

# IMMUNOHISTOCHEMICAL STUDY OF HUMAN PAPILLOMA VIRUS AND EPSTEIN–BARR VIRUS IN PATIENTS WITH LYMPHOEPITHELIOMA-LIKE CARCINOMA OF THE UTERINE CERVIX

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## ABSTRACT

**Introduction.** Lymphoepithelioma-like carcinoma (LELC) of the uterine cervix is a rare type of squamous cell carcinoma (SSC). It differs from the usual SSC of the cervix in its morphology and clinical behavior and shows a better prognosis than the more common SSC of the cervix. It is considered that LELC is associated with Epstein–Barr virus (EBV) infection in Asian and with human papilloma virus (HPV) or no infection in Caucasian patients.

**The aim of the study** was to confirm whether or not LELC is more common in Caucasian patients with EBV/HPV infection or whether there is no correlation to the previous viral exposure.

**Material and methods.** A retrospective research has been done on 775 female patients for a period of 8 years, who have been operated for cervical cancer

## RÉSUMÉ

**Étude immunohistochimique des Papilloma virus et Epstein-Barr virus chez les patientes avec carcinome de type lympho-épithélioma du col utérin**

**Introduction.** Le carcinome de type lympho-épithélioma (LELC) du col utérin est un type rare de carcinome à cellules squameuses (SSC). Il diffère du SSC habituel du col de l'utérus par sa morphologie et son comportement clinique et présente un meilleur pronostic que le SSC plus commun du col de l'utérus. On considère que la LELC est associée à l'infection par le virus Epstein-Barr (EBV) chez les asiatiques et au virus du papillome humain (VPH) ou à l'absence d'infection chez les patients de race blanche.

**L'objectif de l'étude** est de confirmer si la LELC est plus fréquente chez les patients de race blanche

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in the Onco-gynecological Department of UMHAT „Doctor Georgi Stranski“ -Pleven, Bulgaria. A group of 16 women with LELC has been identified by clinical data. Morphologically, 13 of them have been examined by routine histological and immunohistochemical tests, for assessment of the viral status, with monoclonal antibodies against EBV/HPV by DAKO protocol.

**Results.** Two of the women have been proven to have EBV, tree-HPV infection and two – both viruses. In the other six cases no viral infections have been identified.

**Conclusion.** Our results show a stronger correlation between LELC in Caucasian women and a previous HPV infection or no viral infection, rather than association with EBV infection.

**Keywords:** Lymphoepithelioma-like carcinoma, Human papilloma virus, Epstein-Barr virus, Immunohistochemistry.

#### List of abbreviations:

LELC – Lymphoepithelioma-like carcinoma

SSC – squamous cell carcinoma

EBV – Epstein-Barr virus

HPV – Human Papilloma virus

## INTRODUCTION

Lymphoepithelioma is described for the first time as a neoplasm of the nasopharynx. The histological features of this tumor are a syncytial growth pattern of undifferentiated malignant cells with prominent lymphoplasmacytic stromal infiltration<sup>1</sup>. Later the similar tumors have been described in salivary gland<sup>2</sup>, lung<sup>3</sup>, stomach<sup>4</sup> and thymus<sup>5</sup> and have been called lymphoepithelioma-like carcinoma (LELC). In the uterine cervix, it was reported for the first time by Hamazaki et al in 1968<sup>6</sup>. Although it is a very rare tumor, it is necessary to be differentiated from the squamous cell carcinoma because of its better prognosis. It is considered that LELC is associated with Epstein-Barr virus (EBV) infection in Asian women and with Human Papilloma virus (HPV) or no infection in Caucasian patients.

**THE OBJECTIVE OF THE STUDY** was to confirm whether or not LELC is more commonly associated with HPV infection rather than with EBV infection in Caucasian patients or whether there is no correlation to the previous viral exposure.

infectés par le virus EBV / HPV ou s'il n'y a pas de corrélation avec l'exposition virale antérieure.

