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Association of Epstein-Barr virus with Esophageal Squamous Cell Carcinoma

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Abstract

Introduction: Esophageal squamous cell carcinoma is among the leading causes of cancer related deaths within gastrointestinal tumors. There is a growing body of evidence that shows an association between Epstein Barr virus infection and the development of malignancies such as B-cell non-Hodgkin's lymphoma, Hodgkin's disease, and Burkett's lymphoma. However its potential association with esophageal squamous cell carcinoma is controversial. Therefore, in the present study, we have explored the association of Epstein Barr virus with pathological information and clinical outcomes of 108 esophageal squamous cell carcinoma patients.

Methods: There were 48% female and 52% male patients with a mean age of 59.2±11.1 years who enrolled in this study. Patients had the following tumor stages: T1 (5.6%), T2 (21.3%), and T3 (71.3%). A total of 32.4% had lymph node metastases. In order to explore whether patient characteristics might influence clinical outcome, we analyzed data on progression-free survival and overall survival according to patients' clinicopathologic features.

Results: An association existed between tumor size, node and metastasis status, and stage with shorter overall and progression-free survival. We observed that 6.5% of patients had Epstein Barr virus. All patients infected with Epstein Barr virus had T2 and T3 disease.

Conclusion: Our findings demonstrated the presence of Epstein Barr virus in 6.5% of Iranian patients and its potential link with tumor size. Additional studies in multi-center settings should be conducted to determine the association of Epstein Barr virus with development and progression of esophageal squamous cell carcinoma.

Keywords: Esophageal squamous cell carcinoma, Epstein Barr virus, PCR

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Introduction

Esophageal squamous cell carcinoma (ESCC), the most common type of esophageal carcinoma, is the sixth leading cause of cancer-related deaths worldwide.[1,2] This cancer often arises in the upper and middle part of the esophagus, with a more frequent presentation in men.[3,4] Esophageal squamous cell carcinoma causes no signs or symptoms in its early stages; however, in advanced disease, presentation may include dysphagia, weight loss, cough, and chest pain.[5] Although the exact etiology of ESCC is poorly understood, smoking, alcohol abuse, hot foods, and some dietary deficiencies may increase the risk for ESCC.[6,7] There is a significant association between ESCC and viral infections.[8] Human papilloma virus (HPV) and Epstein Barr virus (EBV) infections have been identified as contributing factors to esophageal cancer.[9-11] Epstein Barr virus is a member of the human herpes virus family that infects 95% of the world's population.[12] It is an oncogenic virus which has been linked to several malignancies - African Burkitt's lymphoma, Hodgkin's disease, non-Hodgkin's lymphoma, post-transplant lymphoma, AIDS-associated lymphomas, sinonasal angiocentric T-cell lymphoma, nasopharyngeal carcinoma, gastric carcinoma, leiomyosarcoma, and breast cancer.[13] Epstein Barr virus encodes several proteins which can induce oncogenic properties via activation of a number of signaling pathways involved in cell proliferation, differentiation, survival, and death.[14] Due to the role of EBV in epithelial-derived human malignancies, it is conceivable that EBV may contribute to the pathogenesis of ESCC. However, the association between EBV and ESCC is controversial. Some researchers have reported evidence of EBV infection in ESCC patients, whereas other investigators indicated that EBV had no association with ESCC. The prevalence of EBV and possible mechanism by which EBV may contribute to ESCC oncogenesis remains to be clarified.[15] This study aims to determine whether EBV is associated with ESCC in the Iranian population, as well as with patients' clinicopathologic characteristics.

Materials and Methods

Patients

We recruited 108 ESCC patients based on the diagnosis of histologically confirmed locally advanced or metastatic ESCC. Patients were recruited from Omid Hospital of Mashhad University of Medical Sciences from May 2006-August 2015. All eligible participants were chemo naive patients treated in Omid Hospital. There were 9 mm sections were serially cut from the formalin-fixed paraffin-embedded (FFPE) blocks followed by DNA extraction. The local Hospital Ethics Committee of Mashhad University of Medical Sciences, Mashhad, Iran approved this study.

DNA extraction and Epstein Barr virus (EBV) detection

Genomic DNA was extracted from tissue (ESCC patients) samples using the QIAamp® DNA Mini Kit according to the manufacturer's protocol (Qiagen, San Diego, CA). DNA concentration and purity was determined with the NanoDrop®-ND-1000 Detector (NanoDrop Technologies, Wilmington, DE, USA). We evaluated the presence of EBV in approximately 50 ng of genomic DNA using an Epstein-Barr virus PCR detection kit. Epstein Barr virus positive DNA was utilized as a positive control (Figure 1).

