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Original Article

Epstein–Barr Virus Infection in Adult Patients with Gastric Cancer in Northeast of Iran

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Abstract

Background: Epstein–Barr virus (EBV) is a DNA virus from human herpes virus that associates with many of the human cancers including gastric cancer (GC). **Aims:** The aim of the present study was to report infection of EBV in adult patients with GC in Northeast of Iran and the correlation between a number of clinicopathology factors with EBV status. **Materials and Methods:** In a case–control study in 2016, 56 GC patients and 56 controls were selected for the analysis. All patients had gastric adenocarcinoma untreated patients with age >18 years. EBV status detected by the polymerase chain reaction method. **Results:** Out of 56 GC patients, 35 (62.5%) were EBV positivity and out of 56 controls 3 (5.4%) were EBV positivity ($P < 0.001$). There is not a significant correlation between the variables with the EBV status ($P > 0.05$). Furthermore, the progression-free survival rate for the patients with EBV negativity was 95.2% compared with 82.9% for EBV positivity ($P = 0.174$). **Conclusions:** This study reported a very high prevalence of EBV-associated GC in the Northeast of Iran compared with other areas of the World and showed a significant correlation between EBV infection and incidence of GC.

Keywords: *Epstein–Barr virus, gastric cancer, polymerase chain reaction, survival*

Introduction

Gastric cancer (GC) is the third most common cause of malignancy death worldwide that has estimated more than 700,000 deaths in 2012.[1] Advanced stage, older age, cardiac tumor localization, and less differentiated histology are adverse prognostic indicators in the patients with GC.[2-4] Epstein–Barr virus (EBV) is a ubiquitous double-stranded DNA virus from human herpes virus family, which has B-lymphotropism[5] and this virus has been shown to associate with many of the human malignancies including GC.[6] Around 10% of the GCs throughout the world are monoclonal proliferations of EBV-carrying tumor cells[7] that the lowest prevalence is in Papua New Guinea, Pakistan, and Korea, between 1% and 3% and the highest in Germany and the USA, between 16% and 18%.[6] Although the polymerase chain reaction (PCR) had been used in the first detection of EBV in GC, the majority of studies have used RNA *in situ* hybridization (ISH) due to the possibility of viral genome amplification from infected lymphocytes infiltrating the tumor, resulting in a false positive. Still, there are few studies that use and compare both techniques for EBV detection.[8] The aim of the present study was to report infection of EBV in adult patients with GC in Northeast of Iran and the correlation between a number of clinicopathology factors with EBV status.

Materials and Methods

Patients

In 2016 and in a case-control study, out of all cancer patients referred to Emam Reza Hospital, Mashhad, Iran, the GC was confirmed in 56 patients after endoscopy and biopsy, and after gastrectomy, these patients were selected as case group. We selected 56 controls that had no GC. The inclusion criteria: all of gastric adenocarcinoma untreated patients with age >18 years. The exclusion criteria: The patients with severe gastritis or atrophic and nonadenocarcinoma cancer and treated patient. The progression-free survival (PFS) is defined as the start time of treatment to disease progression or death from any cause.

Hematoxylin and eosin staining

At first, all of the samples were evaluated by a pathologist and hematoxylin and eosin

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staining was done on 3–4 micron slicing of the sample to confirm adenocarcinoma that after that, adenocarcinoma sampling selected as the proper paraffin blocks or sections for PCR detection.

Polymerase chain reaction detection

Paraffin blocks were sectioned by microtome 2 μm about 20 times. Then, these materials gathered in a microtube and are done deparaffinization and rehydaration. After that, DNA extraction by columnar device (Takapozist, Iran) was done and DNA prepared for real-time PCR. EBV DNA detection was fulfilled through real-time PCR system (Bioneer, South Korea), with the real-time PCR kit (Takapozist, Iran) and according to positive and negative control, the data were acquired.

Statistical analysis

The data analyzed with SPSS version 22 software (IBM Corp., Armonk, NY, USA). *T*-test was used for comparison of means between two groups and Chi-square test for other variables. The results of PCR were also checked by univariate and multivariate logistic regression. $P < 0.05$ was considered statistically significant.

