American Journal of Biology and Natural Sciences
https://biojournals.us/index.php/AJBNS
ISSN: 2997-7185

Potential Role of Epstein Barr Virus in Oral Squamous Cell Carcinoma Development

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Received: 2024, 15, Dec
Accepted: 2024, 21, Dec
Published: 2025, 30, Jan

Annotation: Background: Epstein Barr virus is one of the most commonly affective oncogenic viruses that may cause several types of cancer such as oral cancer. **Aim of the study:** This study aims to assess the role of EBV in making a progression in OSCC and its deterioration to cases with OSCC. **Novelty:** This is the first study on patients with OSCC was conducted in Iraq and trying to find the correlation between EBV and OSCC incidence and exaggeration. **Methods:** A case control study was conducted on 70 cases with OSCC and 70 subjects as controls for 8 months of investigation and follow up at Musol hospital in Iraq. **Results:** Among 70 cases were diagnosed with OSCC and 70 were considered as controls and mean age among cases and controls was 41.57±7.4 and 40.7±6.2, respectively. The risk factors reported were distributed as smoking which was more among controls than cases while alcoholism and betel quid chewing was higher among cases (n=7 and 1). The site of tumor among OSCC cases was reported as the tumors in buccal mucosa were the most predominant (n=26, 37.14%). The most cases are EBV positive (n=41). The association

between risk factors and prevalence of EBV among cases showed that all risk factors are significantly associated with incidence of EBV ($p < 0.001$) and the site of tumor reported that all sites except for palate were significantly associated with prevalence of EBV ($p < 0.05$). **Conclusion:** EBV is readily and significantly correlated with development and prediction of OSCCs among different cases.

Keywords: Epstein Barr Virus, OSCC, oncogenic virus, neck cancer, head cancer.

Introduction

In 2018, head and neck cancer were ranked as the seventh most prevalent cancer globally, with a total of 890,000 newly diagnosed cases and 450,000 recorded deaths [1]. Oral squamous cell carcinoma is the most common type of head and neck cancer in males worldwide [2]. It mostly affects several areas in the mouth, including the lips, tongue, hard palate, and buccal mucosa [3]. It ranks ninth in terms of prevalence among boys globally. It is a significant contributor to illness and death on a global scale, particularly in Asia [4]. The development of this condition is a result of various risk factors, such as tobacco and alcohol use, chronic inflammation, exposure to ultraviolet radiation (which can lead to lip cancer), human papilloma virus (HPV) infection [5], Candida infections [6,7], Epstein–Barr virus (EBV) infection, and Hepatitis B virus infection [8]. These factors have become significant contributors, accounting for 10-15% of the global cancer burden [9]. Nevertheless, the connection between EBV and OSCC remains a subject of debate, alongside factors such as immunosuppression, genetic susceptibility, and nutrition [10].

EBV was the initial human virus documented to be linked with the development of several forms of malignancy [11]. EBV infection is prevalent in about 90% of the global population. The EBV virus is primarily spread by saliva and typically does not cause any symptoms during childhood [12]. The International Agency for Research on Cancer (IARC) officially identified EBV as the primary cause of nasopharyngeal carcinoma in 1997. EBV is associated with the formation of different forms of malignancies, including Hodgkin lymphoma [13], gastric cancer [14], lymphoproliferative diseases, and natural killer cell lymphoma/T lymphocytes (NK/T) [15]. The EBV infection begins in the oropharyngeal epithelial cells and then gradually spreads to the subepithelial B-cells [16]. Acquired immunity does not result in the eradication of EBV infection. Similar to other herpesviruses, the infection remains in a dormant state throughout the person's lifetime[17-20]. There is a hypothesis that EBV disrupts several cellular functions [21], resulting in genomic instability, which plays a vital role in the development of viral-induced cancer.

Studies in epidemiology have shown that rates of EBV infection in OSCC vary greatly, perhaps due to changes in geography and ethnicity. Studies have demonstrated that OSCCs that are positive for EBV exhibit a more unfavorable level of tumor differentiation. Epstein-Barr virus (EBV) infection causes a delay in the process of epithelial differentiation and encourages the development of a more aggressive and invasive behavior in epithelial cells [22-27]. Furthermore, the persistent ability to differentiate at a later stage and the enhanced ability to invade surrounding tissues were observed in epithelial cells even after the elimination of EBV, suggesting that a stable alteration in gene

expression patterns occurred following EBV infection [28,29]. Therefore, EBV infection may have a role in the development of OSCC by causing epigenetic changes in the infected cancer cells. Therefore, this study aims to assess the role of EBV in making a progression in OSCC and its deterioration to cases with OSCC.