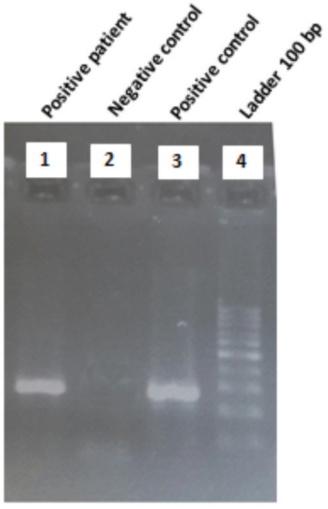

Figure 1. Representative image of the PCR results.

Statistical analysis

Demographic and clinical information were compared across genotype using Pearson's tests. Overall survival (OS) and progression-free survival (PFS) curves were analyzed from the day of treatment onset to the end point (death or censoring) according to the Kaplan-Meier method, and compared by the log-rank and Wilcoxon tests.

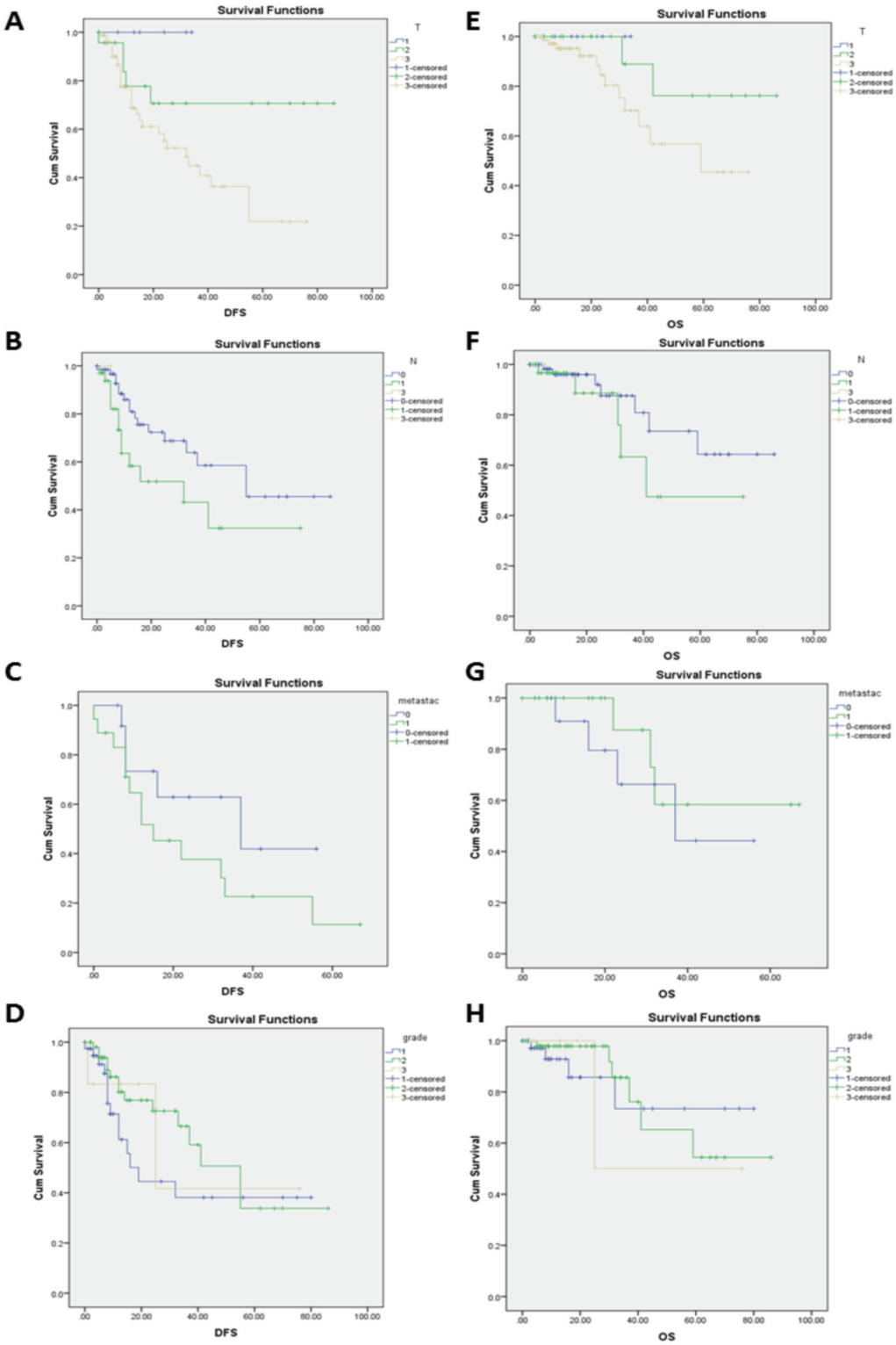

Figure 2. Kaplan-Meier survival curves. (A-D) Progression-free survival (PFS) and (E-H) overall survival (OS) according to tumor (T), node (N), and metastasis (M) grade. *P*-values were calculated with the log-rank test.

