association between various potential risk factors including EBV infections and HL. Through the univariate analysis by backward stepwise logistic regression of risk factors with a P-value%0±25, Statistical analyses were based on non-parametric methods and a p value <0.05 was considered significant.

**Results**

**Demographic characteristics**

The table 1 below illustrates some of the main Demographic characteristics of the HL cases. No significant differences were found between male 30 (54.45%) and Females 25(45.45%) of the distribution of the HL cases and both of them were almost equally affected (P > .5) by the Pearson chi-square test As shown in table 1, 2.

The results of the mean age and age group are summarized in Table 1,2. The range of age was from 17 day to 54 years. The mean age was (29.33±7.278 years). Most of the patients presented in age group (18 – 29) 29 (52.7%), 21 (38.2%) of them were in age group (30 – 42) and 3 (5.5%) in age group(<= 17), while the remaining 2 patients (3.6%) were in age group (43+). No significant differences were found between different age group of HLcases(p>0.05).
Table 1: Demographic data of patients with Hodgkin lymphoma (HL), n=55.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Male, n (%)</td>
<td>30 (54.55%)</td>
</tr>
<tr>
<td>Female, n (%)</td>
<td>25 (45.45%)</td>
</tr>
<tr>
<td>Age at diagnosis of Hodgkin's disease</td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>17 Years - 54 years</td>
</tr>
<tr>
<td>Mean of Age (±SD)</td>
<td>29.33 (±7.27) years</td>
</tr>
<tr>
<td>Age Group</td>
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</tr>
<tr>
<td>&lt;= 17</td>
<td>3 (5.5)</td>
</tr>
<tr>
<td>18 - 29</td>
<td>29 (52.7)</td>
</tr>
<tr>
<td>30 - 42</td>
<td>21 (38.2)</td>
</tr>
<tr>
<td>43+</td>
<td>2 (3.6)</td>
</tr>
</tbody>
</table>

**Detection of the EBV genome by PCR**

The results of the agarose gel electrophoresis stained with Red safe revealed the presence of a small DNA bands with product size of about 316 bpwt-LMP1 under ultra violet-illuminated agarose gels under UV transilluminator (Fig 1). Positive PCR results for EBV DNA were detected in 51/55 (92.73%) HL cases and 85/110 (77.27%) healthy control cases.

![Fig. 1: Detection of EBV in HL cases and healthy cases on Agarose gel electrophoresis stained with Rad safe. Lane M shows a molecular size marker. Lanes 1, 2 and 3 show positive bands of EBV-wt-LMP1 with product size 316 bp.](image)

Potential risk factors for HL illness (i.e. those with a matched odds ratio > 1) are shown in Table 2. Females were less likely to be EBV positive than males (24 of 25 compared with 27 of 30 respectively); however, this difference was not statistically significant and it was not risky (odds ratio = 0.375 with 95% confidence limits, 0.37-3.850. P-value =0.394). Exposure to EBV was risky and independently associated with HL (OR 3.7500, CI 1.2345–11.3916, P-value = 0.016).
Table 2: Comparison between the HL cases and the healthy control tissues as regard potential risk factors

<table>
<thead>
<tr>
<th>Gender</th>
<th>Hodgkin's disease (HL) Cases No (%)</th>
<th>Healthy control tissues No (%)</th>
<th>P. Value</th>
<th>Matched Odds Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Males</td>
<td>30/55 (54.45)</td>
<td>60/110 (54.45)</td>
<td>&gt;0.05</td>
<td>1.000</td>
</tr>
<tr>
<td>Females</td>
<td>25/55 (45.45)</td>
<td>50/110 (45.45)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>EBV Positivity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>51/55 (92.73)</td>
<td>85/110 (77.27)</td>
<td>0.0249</td>
<td>3.7500</td>
</tr>
<tr>
<td>Negative</td>
<td>4/55 (7.27)</td>
<td>25/110 (22.73)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**DISCUSSION**

The results of this study show that 53 cases (96.4%) of HL illness presented in the age between 17-42 year (Table 2). In accordance with the present results, previous studies have demonstrated that HL has a bimodal incidence curve; that is, it occurs most frequently in two separate age groups, the first being young adulthood (age 15–35) and the second being in those over 55 years old although these peaks may vary slightly with nationalities. Unlike some other lymphomas, whose incidence increases with age (15).

This outcome is contrary to that of Alexander et al. (1991) who supported the "two-disease" hypothesis in adult HD; however, there is little additional data available for the pediatric age group. However, the findings of the current study do not support the previous research, which showed that, in developing countries, the first age incidence peak HL occurs in childhood between the ages of 7 and 12 years, and no young adult age peak is observed (16) and this may be due to reflect differences in the age of exposure to infectious agents involved in the development of HL or may suggest different etiological agents (17).

The current study found that, there was equally distributed among male and female and there was no statistically significant relationship between HL and gender (Table 2). These results are in agreement with those obtained by Canadian Cancer Statistics (4) that reported HL accounts for 0.6% of all male cancer cases, and 0.4% of all female cancer cases in Canada (18). However, the findings of the current study do not support the previous research that show, Overall, it is more common in males, except for the nodular sclerosis variant, which is slightly more common in females, current result was contrary to that of Mack et al 1995 (19) who found male predominance of the disease. Kutok et al. (20) and Ocheni et al. (2) showed that showed HL is more common in males.

The current study found that risky and significance of EBV in development of HL (Table 2). These results reflect those of Hjalgrim et al. (2007) (21) who found that mononucleosis cause by EBV is followed by an increased risk of HL. This also accords with our earlier observations, which showed that elevated levels of anti-EBV antibodies have repeatedly, although not unanimously, been demonstrated in HL patients and may be associated with an increased risk of later HL (2) (22).

This study supports evidence from previous observations (21) (23) (e.g. Hjalgrim, 2007; Chang et al., 2008) who also showed that the increased HL risk to be specific to EBV-positive HL with no increased risk of EBV-negative HL. It is difficult to explain this result, but it might be related to tumour EBV status may be a more appropriate marker for.
aetio logically diverse HL subtypes than age and histological subtypes (23) or may be due to presence of EBV in the malignant Hodgkin/Reed-Sternberg cells, the vast majority of which are of B cell origin is consistent with the normal course of EBV infection (22). The possibility that EBV can cause lymphoma is supported by laboratory evidence, including the fact that EBV readily transforms B-lymphocytes in vitro through the expression of various EBV proteins, among which latent membrane protein 1 (LMP-1) is thought to be the most important(24)(6).

This study confirms that gender of EBV infected patients is not associated and not risky for the development of HL. This finding is contrary to previous studies which have suggested that several epidemiological factors such as sex, age, ethnicity country and histological subtype have roles to play in the association between EBV in Peru and Kenya HL(25). However, the findings of the current study do not support the previous research, in particular, in developed populations, the association between EBV and HL is less, with percentages of between 20% and 50% for North American and European cases(23).

References