Results

Out of 56 GC patients with mean age of 66.2 years (±9.8), 45 patients (80.4%) were males and 35 (62.5%) were EBV positivity based on PCR. In addition, out of 56 controls with the mean age of 57.9 years (±14.4), 21 (37.5%) were males and 3 (5.4%) were EBV positivity. There was a significant difference between two groups for EBV status ($P < 0.001$). Therefore, EBV positivity in the patient group was more than the control group. Table 1 shows the clinicopathological characteristics of GC patients based on the results of PCR for EBV. There is not a significant correlation between the variables with the EBV status ($P > 0.05$).

The results of PCR based on univariate and multivariate logistic regression analysis have been shown in Table 2. These analyses did not show significant differences in the results of PCR in patients.

Figure 1 shows the 5-year PFS in GC patients based on EBV status. The PFS rate for the patients with EBV negativity was 95.2% (mean: 58.1 months) and for EBV positivity was 82.9% (mean: 50.5 months) that difference was not statistically significant ($P = 0.174$).

Discussion

This case–control study showed that the prevalence of EBV in GC patients was significantly more than controls in Northeastern Iran (62.5% vs. 5.4%). Furthermore, the analyses did not show a significant correlation between clinicopathological figures of GC with EBV status. A meta-analysis among 39 case–control studies published up to October 2015, reported that EBV infection increases significantly the risk of GC,[9] but other meta-analysis by Lee *et al.*[10] reported that EBV infection is not associated with the incidence of this cancer in Asians on the analysis of 48 studies published up to December 2007. Murphy *et al.*[11] showed that the prevalence of EBV-positive GC did not differ according to a geographical area. A systematic review from Chen *et al.*[12] on published articles up to September 2014, showed that evidence based on ISH method strongly suggests an association between EBV infection and GC risk, but the PCR method alone is invalid to confirm such association. Several studies[7,13,14] indicated that EBV-associated GCs include about 10%

Table 1: The characteristics of gastric cancer patients based on the results of polymerase chain reaction for Epstein-Barr virus (*n*=56)

Variables	EBV-positive (*n*=35), *n* (%)	EBV-negative (*n*=21), *n* (%)	χ^2	*P*
Age (years)	65.9±10.4	66.76±8.74	0.30	0.765
Sex			9.52	0.999
Male	28 (80)	17 (81)		
Female	7 (20)	4 (19)		
Anatomic location			0.15	0.999
Cardia and fundus	4 (11.4)	2 (9.5)		
Antrum and pylorus	12 (34.3)	7 (33.4)		
Corpus (body)	19 (54.3)	12 (57.1)		
Extension			0.37	0.923
Muscle	5 (14.3)	2 (9.5)		
Serous	7 (20)	5 (23.8)		
Lymph nodes	23 (65.7)	14 (66.7)		
Stage			3.02	0.374
I	6 (17.1)	3 (14.3)		
II	6 (17.1)	3 (14.3)		
III	23 (65.7)	13 (61.9)		
IV	0	2 (9.5)		
Grade			0.17	0.881
High	9 (25.7)	6 (28.6)		
Moderate	17 (48.6)	9 (42.8)		
Low	9 (25.7)	6 (28.6)		

EBV – Epstein-Barr virus

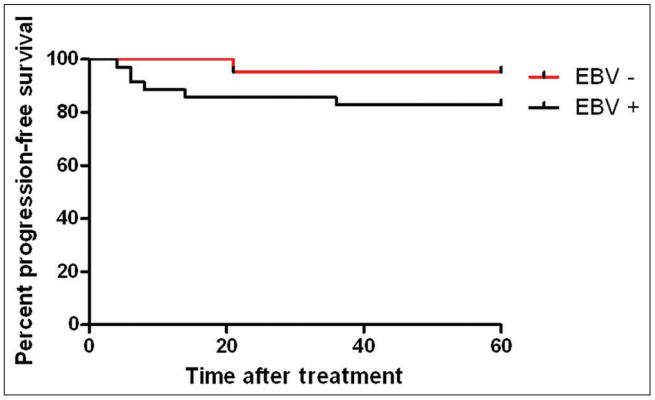

Figure 1: The 5-year progression-free survival for the patients with gastric cancer