We included the significant prognostic variables in the univariate analysis in the multivariate analyses according to Cox's proportional hazards model. Data were expressed as mean±SD, and analyzed by the student's t-test or ANOVA followed by Tukey's multiple comparison. Data were analyzed using SPSS-17 software (SPSS Inc., IL, USA). All analyses were two-sided with a statistical significance of $P<0.05$.

Results

Clinicopathologic characteristics of patients

Table 1 lists participants' demographic and clinical characteristics. Among the 108 patients, 48.1% of patients were female, and 51.9% were male with a mean age of 59.2±11.1 years. There were 5.6% of patients with T1 disease, 21.3% with T2 disease, and 71.3% had T3 disease. A total of 32.4% of patients had lymph node metastases, while 16.7% of patients had M1 (Table 1). In order to evaluate whether patient characteristics might influence clinical outcome, we analyzed data on PFS and OS according to patients' clinicopathologic features. An association existed with tumor size, node and metastasis status, and stage with shorter OS and PFS (Table 2, Figure 2A-H).

Table 1. Clinical and pathological characteristics of the study population.

Variables		Cases (n=108)
Gender	Male	56 (51.9%)
	Female	52 (48.1%)
Age (years)	<50	24 (22.2%)
	≥50	84 (77.8%)
BMI	Mean±SD	20.45±4.06
T	1	6 (5.6%)
	2	23 (21.3%)
	3	77 (71.3%)
N	Negative	63 (58.3%)
	Positive	35 (32.4%)
M	0	90 (83.3%)
	1	18 (16.7%)
Grade (WHO)	G1-2	93 (86.1%)
	G3	6 (5.6%)
Location of lesion		
	Proximal	45 (41.6%)
	Distal	34 (31.4%)
	Other	29 (27%)

T: Tumor; N: Node or lymph node; M: Metastasis; BMI: Body mass index

Association of Epstein Barr virus (EBV) with clinicopathologic characteristics of patients

Our findings showed the present of EBV in 7 patients with a 6.5% frequency, which were mostly stages II and III. According to table 2, our data showed no association between EBV and poor prognosis. However we found that 100% of EBV infected patients and 6.5% of the total population had T2 and T3 disease. There were positive lymph nodes present in 42.8% of EBV infected patients and 2.7% of the total population (Table 2).

Discussion

Squamous cell carcinoma of the esophagus is one of the most common types of esophageal and gastrointestinal cancers. Factors postulated to be involved in the development and progression of this disease include intake of foods contaminated with nitrosamine and fungal toxins; chronic use of alcohol and tobacco; genetic factors;[6,7] and viral infections. One of the most important tumor-related viruses is EBV, which has an association with numerous human malignancies such as Burkitt's lymphoma, Hodgkin's disease, B-cell non-Hodgkin's lymphoma, and lymphoepithelioma-like carcinomas.[8,13-15] Epstein Barr virus infection has been detected in gastric adenocarcinomas and nasopharyngeal carcinomas.[6,16] However, an association of EBV with the carcinogenesis of ESCC and its clinical outcome remains to be elucidated. Reportedly, EBV expresses oncogenic proteins such as latent membrane proteins (LMP 1,2A/2B), nuclear proteins (EBNA1-3), and EBV-encoded early RNAs (EEBER1 and 2) which induce oncogenic effects by disruption of several signaling pathways such as JAK-STAT, MAP kinase, and NF-κB.[15-17]

The present study aimed to elucidate the association of EBV with clinical outcome in Iranian patients with ESCC. In this study, 7 (6%) patients had EBV. The current study findings supported those by Wang et al. who detected EBV DNA by PCR in 11 (35.5%) samples from 31 patients with ESCC in Taiwan. *In situ* hybridization (ISH) confirmed expression of

Table 2. Clinical outcome according to clinicopathologic characteristics and Epstein Barr virus (EBV).

