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Epstein-Barr virus predominance and immunological abnormalities in oral squamous cell carcinoma

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Abstract

BACKGROUND: Both benign and harmful head and neck disorders have been associated with the Epstein-Barr virus (EBV). Many studies have connected EBV to oral squamous cell carcinoma (OSCC). Oral squamous cell carcinoma is the most prevalent type of cancer. Fresh tissue samples from patients with OSCC were tested for the presence of Epstein-Barr virus.

OBJECTIVE: To determine the frequency of EBV and IL10 expression in OSCC patients.

MATERIALS AND METHODS: Fifty individuals with OSCC and 25 with clinically healthy oral mucosa were studied. *In situ* hybridization was used for the detection of EBV. Serum IL-10 levels were also evaluated in patients and controls using a human IL-10 ELISA Kit.

RESULTS: EBV was detected in 4 healthy patients, 6 with moderately differentiated OSCC, 10 with poorly differentiated OSCC, and 19 with undifferentiated OSCC. These differences were statistically significant ($p<0.05$). IL-10 expression was more common in OSCC diagnostic groups than healthy controls, and the difference in blood IL-10 levels between patients and controls was statistically significant ($p<0.01$).

CONCLUSION: The prevalence of EBV in OSCC suggests its possible role in oral cavity malignancy. On the other hand, IL-10 is expressed at higher levels in OSCC biopsies; such elevated concentrations may promote viral spread.

KEYWORDS: oral squamous cell carcinoma; Epstein-Barr virus; *in situ* hybridization; EBNA; IL-10

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Introduction

Oral cancer is increasing worldwide, although the annual death toll is higher in low- and middle-income countries. People are living longer, and there are more factors that might lead to cancer [1]. Squamous cell carcinoma (SCC) is the most common kind of oral cancer and poses a significant risk to public health across the world.

Oral cavity SCC has a complex etiology; the incidence rate of this cancer varies considerably based on habits or forms of intake, such as alcohol or cigarette use, exposure to other risk factors, viral infection, and individual genetic susceptibility [2].

The most frequent kind of mouth cancer is oral squamous cell carcinoma (OSCC). OSCC is the most common kind of oral cancer. Although it has been observed in young people, it is more frequent in middle-aged and elderly people [3]. OSCC accounted for 91% of all instances of cancer diagnosed in Iraq between 2000 and 2008, according to the Iraqi Cancer Registry, which

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records all cases in the country except those in the north [4]. An analysis of 1,425 biopsy reports showed that OSCC was found to be frequent in male patients over 50 [5]. Frequency of OSCC in Iraq and Saudi Arabia varies greatly [6-12].

Viruses cause 15% of human cancers worldwide, including head and neck neoplasms [13]. The Epstein-Barr virus (EBV) is the third most prevalent cause of infections associated with cancer in the United Kingdom, and it is present in 90% of nasopharyngeal cancers (NPCs)[14] which is widespread around the world [15]. Host-tumor interactions, which occur through various genetic and cellular variables inside the tumor microenvironment, are a crucial part of the complicated process of oral cancer progression [16].

Interleukin 10 (IL10) is a cytokine that helps stop inflammation. It is also called a human cytokine synthesis inhibitory factor (CSIF). It primarily controls the immunological response, and when it is absent, inflammation is probable. Macrophages are the primary source of IL-10 production. IL10 performs various tasks, including controlling angiogenesis, cytokine synthesis, T-cell proliferation, and inflammatory responses. Whether IL10 has a dynamic function in carcinogenesis and tumor inhibition is debatable. Oral malignancies and solid tumors have been shown to have elevated amounts of IL-10 production [17,18]. Macrophages produce most IL-10. Tumor tissues, serum, and saliva showed increased IL-10 levels in several cancers. Moreover, this increased concentration has been recommended as a sign of a bad prognosis [19].

The aim of this work was to define the existence of EBV and IL10 expression in OSCC patients.

Material and Methods

Fifty formalin-fixed paraffin-embedded archival tissues were used. They included 25 OCSS tissues as well as 25 biopsies from apparently normal tissues. According to the manufacturer's protocol, 4-5 mm sections of the studied tissue block were stuck on "positively charged slides" and used for *in situ* hybridization for the detection of the expression of EBV-EBNA (from Abcam Company, United Kingdom). Each test included positive and negative controls, and samples were inspected under a microscope at 10 and 40 magnification [20]. Following the manufacturer's instructions, a Human IL-10 ELISA kit (BioLegend, San Diego, USA) was used to measure the concentration of IL-10 in the serum.

Statistical analysis

The SPSS 24 program was utilized for all the analyses. Pearson's chi-square or Spearman's rho test were used in all comparisons with an alpha of 0.05.

