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THE ONCOGENIC EFFECT OF EBV/HPV CO-INFECTION IN A GROUP OF IRAQI WOMEN WITH CERVICAL CARCINOMA

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ABSTRACT : The current paper was designed to find the possible synergic effect of EBV infection with the HPV-16 in Iraqi women suffering from cervical carcinoma. This retrospective study involved paraffinized blocks of two groups. The research included 30 carcinomatous cervical tissues and 15 samples from normal cervical biopsies. After sectioning using positively charged slides, immunohistochemistry (IHC) was performed to detect anti-Epstein Barr Virus LMP1 and Human papillomavirus type 16 primary antibodies. 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 co-infection of the EBV and HPV-16 supports the hypothesis regarding the possible role of the EBV infection to increase the burden of the cervical squamous carcinogenesis.

Key words : Health, biochemical changes, critical carcinoma.

INTRODUCTION

Among different cancers, cervical cancer represents the leading cause of mortality among women globally, and the burden of disease occurs in the developing countries with a percentage reach to 85% of the global deaths (Elmi *et al*, 2017).

The hallmark ZurHausen discovery of the connection between high-risk types of human papillomavirus (especially HPV16) infections and the involvement of the viral oncogenes in the development of cervical malignancy is well established as a definite etiology, which represents the causal of five percent of all human cancers and 70–80% of cervical cancer (Khashman *et al*, 2019). In patients with H-SIL biopsy revealed genotypes 16 and/or 18. The CH2 technique is useful as a screening procedure, while PCR is interesting to identify HPV-HR genotypes (Hachim *et al*, 2020).

After the infection with HPVs, it takes several years to develop cervical cancer which increases the suggestion of the involvement different etiological factors that add synergic effects during the carcinogenesis of cervical cancer (Khenchouche *et al*, 2013; Nichols *et al*, 2011; Ekalaksananan *et al*, 2011) and in this context, the synergism between HPVs and Epstein-Barr virus EBV considered on of the most intriguing research issues (Vranic *et al*, 2018).

EBVis a member of eight known human viruses that belong to theherpesviridae,the viral oncogenicity was first discovered in 1964 through identifying the virus as the causal of Burkitt lymphoma (Mui *et al*, 2017).

The main oncogenic protein of the EBV is the late membrane protein-1 (LMP-1) and besides its ability to transform resting primary B cells, it is also able to transforme epithelial cells and fibroblasts (Kieser and Sterz, 2015).

For all the above, we try in this paper to study the possible effect of viral coinection in the development of cervical carcinoma.

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 µm thickness were used, for routine hematoxylin and eosin staining and the otheron a positively-charged slide for the immunohistochemical procedure using Abcamanti-EBV LMP1, anti-HPV16 and the Mouse and Rabbit Specific HRP/DAB detection kit.

After dewaxing and rehydration, peroxide blockand

protein block were used for blocking endogenous peroxidase and non-specific binding respectively.To remove the fixative effect, heat mediated antigen retrieving was used with citrate buffer pH 6 before commencing with IHC staining protocol. Slides were then incubated sequentially with diluted primary antibody for 1hour at 37°C and then secondary antibody was applied for ten 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, dehydrated and mounted (Khashman *et al*, 2018).

Fisher exact test was used to find the association between EBV and HPV-16 infectionin both cervical and normal groups. P value (<0.05) was considered statistically significant.

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 results of Epstein Barr Virusconfection with Human papillomavirus type 16 in cervical squamous cell carcinoma through detection of EBVLMP1 expression

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

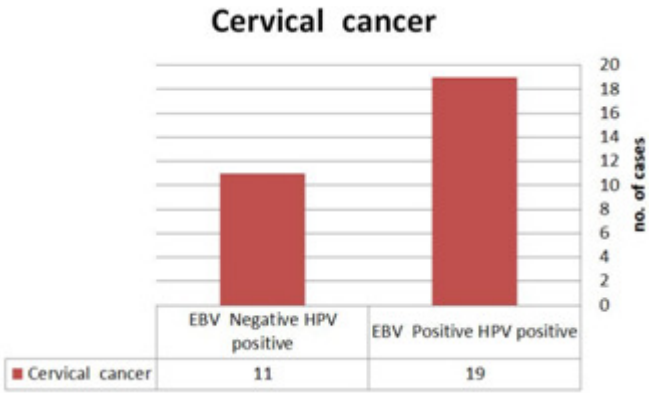

Fig. 2 : The immunohistochemical expression of EBV LMP1 confection in the studied groups. *p= 0.The result is significant at p < 05

showed that 63% (19 out of 30) of the studies group are positive as shown in Fig. 2 with a significant association of the expression with cervical cancer.

