

## EXPRESSION OF EBV LATENT MEMBRANE PROTEIN 1 (LMP1) IN IRAQI WOMEN WITH CERVICAL CARCINOMA

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**ABSTRACT :** The design of this paper is to find the possible correlation of Epstein Barr virus infection in a group of Iraqi women with cervical carcinoma through detection of Latent Membrane Protein 1 (LMP1) in these cervical tissues. Paraffinized blocks of two groups were included. The first sample of 30 cervical carcinomatous tissues and 15 biopsies from an apparently normal cervical tissues. All the samples were sectioned on a positive charged slides with 4 mm – thickness then submitted for immunohistochemical (IHC) staining to detect viral LMP1 expression. Sixty three percentage (19 out of 30) of the studies group showed positive overexpression as shown in with a significant association of the expression with cervical cancer with a significant association ( $p = 0$ ). The infection with EBV may be a possible causative agent affected the development of cervical cancer. Further studies with large samples are recommended.

**Key words :** Epstein Barr Virus, LMP1, immunohistochemistry, cervical cancer.

### INTRODUCTION

Cervical malignancy positioned as the fourth most prevalent disease among women around the world and it was the most widely recognized female cancer in many low-income societies (Ferlay *et al*, 2012). The early detection programs of cervical cancer is the most efficient screening comparing with other cancers, as it was able to detect pre-cancerous lesions in the cervix by using Pap smear, which can also detect high-risk types of HPV that are responsible of 70% cervical tumors (WHO, 2012; Nwabichie *et al*, 2017).

Globally, infectious agents are responsible of the development 20% of the all cancers including Cervical cancer in which the vast majority of cases are attributed the viral infection (mainly high-risk types of HPVs) (Vedham *et al*, 2015).

Epstein-Barr virus, which is one of the most common human viruses, belongs to the herpesvirus family. The EBV is the most interested member of Gamma herpesviruses due to its wider spreading infection in more than 90% of the adult population globally (Avgil and Ornoy, 2006; Tsai *et al*, 2017).

Although, the Epstein-Barr virus (EBV) is a causative for a persistent asymptomatic infection, it is also the etiology of two premalignant tumors and about nine B

and non-B cell tumors with a worldwide burden of 200,000 new cancers per year (Shannon and Rickinson, 2019). Previous data indicate suggested involvement of infectious agents other than HPVs in the cervical carcinogenesis and among these etiologies, EBV considered one of the most relevant (de Lima *et al*, 2017).

For all above, this study was designed to find the possible correlation of EBV infection with the Iraqi women with cervical carcinoma through detection of Latent Membrane Protein 1 (LMP1) in these cervical tissues.

### MATERIALS AND METHODS

Two groups were used in this study, the apparently healthy group of fifteen archival blocks and the study group of thirty archival tissues of cervical cancer. All these samples were collected from different governmental and private laboratories in Baghdad.

For each block, two slides with 4  $\mu$ m thickness were used, for a routine hematoxylin and eosin staining and the other on a positively-charged slide for immunohistochemical procedure using anti-EBV LMP1 and staining kit from (ABCAM) company.

After dewaxing and rehydration, the endogenous peroxidase activity and non-specific binding were blocked by incubation with peroxide block and protein block ready

to use reagents, respectively. To remove fixative effect, heat mediated antigen retrieving was used with citrate buffer pH 6 before commencing with IHC staining protocol. Slides were then incubated with diluted primary antibody (1/100) for 1 hour at 37°C, after washing the secondary antibody was applied for 10 minutes at room temperature followed by incubation with Streptavidine-HRP for 10 minutes at 37°C. Diaminobenzidinehydrochloride (DAB) was used as the chromogen to visualize peroxidase activity. Sections were counterstained with Mayer’s hematoxylin for 30 seconds then dehydrated and mounted (Khashman *et al*, 2018). Fisher exact test was calculated using Social Science Statistic (<http://www.socscistatistics.com>) considering the statistical significance when P value (<0.05).

**RESULTS**

Fifty four year is the mean age of the cervical cancer patients with a percentage of 57% for the patients with age above 50 years and 43% for those less than 50 years



**Fig. 1 :** The distribution of age groups of the studied samples.

**Table 1 :** Immunohistochemical expression of LMP1 in the studied groups.

IHC expression	Cervical cancer	Healthy tissue	Totals
EBV Positive	19	0	19
EBV Negative	11	15	26
Totals	30	15	45

\*(p= 0).significant at p < 05

(Fig. 1).