Results and Discussion

Immunological profile of OSCC

Immunostaining for IL-10 antibody was associated with OSCC (Table 1, $p<0.05$). Mean IL-10 immune signaling expression levels were 19.3, 20.7, 23.6, and 29.2, moderately, badly, and undifferentiated grades of OSCC respectively.

In contrast to the findings of the present study, interleukin-10 was not detected in the blood of patients with SCC and adenoid cystic carcinoma of the head and neck in a previous study [21]. Serum IL-10 overproduction was seen in individuals with various solid and hematopoietic tumors [22], suggesting it may be frequent in some cancers. Increased blood IL-10 levels were seen in patients with more advanced malignancy [23]. Salivary IL-10 levels in OSCC patients were higher than in healthy participants in the current study, which was shown before [24]. Our findings suggest that IL-10 in saliva likely serves as a physiological purpose but is not a helpful salivary biomarker for OSCC [25]. Variables in the oral environment, like periodontal disease and oral microbial flora, could cause protein artifacts to be found. This makes it hard to compare people based on their salivary composition. This can be seen as a limitation of this study. Acute stress, which was not measured here, elevates serum and salivary

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IL-10 levels, suggesting that psychosocial variables may influence the present results [26].

Table 1: Immunological detection of IL-10 in OSCC

Cytokine	Diagnosis	OSCC Affected	Unaffected	Pearson's chi-square (*P*-value)
IL-10 pg/mL	Well differentiated	19.3±5.12	11.23±2.11	P<0.01
	Moderate differentiated	20.7± 9.03	11.23±2.11	
	Poorly differentiated	23.6± 11.23	11.23±2.11	
	Undifferentiated	29.2±5.17	11.23±2.11	

In situ *hybridization detection of the Epstein-Barr virus EBNA gene among studied samples*

The expression patterns of EBV-EBNA levels in all groups of OSS cancer tissues are shown in Table 2 and Figure 1. This investigation revealed that EBV was more prevalent in OSCC tissues. In a previous study, EBV infection was identified in all 36 (100%) OSCCs [27], indicating the high incidence of EBV infection in OSCC samples. A similar finding was unveiled with the use of an EBV genomic microarray. Most samples (82.5%) of biopsy tissues from 57 OSCC patients were infected with EBV [28].

Table 2: EBV prevalence in OSCC

Study group	Diagnosis	Negative	EBV-EBNA Expression Pattern	Pearson Chi-Square (P-value)
OSCC	Well differentiated	0 (0.0%)	4 (10.26%)	P=0.04
	Moderately differentiated	7 (63.64%)	6 (15.38%)	
	Poorly differentiated	3 (27.27%)	10 (25.64%)	
	Undifferentiated	1 (9.09%)	19 (48.72%)	
	Total	11 (100.0%)	39 (100.0%)	

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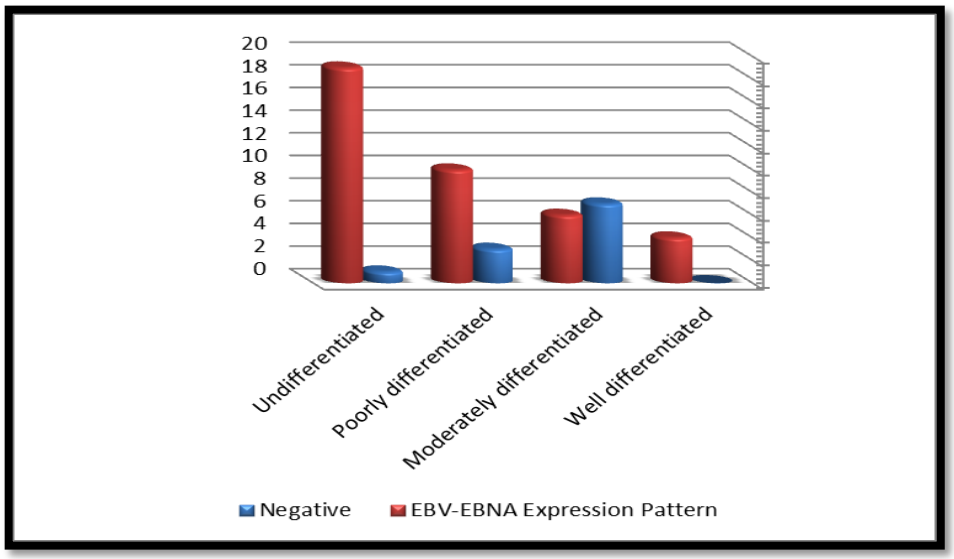

Figure 1. *Expression of EBNA-EBV in diagnostic tumors.*

Conclusion

This study found more often EBV in OSCC tissues in comparison to OSCC-unaffected tissues, suggesting that EBV infection may contribute to oral tissue carcinogenesis. Nevertheless, further investigation is required to identify the role of EBV in the development of OSCC. In contrast, OSCC biopsies had antibodies to IL-10, whereas normal tissues were immunonegative.

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