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The impact of type 2 diabetes on selected clinical parameters and serological markers in patients with EBV-related oropharyngeal cancer

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Abstract

Introduction and Objective. Diabetes can lead to many complications and may also increase the risk of malignant tumours. Numerous studies have shown that diabetes not only increases the incidence, but also affects the prognosis of many cancers, including those of the head and neck. The aim of the study was to assess the possible impact of diabetes on the clinical course of EBV-related oropharyngeal cancer. For this purpose, the frequency of G and TN features was analyzed among diabetic EBV-related oropharyngeal cancer patients compared with those without diabetes. The study also took into account the duration of diabetes (in years) and the level of anti-EBV antibodies.

Materials and Method. The study included a total of 115 patients diagnosed and histologically confirmed oropharyngeal squamous cell carcinoma (OPSCC). The research used tumour tissue samples taken during surgery and sera. EBV DNA was detected by PCR in the tissue and anti-EBV antibodies were detected in the serum (ELISA test).

Results. Poorly differentiated tumours (G3) and more advanced clinical stages (T3 – T4) as well as more involved lymph nodes (N) were observed in diabetic patients. The frequency of the mentioned clinical parameters depended on the duration of diabetes. The level of anti-EBNA and anti-EBVCA antibodies was significantly lower in the group of patients with diabetes (both in IgA and IgG classes). Patients suffering from diabetes for over 6 years had significantly lower levels of tested antibodies. The obtained results encourage further, in-depth research in this area.

Conclusions. More advanced stages of EBV-related OPSCC and lower levels of anti-EBV antibodies are observed in diabetic patients.

Key words

diabetes, EBV, oropharyngeal cancer

INTRODUCTION

Cancers, including head and neck cancers (HNCs), are trending upwards in terms of morbidity and mortality, constituting a significant problem for public health worldwide. HNCs can be located in the mouth, pharynx and laryn, with the majority classified as head and neck squamous cell carcinoma (HNSCC) [1]. In the multifactorial etiology of these cancers, the role of persistent infections with oncogenic viruses, especially human papillomavirus (HPV) and EBV, is also emphasized [2, 3].

Cancer patients very often have other comorbidities. One of the more common comorbidities is diabetes, a complex and chronic disease in which the beta cells of the pancreas do not produce enough insulin, or when the body's cells cannot respond effectively to the hormone insulin (insulin resistance) [4]. High blood glucose levels (hyperglycaemia) are a systemic undesirable condition that negatively affects patient's overall health. Therefore, diabetic patients require constant medical care and regular glycaemic control.

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Type 2 diabetes (T2D) is a very important public health problem of the 21st century, constituting the first non-communicable epidemic in the world [5]. According to the International Diabetes Federation (IDF), 537 million people have type 2 diabetes, with an increasing tendency to 783 million by 2045 [6]. In Poland, 8% of the population have been diagnosed with diabetes, a percentage that is expected to increase to 11% by 2040. A disturbing fact is that approximately 25–30% of adults do not know that they have diabetes [7].

Diabetic patients are at risk of infections because hyperglycaemia increases the virulence of various microorganisms [8, 9]. Moreover, diabetes can lead to many complications and may also increase the risk of malignant tumours.

Previous studies by the authors of the current article on the prevalence of herpes viruses in diabetic patients, showed that EBV DNA was detected much more frequently in these patients than in healthy controls, and depended on the duration of the disease [10]. Therefore, it was decided to check whether diabetes has an impact on the course of EBV-related oropharyngeal cancer. For this purpose, the prevalence was investigated of G and TN features among EBV-related oropharyngeal cancer diabetic patients, compared with

patients without diabetes. The study also took into account the duration of diabetes (in years) and the level of anti-EBV antibodies.

MATERIALS AND METHOD

The study included a total of 115 patients diagnosed and histologically confirmed with oropharyngeal squamous cell carcinoma (OPSCC), hospitalized in the Department of Otolaryngology, Head and Neck Cancer, of the Casemiro Pulaski University in Radom, Poland.

The basic criterion for selecting patients for the study was the presence of EBV DNA in tumour tissue. Exclusion criterion was the presence of HPV DNA in the tumour tissue. Only HPV-negative patients were included in the study group. The 8th edition of the American Joint Committee on Cancer TNM recommends stratification of all OPSCC cases according to HPV status [11]. None of the patients had previously received radiotherapy or chemotherapy.

Guided by the above-mentioned criteria, two groups of EBV-related oropharyngeal cancer patients were selected who were similar in terms of gender and age: Group 1 – diabetic patients (58 people), Group 2 – non-diabetic patients (57 people). The socio-demographic characteristics of the study patients are presented in Table 1.