Methodology

Design and setting

This study followed a case control design with OSCC cases and noncancerous controls in Musol hospital in Iraq.

Sample and population

A random sampling technique was performed on the cases with EBV who are visiting the clinics for one month to be involved in the study when they are eligible to inclusion criteria. All clinical history for cases and controls were obtained in addition to their demographic data such as gender, age, educational level, family history of cancer as well as job status and characteristics as well as the risk factors for OSCC were get too such as smoking, alcoholism, and betel quid chewing.

Inclusion and exclusion criteria

All cases must be diagnosed with OSCC for one year at least without tumor in salivary glands as well as their approval and signing a consent to participate in this study.

Diagnosis for OSCC

The conical cytobrush was used to get cells from lesions and layers of cells among cases and controls by scratching 10-12 rounds at buccal cavity, a tube with 25 ml phosphate buffer saline was used to be a vehicle for brushed cells which was obeyed to centrifugation at 3000 g for 15 minutes and then the pellets were stored at -80 °C

Screening for EBV

After making DNA extraction using DNA extraction kit (Intron, USA) and carried out according to manufacturer's instructions, the DNA was isolated from the cells of lesions in cases and from oral mucosal membranes from controls. Using endpoint PCR for detecting EBV DNA was performed with a primer (f:5′-ACCCGGAGCCTGTTTGTAGC-3′, r:5′-GGAGAAGGTCTTCTCGGCCTC-3′) as described previously by Pozo and Tenorio (1999), with final volume of 25 of PCR product which contains 0.2 μM of each primer, 0.2 mM of each dNTP, 4 mM of MgCl2, 0.025 U of Taq DNA polymerase, in buffer. The annealing temperature was 47°C for 1 min. Then the final PCR product was run on gel electrophoresis using cell line contains positively EBV- B95-8 as a positive control [30].

Ethical Considerations

This study gets an ethical approval to be conducted from Musol hospital with IRB number 314-c23 at year of 2024.

Statistical analysis

SPSS version 21.0 was used as statistical test to analyze the data extracted using chi square and unpaired t-test at level of significance 0.05 for inferential data statistics and using mean, frequencies, percentages, and standard deviation to analyze descriptive data while the multivariate analyzing tool was used to find the correlation between EBV and OSCC incidence among cases in regard to risk factors.

Results

Among 140 participants in this study which were distributed as 70 were diagnosed with OSCC and 70 were considered as controls, the demographic data showed that mean age among cases and

controls was 41.57±7.4 and 40.7±6.2, respectively and there was an approximate equality in their gender distribution, other demographic data is presented (Table 1, Figure 1 and 2).

Table 1 The demographic data among cases with OSCC and controls

Demographic characters	Cases (n=70)	Controls (n=70)
Mean Age (years)	41.57±7.4	40.7±6.2
Gender		
Male	31	43
Female	39	27
Educational Level		
Below secondary	3	4
Secondary	25	16
Bsc, Msc, or PhD	42	50
Job status		
Employed	28	30
Unemployed	42	40

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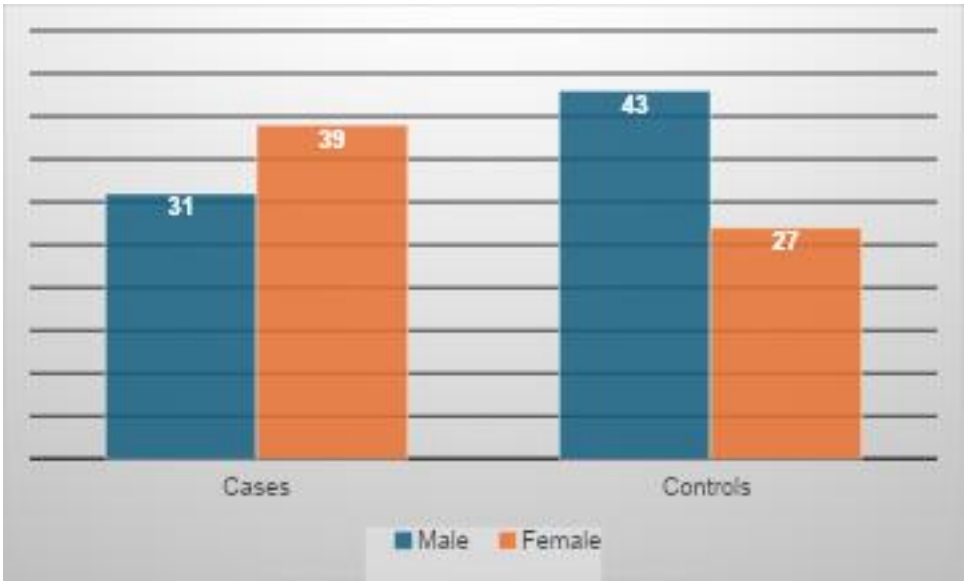