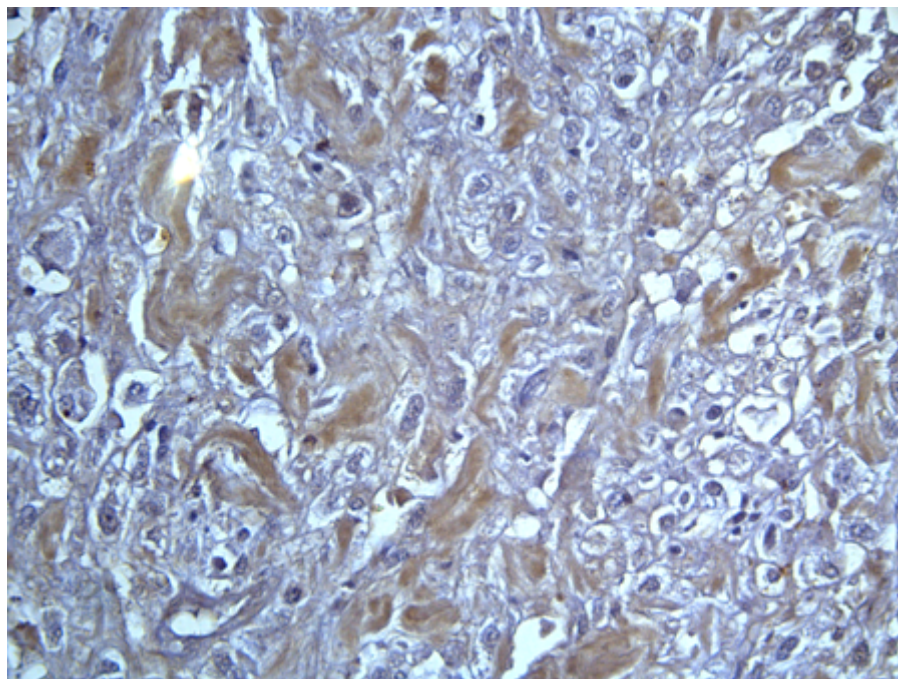
Regarding EBV LMP1 expression, 63% (19 out of 30) of the studies group showed positive overexpression as shown in Table 1 with a significant association of the expression with cervical cancer.

**DISCUSSION**

The life cycle and natural history of infectious agents and their and impacts on initiation and prevention of different cancers should have the priority in different research focal (Parkin, 2006).

Despite the undoubtedly role of the HPV in the transformation of the cervical tissue (Saad *et al*, 2011) several studies referred to the involvements of different infectious agents including Herpesviridae in the initiation and development of cervical carcinoma (de Lima *et al*, 2017). The rationale of choosing EBV in this study is its increased evidences of the presence and herpesviruses in both precancerous and cancerous cervical tissues (Vranic *et al*, 2018; Hussain *et al*, 2019).

In the present study, our findings point out that Latent Membrane Protein 1 expression was detected in 63%



**Fig. 2 :** The expression of EBVLMP1 in Cervical cancer tissues. The DAB produced (Brown) signals while the Harris Hematoxylin produced (purple) color: Nuclear & Cytoplasmic positive results (40x).

(19 out of 30) with a significant association (P value = 0) Table 1 with the squamous cell cervical cancer samples which is completely compatible with Se Thoe *et al* (1993) using *in situ* Hybridization technique. In this respect, the present results support other literatures in that respect, who have found significant expression of EBV genes in cervical carcinoma (Mohammed Ali, 2015).

The discrepancy in the data of EBV infection could be attributed to the different diagnostic techniques and whether it is manual or automated besides the technical issues regarding type and tissue preparation (Vranic *et al*, 2018).

LMP1 considered as the major transforming viral protein of EBV, which expressed in different types of cancers and affects different cellular genes leading to immortalization in both lymphocytic and epithelial cells (Young and Dawson, 2014; Miller *et al*, 1995).

In conclusions, the present result suggests that the infection with EBV may be a possible causative agent affected the development of cervical cancer. Further studies with large samples are recommended.