Table 1. Baseline characteristics of EBV-related oropharyngeal cancer patients

		Patients				p
		diabetic N = 58		non-diabetic N = 57		
		N	%	N	%	
Gender	Female	8	13.8	7	13.5	0.9999
	Male	50	86.2	50	86.5	
Age	50–59	27	46.6	28	46.2	0.8527
	60–79	31	53.4	29	53.8	
Place of residence	Urban	41	70.7	39	69.2	0.8411
	Rural	17	29.3	18	30.8	
Smoking	Yes	38	65.6	38	66.7	0.7826
	No	20	34.4	19	33.3	
Alcohol abuse	Yes	28	48.3	27	47.4	0.9376
	No	30	51.7	30	52.6	
Duration of diabetes (years)	1–5	18	31.1			
	6 and more	40	68.9			

Chi-square test with Fischer's exact test

Clinical materials. The research used tumour tissue samples taken during surgery, and sera. EBV DNA was detected in the tissue and anti-EBV antibodies were detected in the serum. Tissue samples from all patients were frozen at −80 °C and stored until analysis. During primary diagnosis, the classification of the tumour, node and metastases (TNM) was determined according to the 8th edition of the TNM classification of head and neck cancer [10]. Histological grading was performed according World Health Organization (WHO) criteria, which divide tumours into 3 types: well differentiated (G1), moderately differentiated (G2), and poorly differentiated (G3) [12].

Venous blood samples collected from all patients were centrifuged at 1,500 rpm for 15 min at room temperature, and the serum frozen at −80 °C until analysis.

EBV DNA extraction and detection. Freshly frozen oropharyngeal squamous cell carcinoma (OPSCC) tissue (20 mg) was cut and then homogenized using an Omni TH/Omni (International/Kennesewa, GA, USA) manual homogenizer. DNA was extracted using the QIAampDNA Mini Kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions.

A commercially available diagnostic kit was used to detect the EBV DNA Gene Proof EBV virus (Brno, Czech Republic), in accordance with the manufacturer's protocol. All samples were analyzed in duplicate. A specific conserved DNA sequence for the EBV nuclear antigen 1 gene (EBNA-1) was amplified using Light Cycle 2.0 Software Version 4.1 (Roche Applied Science System, Penzberg, Germany).

Antibodies detection. Serum anti-EBNA and anti-EBVCA antibodies both in IgA and IgG classes were determined by the commercially available Microblot-Array test (TestLine Clinical Diagnostics Ltd. Brno, Czech Republic), according to the manufacturer's recommendations.

Ethics. The study was performed according to the guidelines of the Declaration of Helsinki, and approved by the Ethics Committee at the Medical University in Lublin, Poland (Approval No. KE-0254/295/2019, dated 26 September 2019).

Statistical analysis. GraphPad Prism software version 10.4.0. (San Diego, California, USA) was used to conduct data analysis. The normal distribution of continuous variables was checked using the Shapiro–Wilk test. The relationship between clinical and demographic parameters was calculated using the Pearson chi-square test. Pearson's chi-square test and Fisher's exact test were used to compare the frequency of the examined clinicopathological features in both groups. The Mann–Whitney U test was used to compare differences in antibody levels between the study groups. Results were considered significant at $p \leq 0.05$.

RESULTS

The first stage of the study was to check whether diabetic and non-diabetic patients differed in terms of the degree of histological differentiation (grading) and TN features (Tab. 2). None of the studied patients had distant metastases (M0). The highest frequencies of moderately differentiated (G2) and poorly differentiated tumours (G3) were observed in diabetic patients. The difference was statistically significant ($p = 0.0192$). Similarly, more advanced clinical stages (T3 – T4), as well as more involved lymph nodes (N), were found in diabetic patients. These differences were also statistically significant.

It was then checked whether the frequency of the mentioned clinical parameters depended on the duration of diabetes (Tab. 3). As these data show, in the group of patients suffering from diabetes for 6 years and more, less differentiated tumours (G3) as well as T3/T4 and N2/N3 features were diagnosed more often compared to non-diabetic patients.

Table 2. Clinical parameters in EBV-related oropharyngeal cancer in diabetic and non-diabetic patients, in relation to grade and TNM classification.

		Patients				p
		Diabetic; N = 58		Non-diabetic; N = 57		
G	G1	9	15.5	21	32.7	0.0192*
	G2	33	56.9	28	51.9	
	G3	16	27.6	8	15.4	
T	T1	7	12.1	8	15.4	0.0491*
	T2	16	27.6	27	42.3	
	T3	18	3.0	16	28.8	
	T4	17	29.3	6	12.1	
N	N0	5	8.6	23	42.3	0.0008*
	N1	16	27.6	11	19.2	
	N2	18	31.0	13	21.2	
	N3	19	32.8	10	17.3	
M	M0	58	100.0	57	100.0	

*statistically significant; Chi-square test with Fischer's exact test

The last point of the analysis was assessment of the level of anti-EBV antibodies, i.e. EBVCA and EBNA in the IgA and IgG classes in the group of diabetic and non-diabetic patients (Fig. 1). It was observed that the level of anti-EBNA and anti-EBVCA antibodies was significantly lower in the group of patients with diabetes. This applied to both classes of antibodies.