Figure 1 The gender distribution among cases and controls

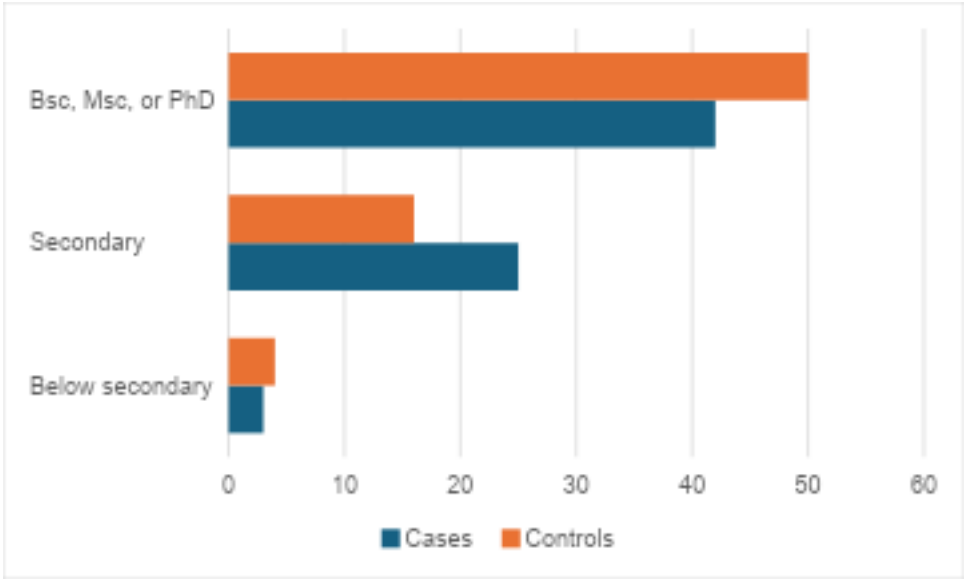

Figure 2 The educational level among both cases and controls

The risk factors reported were distributed as smoking which was more among controls than cases while alcoholism and betel quid chewing was higher among cases (Table 2). The site of tumor among OSCC cases was reported as the tumors in buccal mucosa were the most predominant (n=26, 37.14%) (Table 3).

Table 2 The reported risk factors among all participants in this study

Risk Factors	Cases (n=70)	Controls (n=70)
Smoking	32	41
Alcoholism	14	2
Betel quid chewing	7	1

Table 3 The site and anatomical localization of SCC among patients with cancer and EBV prevalence

	OSCC cases		EBV positive	
	F	**%**	**F**	**%**
Tongue	2	2.86	1	50.00
Gum	12	17.14	5	41.67
Lip	3	4.29	0	0.00
Palate (Hard and soft)	12	17.14	6	50.00
Tonsil	15	21.43	11	73.33
Buccal mucosa	26	37.14	18	69.23

The visualization of DNA in gel electrophoresis was shown effectively to report that cases were positive EBV (Figure 3) to report that most of cases are EBV positive (n=41) (Figure 4). The association between risk factors and prevalence of EBV among cases showed that all risk factors are significantly associated with incidence of EBV (Table 4) and the site of tumor reported that all sites except for palate were significantly associated with prevalence of EBV (Table 5).