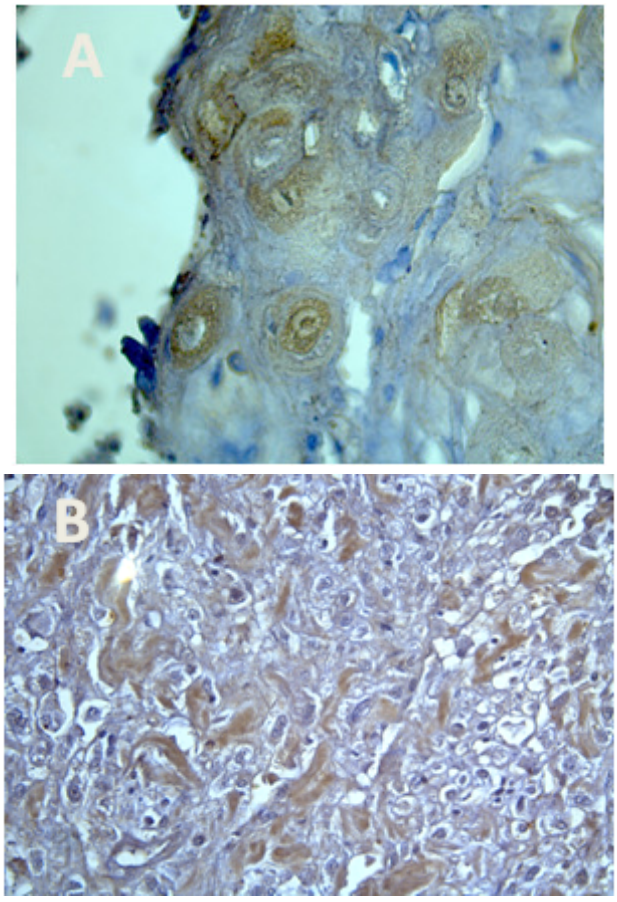

Fig. 3 : Immunohistochemstry Nuclear & Cytoplasmic positive results in cervical cancer tissues for **A**. HPV16 (100x) **B**. EBVLMP1 (40x). The DAB produce (Brown) signal, Harris Hematoxylin (the counter stain) produce (purple) color.

DISCUSSION

Although, HPV live cycle is well studied as a main causative agent of cervical cancer, there is increased interesting about the possibility of synergism of HPVs and EBV confection and its impacts on the progression of cervical oncogenesis and in this regard many researchers observed this synergic effect in the oral squamous cell carcinoma (Al Moustafa *et al*, 2009; Al Moustafa *et al*, 2017; Vranic *et al*, 2018).

In the present study, our findings point out that EBV (LMP1) expression was detected in 63% (19 out of 30) of the HPV infected cervical carcinoma. Our findings of the confection with both HPV and EBV in the this study coincides with the fact that points out that the developing cervical cancer after exposure to the HPV infection takes several years which supports the idea of the ethiopathogical mechanism of both viruses in the progression of cancer (Khenchouche *et al*, 2013).

The correlation between the infection with EBV and the development of the major changes in the cervical epithelia are supported by meta-analysis study done by de Lima *et al* (2018), which indicates synergic effect with HPV infection in the initiation of cervical carcinogenesis.

There are a body of researchers' evidences support the synergism between HPV and Epstein-Barr (EBV) in the carcinogenesis of the cervix and this context,Sixbey et al suggested EBV sexual transmission due to the observation of the viral shedding from the genital tract (Sixbey *et al*, 1986), isolation of viral EBV from cervical pre-malignant and malignant specimens (Bevan *et al*, 1989; Landers *et al*, 1993; Ammatuna *et al*, 2000) and even the detection of EBV during pregnancy refers to the establishment of this virus in the cervical tissue (Hussain and Khashman, 2018).

Interestingly, the most plausible mechanism of EBV to transform cells is through EBV/C3d or CD21 receptor and since this receptor is also found in the cervical epithelium, this make these cells site of the viral shedding and confirming the possibility of its direct involvement in carcinogenesis or synergy with papillomavirus (Young *et al*, 1989; Sixbey *et al*, 1987; Zhang *et al*, 1992; Kahla *et al*, 2012).

CONCLUSION

Alongside with the increased evidences of virological synergic effect that increased the progression of the carcinogenesis, the present study showed that the coaction of human papilloma virus type 16 and Epstein Barr virusin the cervical cancer supports the hypothesis regarding the possible role of the EBV infection to increase the burden of the cervical squamous carcinogenesis.

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