Table 3. Prevalence of G, T and N features in oropharyngeal cancer patients, according to duration of diabetes

Parameter		Duration of diabetes (years)				p
		1 – 5; N = 18		6 and more; N = 40		
G	G1	7	38.8	2	5.0	0.0032*
	G2	9	50.0	24	60.0	
	G3	2	11.1	14	35.0	
T	T1/T2	12	66.7	11	27.5	0.0083*
	T3/T4	6	33.3	29	72.5	
N	N0/N1	12	66.7	9	22.5	0.0025*
	N2/N3	6	33.3	31	77.5	

*statistically significant; Chi-square test with Fischer's exact test

The level of anti-EBNA and anti-EBVCA antibodies was also analyzed depending on the duration of diabetes (Fig. 2). Observations showed that patients suffering from diabetes for over 6 years had significantly lower levels of the tested antibodies.

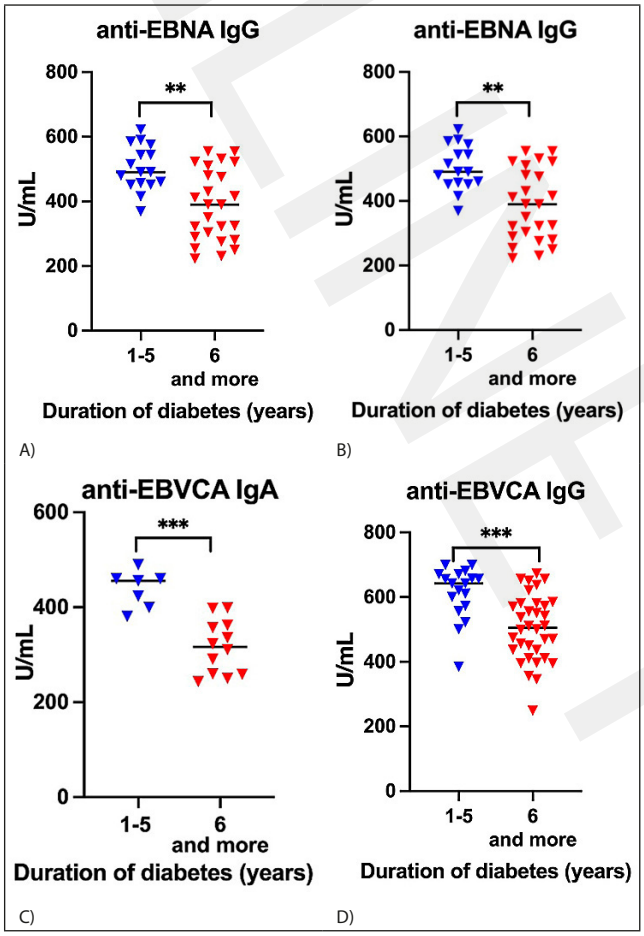

Figure 1. Level of anti-EBV antibodies: A) anti EBNA IgA (p = 0.0002), B) anti-EBNA IgG (p = 0.0041), C) anti-EBVCA IgA (p = 0.0084), D) anti-EBVCA IgG (p < 0.0001), in diabetic (red colour) and non-diabetic (blue colour) patients; (Mann-Whitney Test)