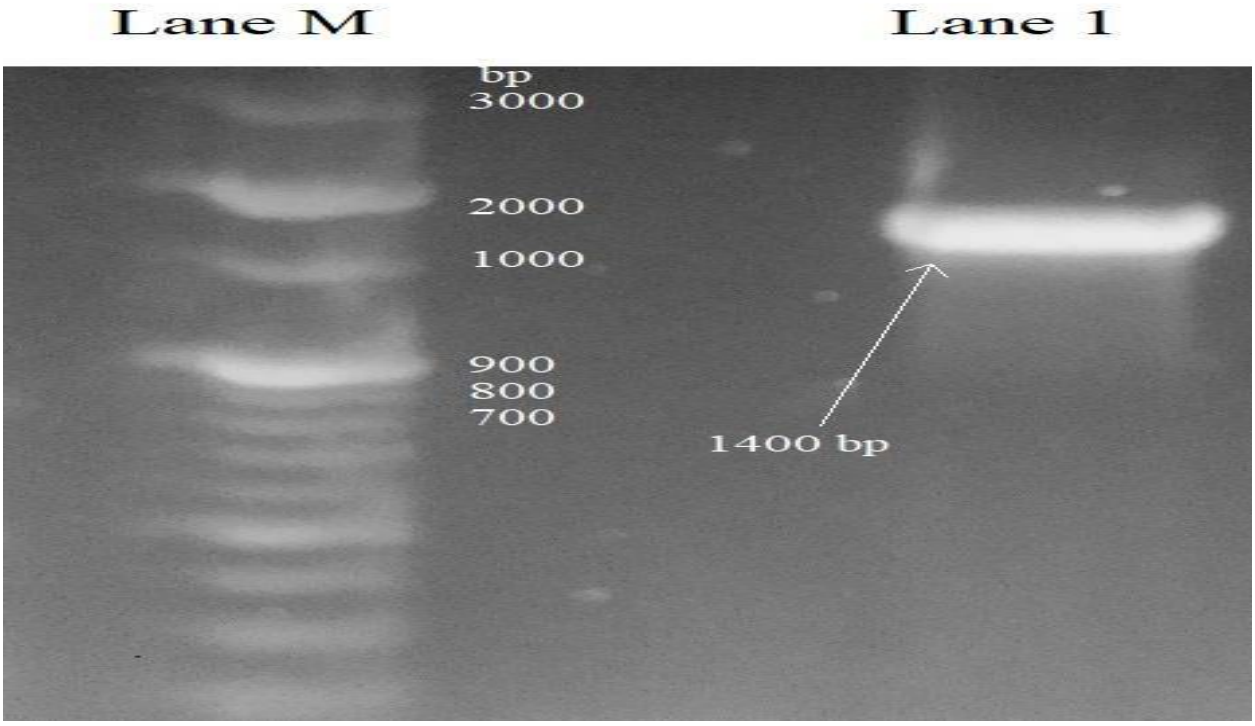

Figure 3 The gel electrophoresis detecting EBV DNA gene using endpoint PCR

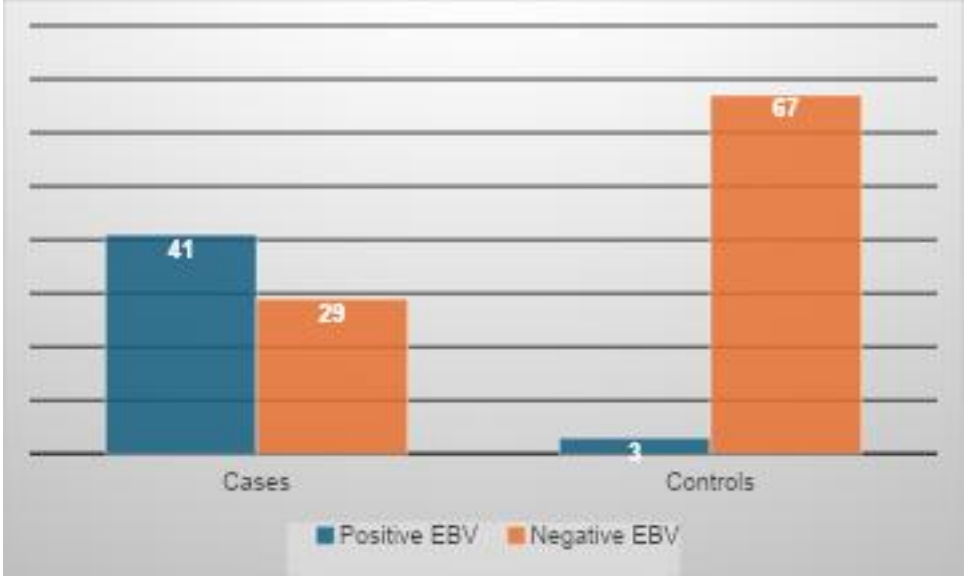

Figure 4 The EBV prevalence among cases with OSCC and controls

Table 4 The association between risk factors and prevalence of ENBV infection among subjects in this study