**Méthodes.** Une recherche rétrospective a été menée sur 775 patientes pendant une période de 8 ans qui avaient été opérées pour un cancer du col utérin dans le département d'oncologie-gynécologie de l'UMHAT «Docteur Georgi Stranski» -Pleven. Un groupe de 16 femmes avec LELC a été identifié par les données cliniques. Sur le plan morphologique, 13 d'entre elles ont été examinées de manière histologique et immunohistochemie de routine, afin d'évaluer le statut viral, avec des anticorps monoclonaux anti-EBV / HPV selon le protocole DAKO.

**Résultats.** Il a été prouvé que deux des femmes avaient le virus EBV, une infection par le HPV des arbres, et deux – les deux virus. Dans les six autres cas, aucune infection virale n'a été identifiée.

**Conclusions.** Nos résultats montrent une corrélation plus forte entre le LELC chez les femmes de race blanche et une infection à HPV antérieure ou l'absence d'infection virale, plutôt qu'une association avec une infection à EBV.

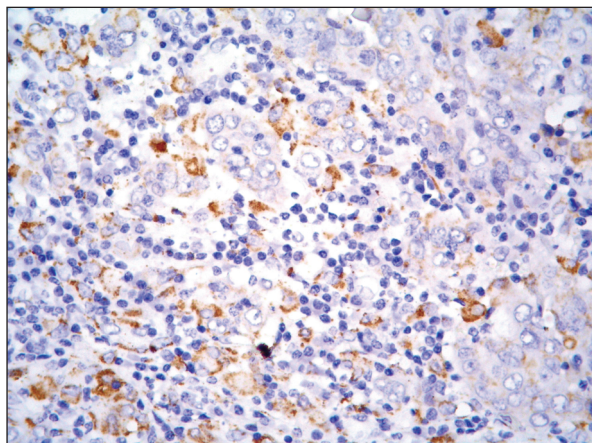
**Mots-clés:** carcinome de type lympho-épithélioma, virus du papillome humain, Epstein-Barr virus, immunohistochemie.

## MATERIAL AND METHODS

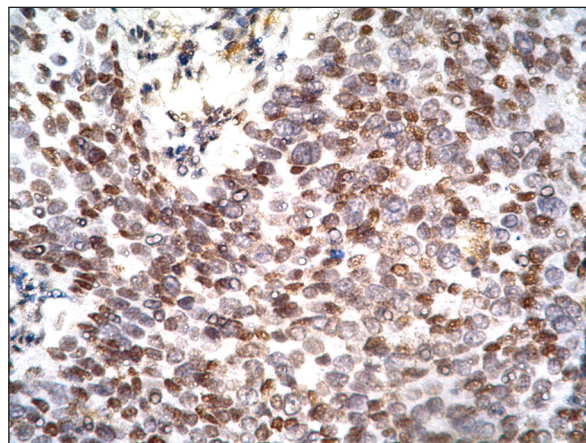
A retrospective research has been done on 775 female patients for a period of 8 years (2008- 2015), who have been operated due to cervical cancer in the Onco-gynecological Department of UMHAT „Doctor Georgi Stranski“ -Pleven, Bulgaria. A group of 16 Caucasian women with LELC has been identified by clinical data. Only 13 of them were included in the trial because the paraffin blocks of the last 3 women had not been found, making thus impossible their examination. The patients have been examined by routine histological and immunohistochemical tests for assessment of the viral status, with monoclonal antibodies against EBV/HPV by DAKO protocol.

## RESULTS

The frequency of LELC in our group was 2.06%. The conventional immunohistochemical stain proves viral presence (HPV/EBV) in seven cases, but generally the staining intensity and distribution were very weak and limited. Immunohistochemistry proves the presence of only HPV in three (23.07%) (Fig. 1) and only EBV in two cases (15.38%) (Fig. 2).



**Figure 1.** Moderately intensive cytoplasmic immunostaining for HPV in individual neoplastic cells and clusters of neoplastic cells



**Figure 2.** Weak to moderately intensive nuclear immunostaining for EBV in neoplastic cells

In two cases it was found the presence of both viruses (15.38%) and the remaining six samples did not show the presence of either HPV or EBV.