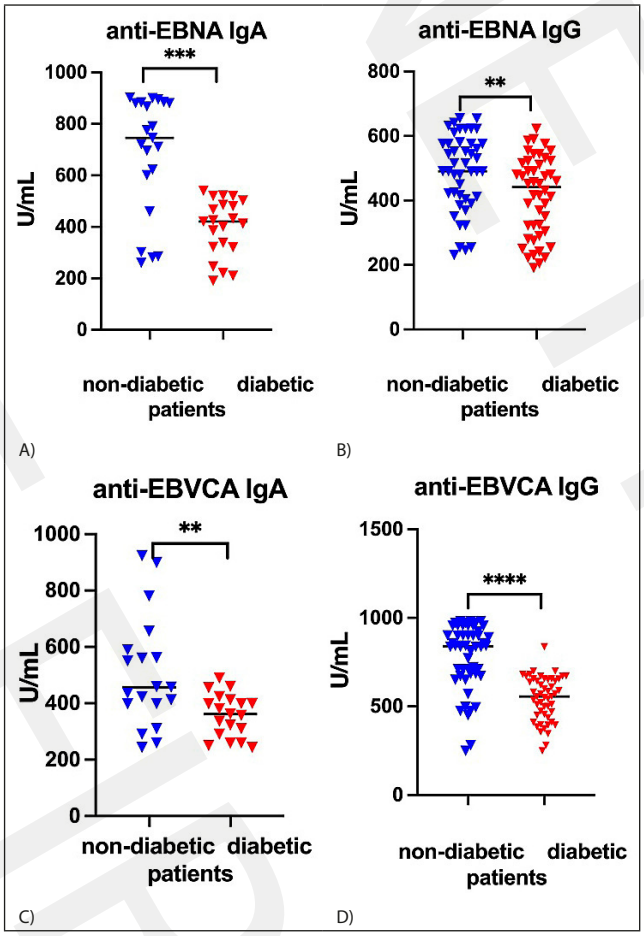

Figure 2. Level of anti-EBV antibodies in diabetic patients according to duration of diabetes (1 – 5 years – blue colour, 6 and more years – red colour); EBNA IgA p < 0.0001;
EBNA IgG p = 0.0012; EBVCA IgA p = 0.0002; EBVCA IgG p = 0.0002 (Mann-Whitney Test)

DISCUSSION

Numerous studies have shown that diabetes increases not only the incidence but also affects the prognosis of many cancers, such cancer of the breast, liver and lung [13, 14]. Hyperglycaemia promotes cancer development and proliferation. In the available literature on the subject, some authors emphasize the role of diabetes in the development of NPC [15, 16]. Studies by Peng et al. [17] showed an association of diabetes with a poor prognosis. Very interesting research results were presented by Midorikava et al. [18] who analyzed the impact of T2D on virus-related NPC. They observed a high prevalence of EBV in NPC patients with diabetes. Moreover, their results clearly demonstrated the negative impact of T2D on the prognosis of nasopharyngeal cancer. Many studies show that during malignant transformation of nasopharyngeal epithelial cells, glucose metabolism increases, which contributes to latent EBV infection and the development of NPC [19].

Although the current study concerns a different location, i.e. oropharynx, the results obtained are similar to those of the above-cited authors. To the best knowledge of the authors of the current study, it is the first study to examine the possible impact of diabetes on the clinical course of EBV-related oropharyngeal cancer.

Innate immunity, as well as acquired immunity (including T cells and B cells), play an important role in the progression of type 2 diabetes [20]. Many studies have shown that people with diabetes have an impaired immune response against various pathogens, including viruses [21, 22]. This concerns mechanisms such as suppression of cytokine production, phagocytosis defects, and immune cell dysfunction. For example, lower levels of anti-SARS-CoV-2 antibodies were found in diabetic patients than in those who were non-diabetic [23].

EBV is widespread in the human population. In the diagnosis of EBV infections, the assessment of the level of anti-EBV antibodies is commonly used. Routine screening is based on serological tests, including antibodies against EBV capsid antigen (VCA), and EBV nuclear antigen (EBNA) in both IgA and IgG classes. Hence, the current study assessed the level of these antibodies in relation to clinico-pathological features.

The results obtained show that the level of anti-EBNA and anti-EBVCA antibodies (both in IgA and IgG classes) was lower in patients with EBV-related oropharyngeal cancer with co-existing diabetes, than in patients with the same cancer, but without diabetes. The duration of the diabetes was also important: in patients with diabetes for 6 years or more, the level of antibodies tested was lower than in people with diabetes for 5 years or less.

Limitations of the study. The authors are aware that the research conducted has certain limitations. Firstly, the size of the research group was not very large, due to the low incidence of this type of cancer in Poland. Therefore, the number of cases in each subgroup was also small, making it necessary to combine them. Therefore, feature T was analyzed as T1–T2 and T3–T4, and feature N was analyzed as N0–N1 and N2–N3. Secondly, the concentration of glycated haemoglobin was not included in the study because the information about diabetes was taken from the medical histories. When examining this issue in the future, this will be taken into account to check whether glycaemic instability has an impact on the clinical course of EBV-related oropharyngeal cancer.

These limitations will be supplemented in future with specially planned studies on a much larger group of patients, which will certainly allow for the verification of the observed trends. Nevertheless, despite these limitations, performance of the presented study seems fully justified, and the obtained results encourage further, in-depth research in this area.

CONCLUSIONS

More advanced stages of cancer have been observed in diabetic patients. Moreover, the obtained results show that the level of anti-EBV antibodies was significantly lower in the group of EBV-positive OPSCC diabetic patients, compared to non-diabetic patients.

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