Risk Factors	EBV Positive	EBV negative	p-value
Smoking	7	1	< 0.001
Alcoholism	15	2	
Betel quid chewing	12	1	

Table 5 The association between localization of OSCC and prevalence of ENBV infection among cases

	EBV positive	EBV negative	p-value
Tongue	1	0	0.002
Gum	3	1	0.001
Lip	0	0	0.04
Palate (Hard and soft)	4	1	0.05
Tonsil	9	1	0.001
Buccal mucosa	15	2	0.002

Discussion

Among 140 participants in this study which were distributed as 70 were diagnosed with OSCC and 70 were considered as controls, the demographic data showed that mean age among cases and controls was 41.57±7.4 and 40.7±6.2, respectively and there was an approximate equality in their gender distribution, to show and affirm that most of cases with OSCC are aged more than 40 years as previously reported in Rahman et al. [31] and Heawchaiyaphum et al. [32] studies.

The risk factors reported were distributed as smoking which was more among controls than cases while alcoholism and betel quid chewing was higher among cases and the site of tumor among OSCC cases was reported as the tumors in buccal mucosa were the most predominant (n=26, 37.14%) which agrees with Núñez-Acurio et al. [3] study who focused on the role of the oral cavity as the primary site for harboring EBV and their potential to cause cancer with showing an overview of the current knowledge regarding the involvement of these microorganisms in oral carcinogenesis, explain the mechanisms through which EBV can independently or together contribute to cancer development, and propose a model illustrating the interaction between these two microorganisms and with Acharya et al. [34] study, who found that there was a statistically significant connection

between chewing betel quid and the prevalence of EBV, while there was no association with tobacco smoking and alcohol intake. There was a significant association between alcohol intake and betel quid chewing with OSCC, while tobacco smoking was not linked. Notably, there was a strong correlation between EBV and OSCC, with an odds ratio of 3.76 as well as Reddy et al. [2], who reported that the results are inconclusive and rely on multiple factors, including geographical and regional differences, sociocultural lifestyles, nutritional patterns, and the habit of chewing or smoking tobacco.

It was reported that report that most of cases are EBV positive (n=41), which agrees with Zebardast et al. [20], who found that from a total of 88 samples from the oral cavity were analyzed using the Real-Time PCR technique. Of these, 23 were diagnosed with OSCC, and the sequencing was performed on some of the samples to find that there is an average of EBV copy number in OSCC samples (1.2 copies per each cell) and all of cases exhibited a high viral load of Epstein-Barr virus (EBV).

The association between risk factors and prevalence of EBV among cases showed that all risk factors are significantly associated with incidence of EBV ($p < 0.001$) and the site of tumor reported that all sites except for palate were significantly associated with prevalence of EBV ($p < 0.05$) and it is in agreement with Guidry et al. [33] study, who found that EBV is most likely responsible for advancing the growth of tumors rather than initiating them. This results in the development of malignant characteristics seen in malignancies that are positive for EBV. In this analysis, we will examine the prevalence of EBV in oral malignancies and explore the mechanisms via which EBV contributes to the advancement of tumors, considering that the mouth cavity is the primary location for EBV residence and transmission as well as agreement with Acharya et al. [34] study, when Oral exfoliated cells were obtained from individuals diagnosed with OSCC as well as from individuals without cancer (non-cancer controls). Cells extracted from tumor lesions were collected from patients with OSCC to provide robust evidence of the link between EBV and OSCC and finally it was suggested that EBV was present in the oral exfoliated cells of OSCC patients, compared to the non-cancer control group ($P < 0.001$).

Conclusion

EBV is readily and significantly correlated with development and prediction of OSCCs among different cases who are visiting Musol hospital as well as there is a significant observation that the EBV infections may be a predictor for OSCC occurrence, the rapid and current screening for EBV among population is a must and more advanced and cheap criteria for OSCC diagnosis must be implemented.

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