		EBV			Clinical outcome			
		Negative patients (n)	Positive patients (n)	*P*-value	OS mean Months (95% CI)	*P*-value	PFS mean Months (95% CI)	*P*-value
EBV	**status**	101	7		63.7 (54.1-73.3)	>0.05	47.2 (38.0-56.3)	>0.05
T	1	6	0	0.745	20.8 (12.1-29.5)	0.234	20.8 (12.1-29.5)	0.388
	2	21	2		32.0 (19.2-44.9)		35.0 (21.0-49.0)	
	3	72	5		21.6 (16.6-26.7)		27.4 (20.6-34.3)	
N	0	59	4	0.869	25.8 (19.6-32.1)	0.080	29.3 (21.9-36.6)	0.108
	1	32	3		18.8 (11.3-26.3)		23.9 (13.7-34.0)	
M	0	12	7	0.419	28.5 (17.4-39.5)	0.456	34.9 (21.7-48.1)	0.157
	1	18	0		27.1 (15.2-38.4)		24.7 (13.7-35.7)	
Grade	1	36	3	0.783	64.2 (49.7-78.7)	0.890	38.4 (24.0-52.8)	0.388
	2	50	4		64.9 (51.3-78.6)		49.6 (36.9-62.3)	
	3	6	0		50.5 (15.1-85.8)		42.2 (9.3-75.1)	

OS: Overall survival; PFS: Progression-free survival; $P<0.05$

EBERs in ESCC, which suggested EBV involvement in ESCC carcinogenesis.[18] In Germany, Awerkiew et al. reported 35% of EBV infections in ESCC.[19] Jenkins et al. observed EBV in 8.3% (5/60) of tumor samples and 1/16 of ESCC cell lines according to PCR analysis.[11] Similarly, EBV EBER and LMP-1 proteins were detected in 10 (6.1%) cases in the Chinese population by ISH and immunohistochemistry. EBV-positive cases were found in poorly differentiated ESCC or undifferentiated carcinomas with severe lymphocytic infiltrate.[20] In contrast, Mizobuchi found no statistically significant association between ESCC and EBV in all 41 surgical specimens and 12 cell lines of ESCC.[21,22] Consistently, two studies reported no EBER-1 expression in Japanese patients with ESCC and no evidence of EBV infection in 51 cases of ESCC from northern China were observed.[23] This difference could be explained by several factors such as environmental factors, geographic variations in EBV infections, genetic susceptibility factors, and variations in sensitivity and specificity of molecular methods used to detect EBV DNA. Lewensohn et al. detected EBV in 2 out of 10 ESCC cases in Sweden by PCR. However all of these cases were negative according to ISH and immunohistochemistry.[24] Lately, the same negative result was reported by Salehzadeh et al. in 30 Iranian patients with ESCC.[25]

We observed that EBV infected patients had mostly stages II and III disease. In this study, 100% of EBV infected patients and 6.5% of the total population had T2 and T3 disease. There were 42.8% of EBV infected patients with positive lymph nodes. However, none of the EBV infected patients were M1. In agreement with our observations, several other studies reported an association of EBV with clinical outcome and tumor progression.[26-30] In particular, Park et al. observed that diffuse large B-cell lymphoma patients with EBER had a more rapidly deteriorating clinical course with poorer outcome, OS, PFS, and more advanced disease stages.[26] In the current study, the association of EBV infection with clinical outcome was not statistically significant. Hu et al. recently reported the contribution of EBV to breast cancer etiology via transformation of mammary epithelial cells to a malignant form.[31] Marrão et al. explored the impact of EBV on the clinical outcome of 85 breast cancer patients in both peripheral blood mononuclear cells (PBMCs) and tumor biopsies. They found an association between EBV infection and poor prognosis. A correlation existed between this relationship and the immune cell TNF-α/IFN-γ response.[28] Another study reported co-infection of EBV with HPV in human tumorigenesis.[32] A recent study by Kunzmann et al. reported the prevalence of EBV in 6% (n=5, 95% CI: 0-27%) of esophageal adenocarcinomas.[33] However this frequency was different for other cancers. Shahani et al. evaluated the frequency of EBV DNA in

nasopharyngeal carcinomas in Iranian patients. They found that EBV in 28% of patients and both WHO histological types II and III showed approximately the same prevalence.[34] Al-Antary et al. observed that 36.27% of colorectal cancer patients had EBV infection, which was associated with a more aggressive cancer phenotype.[35] EBV infection was also reported in 11.1% of gastric cancer patients in an Iranian population.[36]

Our findings have demonstrated the presence of EBV in ESCC patients and its potential link with tumor size. Additional studies in a multicenter setting are necessary to determine the association of EBV with ESCC development and progression.

Conflict of Interest

No conflict of interest is declared.

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