## REFERENCES

- Ferlay J, Soerjomataram I, Ervik M, Dikshit R and Eser (2012) GLOBOCAN 2012 v1.1, Cancer Incidence and Mortality Worldwide: IARC Cancer Base No. 11.
- World Health Organisations (2012) World health organization cancer facts sheet, 2012; Retrieved on 12 January 2017 from <http://www.who.int/mediacentre/factsheets/fs297/en/>
- Nwabichie Chinemerem Cecilia, Rosliza A M and Suriani I (2017) Global burdern of cervical cancer : A literature review. *Int. J. Pub. Hlth. Clin. Sci. (IJPHCS)* **4**(2), 10-18 .
- Vedham V, Verma M and Mahabir S (2015) Early-life exposures to infectious agents and later cancer development. *Cancer Med.* **4**(12), 1908–1922. doi:10.1002/cam4.538.
- Avgil M and Ornoy A (2006) Herpes simplex virus and Epstein- Barr virus infections in pregnancy : consequences of neonatal or intrauterine infection. *Reprod. Toxicol.* **21**(4), 436–445. DOI: 10.1016/j.reprotox.2004.11.014.
- Tsai M H, Lin X, Shumilov A, Bernhardt K, Feederle R, Poirey R, Kopp-Schneider A, Pereira B, Almeida R and Delecluse H J (2017) The biological properties of different Epstein-Barr virus strains explain their association with various types of cancers. *Oncotarget* **8**(6), 10238–10254. <https://doi.org/10.18632/oncotarget.14380>
- Shannon-Lowe C and Rickinson A (2019) The global landscape of EBV-associated tumors. *Front. Oncol.* **9**, 713. doi: 10.3389/fonc.2019.00713.
- de Lima M A P, Neto P J N, Lima L P M, Goncalves Junior J, Teixeira Junior A G and Teodoro I P P (2017) Association between Epstein-Barr virus (EBV) and cervical carcinoma: a meta-analysis. *Gynecol.Oncol.* **148**(2), 317–28. <https://doi.org/10.3389/fonc.2019.00713>
- Khashman B M, Abdul Ghafour K H, Mohammed Ali S H and Mohammed K I (2018) Nuclear targeting of latent membrane protein 1 of epstein barr virus in tissues from patients with pancreatic carcinoma. *Biochem. Cell. Arch.* **18**(Supplement 1), 1293-1297
- Parkin D M (2006) The global health burden of infection-associated cancers in the year 2002. *Int. J. Cancer* **118**(12), 3030–3044. <https://doi.org/10.1002/ijc.21731>
- Saad H A, Al-Hijazi A and Khashman B M (2011) P53-tumor suppressor gene overexpression in human papillomavirus-infected patients with oral squamous cell carcinoma. *J. Baghdad College Dentistry* **23**, 70-76.
- Vranic S, Cyprian F S, Akhtar S and Al Moustafa A E (2018) The Role of Epstein-Barr Virus in Cervical Cancer: A Brief Update. *Frontiers in Oncol.* **8**, 113. <https://doi.org/10.3389/fonc.2018.00113>.
- Hussain A A and Khashman B M (2019) Detection of HPV-16 and HSV-2 among women with Chronic Cervicitis in Baghdad City. *Res. J. Pharm. Technol.* **12**(9), 4443-4446. doi: 10.5958/0974-360X.2019.00766.2
- Se Thoe S Y (1993) Elevated secretory IgA antibodies to Epstein-Barr virus (EBV) and presence of EBV DNA and EBV receptors in patients with cervical carcinoma. *Gynecologic Oncol.* **50**(2), 168-72. doi:10.1006/gyno.1993.1187.
- Mohammed Ali Saad (2015) Molecular Localization of Latent Epstein – Barr Virus Early Repeats (EBERS) in Cervical Tissues with Adenocarcinoma by RNA-In Situ Hybridization. *Iraqi J. Cancer and Med. Gene.* **8** (1), 5-13.
- Young L S and Dawson C W (2014) Epstein-Barr virus and nasopharyngeal carcinoma. *Chinese J. Cancer* **33**(12), 581-590. doi:10.5732/cjc.014.10197.
- Miller W E, Earp H S and Raab-Traub N (1995) The Epstein-Barr virus latent membrane protein 1 induces expression of the epidermal growth factor receptor. *J. Virol.* **69**(7), 4390–4398.