Table 2: The results of polymerase chain reaction based on univariate and multivariate logistic regression analysis in patients

Variables	Unadjusted		Adjusted	
	OR (95% CI)	*P*	OR (95% CI)	*P*
Age (years)	1.005 (0.948-1.06)	0.878	1.001 (0.938-1.06)	0.978
Gender				
Male	Reference		Reference	
Female	0.938 (0.236-3.724)	0.927	1.012 (0.24-4.26)	0.987
Extension				
Lymph nodes	Reference		Reference	
Muscle	1.522 (0.259-8.92)	0.642	0.692 (0.009-55.8)	0.870
Serous	0.852 (0.226-3.20)	0.813	0.266 (0.015-4.81)	0.371
Grading				
Low	Reference		Reference	
High	1 (0.232-4.31)	0.999	0.762 (0.134-4.32)	0.759
Moderate	1.25 (0.339-4.67)	0.730	1.184 (0.24-5.72)	0.834
Stage				
I	Reference		Reference	
II	1 (0.141-7.09)	0.999	2.46 (00.03-154.33)	0.669
III	0.885 (0.18-4.14)	0.876	3.657 (0.154-86.91)	0.423
Location				
Corpus	Reference		Reference	
Cardia and fundus	1.083 (0.333-3.522)	0.895	0.96 (0.21-4.29)	0.960
Antrum and pyloric	1.263 (0.2-7.99)	0.804	3.735 (0.26-53.26)	0.331

OR – Odds ratio; CI – Confidence interval

of all GCs in the World. One study[15] reported that the incidence of EBV-associated GC in all cases of GC is distributed from highest (16%–18%) in the USA and Germany to the lowest (4.3%) in China. Two studies in Iran[6,16] reported that the prevalence of EBV in GC patients was 6.66% and 3%, respectively, and concluded that the frequency of EBV-associated GC in Iran[6,16] and the Middle East[6] was low, but this study reported that the prevalence of EBV in GC was 62.5% by PCR method and very higher than other studies in Iran. Camargo *et al*.[17] among 4599 patients with invasive GC, suggested that tumor EBV positivity is an additional prognostic indicator in GC and 8.2% tumors were EBV-positive overall. Koshiol *et al*.[18] did not find a positive association between prediagnostic EBV seropositivity and GC and in fact, there was some evidence that EBV seropositivity was associated with a reduced risk of malignancy and death after diagnosis of cardia cancer. The unadjusted logistic regression analyses showed tumor EBV positivity was higher in an early stage, cardiac localization, diffuse-type histology, poorer differentiation and men that there was a direct correlation between stage and mortality.[17] On the contrary, a meta-analysis by Li *et al*.[19] found a significant risk for lymph node spread. EBV-positive GC also displays distinct clinical, genetic and demographic features as compared to EBV-negative cancer.[20,21] Differences in prevalence and more generally the EBV infection patterns have never been clearly associated with race, but merely seen as differences in socioeconomic, hygienic, and cultural behavior.[22] Vo *et al*.[23] checked EBV in GCs and suggested that there are ethnic differences in tumor virology and pathogenesis and two other studies confirmed it.[24,25] Camargo *et al*.[26] reported that smoking is associated with risk of EBV positive in GC. Therefore, these differences in EBV-associated GC incidence in different areas may reflect the epidemiologic and clinicopathologic factors; dietary habits and genetic. A meta-analysis revealed that patients with EBV-associated GC had a longer survival than those with EBV-negative GC[17] that two other studies confirmed this result.[27,28] This study showed that EBV-associated GC patients had a low PFS rate compared with EBV-negative GC patients ($P > 0.05$). While some studies have shown significantly better prognosis in EBV-associated GC than in EBV-negative GC.[17,29,30]

Conclusions

This study reported a very high prevalence of EBV-associated GC in the Northeast of Iran compared with other areas of the World and showed a significant correlation between EBV infection and incidence of GC. The further studies need more cases in other areas of Iran by controlling epidemiologic and clinicopathologic factors; dietary habits and genetic that can receive the effect of each of these factors on the prevalence of EBV-associated GC.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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