## DISCUSSION

It is well known that four human viruses can cause cancer in humans – HPV, EBV, hepatitis B virus, and Kaposi sarcoma herpes virus<sup>7</sup>. One of these types of malignancy is cervical cancer. This is the fourth-most common and fourth-most deadly female malignancy worldwide and in developing countries is the most common cancer and the third leading cancer-mortality causes<sup>8</sup>. It is thought that more than 95% of all cases have been caused by infection with high-risk HPVs<sup>9,10</sup>. Some data indicate that other infectious agents may also be actively involved in cervical carcinogenesis<sup>11</sup>. EBV looks to be one of the most relevant and there are indications that EBV is a potentially active cofactor in the cervical cancer pathogenesis and progression<sup>12</sup>.

Cervical carcinoma is divided in squamous cell carcinomas (SCC) and adenocarcinomas. SCC has several subtypes and one of them is LELC, which is very rare.

First, it was thought to be a subtype of poorly differentiated SCC, histologically characterized by nests of undifferentiated epithelial cells with a syncytial growth pattern infiltrated by a severe lymphocytic infiltrate<sup>13</sup>. This type of cervical cancer has low frequency – 5.5% in Asians<sup>14</sup> and even less – 0.7% in Caucasians. In the female genital system it has been reported in the vulva, vagina, uterine cervix and endometrium<sup>15</sup>. It affects mostly younger women than the common cancer of uterine cervix – often less than 40 years old<sup>6</sup> and the tumor size can vary from

no visible lesion to a large exophytic mass. Usually, it is diagnosed in earlier stage and has better outcome compared with conventional cervical carcinoma<sup>14,16</sup>.

The pathogenesis of LELC is unknown, it is suggested that this carcinoma is associated with EBV infection in the Asian population<sup>14,17</sup> and with HPV infection or no virus infection in Caucasians<sup>14</sup>. These findings are not unconditional because some studies failed to confirm this information<sup>13,18-23</sup>. Tseng et al reported that 73.3% (11/15) of Asian women with this type of cervical cancer were positive for the antibody of EBV<sup>14</sup>. In Caucasians, Noel et al did not detect EBV and found HPV in some of their patients<sup>18</sup>. Bais et al<sup>19</sup> also detected HPV in some of their patients. The same results report and Chao et al<sup>16</sup> – in no western women they detected EBV and in 48% of Asian women with LELC they found EBV. They suggest that racial and geographic factors might have role of the pathogenesis of LELC<sup>16</sup>. There are more and more reports showing the role of HPV/EBV coinfection in genesis of cervical carcinoma<sup>11,24</sup>. There is no data for the role of this coinfection in development of LELC but it cannot be rejected.

The LELC has better prognosis than common cervical cancer, such as squamous cell carcinoma and adenocarcinoma<sup>19,25</sup>. Hasumi et al<sup>25</sup> reported that the 5-year survival of such patients is also better than the other squamous cell carcinomas. Probably this is related to tumor antigen-associated cell mediated immunity, which causes a host response in the form of intense lymphocytic inflammation. This can explain the lower incidence of lymph node metastasis and overall improved survival rate, although a larger series is required to confirm the prognosis of this tumor<sup>26</sup>.



In our group, the frequency of LELC was 2.06%, which is significantly higher than in western women. This is explained by the fact that we have included only those patients who have gone through primary surgery, but not those who have had radiotherapy – preoperative or definitive. Our results demonstrate that almost in half of the patients neither HPV nor EBV was detected and this coincides with data of Martorell et al and Kohrenhagen et al, who did not find any relationship between EBV and HPV infection and pathogenesis of LELC of the uterine cervix in Western women<sup>20,27</sup>. In three cases we found HPV and in two EBV. In two cases we found both viruses. This is similar to the results published in the literature<sup>28-30</sup>.

## CONCLUSIONS

Our results show that not every patient with LELC has infection with HPV or EBV. The role of these two viruses in the pathogenesis of the tumor is not fully understood. This could indicate that immunohistochemistry cannot be a sole indicator for the viral presence in LELC of the uterine cervix. The immunohistochemical results should be compared with in situ hybridization and PCR.

## Compliance with Ethics Requirements:

„The authors declare no conflict of interest regarding this article“

„The authors declare that all the procedures and experiments of this study respect the ethical standards in the Helsinki Declaration of 1975, as revised in 2008(5), as well as the national law. Informed consent was obtained from all the patients included in the study“

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