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POSSIBLE ROLE OF HCMV INFECTION ON THE DEVELOPMENT OF HPV POSITIVE CERVICAL CARCINOMA IN A GROUP OF IRAQI WOMEN

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ABSTRACT : The cervical cancer considered as the fourth female prevalent disease worldwide, it was once the most extensively recognized female cancer two in many low-income countries. Human Cytomegalovirus (HCMV) exhibits broader tropism and can cause infection in most of the human body organs. Although, human cytomegalovirus HCMV is not yet considered an oncogenic virus, there is increased evidences of HCMV infection implication in malignant diseases of different cancer types. The present study aims to evaluate the effect of CMV infection on the development of HPV16 positive cervical cancinoma. The current retrospective study enrolled a number of paraffinized cervical cancer tissues .included 30 cervical carcinomatous tissues and 10 biopsies from an apparently normal cervical tissues. Each 4 mm – thick sections of the requested tissue block was stuck on a positive charged slide to be used for an immunohistochemical (IHC) technique for detecting Human papillomavirus (HPV16) and HCMV pp65 using monoclonal primary antibodies against them. The results revealed that there is a significant association in the cervical cancer tissues when compared with normal cervical tissues (p = 0.0003).

Key words : Human papillomavirus, HCMV, pp65, immunohistochemistry, cervical cancer.

INTRODUCTION

The cervical cancer considered as the fourth female prevalent disease worldwide it was once the most extensively recognized female cancer two in many low-income countries (Ferlay *et al*, 2012). Comparing with other cancers; screening for cervical cancer is the most valuable especially for detecting cervical pre-cancerous lesions by using Pap smear technique, which is also used for detection of high-risk types of HPV that are the causative agent of 70% cervical tumors (WHO, 2012; Nwabichie and Rosliza, 2017).

Although, human cytomegalovirus HCMV is not yet considered an oncogenic virus, there is increased evidences of HCMV infection implication in malignant diseases of different cancer types so the concept of "oncomodulation" is the better to define its role in the carcinogenesis, which means that HCMV infects tumor cells and increases their malignancy (Martin *et al*, 2009).

HCMV exhibits broader tropism and can cause infection in most of the human body organs including blood, brain, breast, colon, eye, kidney, liver, and lung (Georges and Kumar, 2014). There is increased attention about the phosphoprotein pp65 (ppUL83) which is an abundant viral tegument components. This protein has an important role in different stages of the viral life cycle (Sabine *et al*, 2010).

The viral pp65 is responsible for modulating/evading the host cell immune response during HCMV infections through counteracting both innate and adaptive immune responses (Kalejta, 2008). The rationale of this study is to evaluate the CMV co-infection with HPV16 on the development of in a group of Iraqi women withcervical carcinoma.

MATERIALS AND METHODS

A forty subjects included in this study were represented by their archival formalin-fixed, paraffin embedded tissue blocks with cervical tissues collected from the archives of Teaching Hospitals in Baghdad and from private laboratories. The sample was divided into two groups; 10 cases from apparently healthy women and thirty cases from patients with cervical carcinoma.

All cervical tissue sections were sectioning at 4 μm and placed on positively-charged slides; one section was stained with hematoxylin and eosin while other use for anti-HPV16 and anti CMVPp65 (Abcam, UK).

Immuno-detection was performed according to manufacture instructions of Cambridge Science Company using EXPOSE Mouse and Rabbit Specific HRP/DAB Detection IHC kit. After de-waxing and rehydration, endogenous peroxidase activity and non-specific binding were blocked by incubation with 3% hydrogen peroxide and protein block, respectively. Heat mediated antigen retrieving was performed with citrate buffer pH 6 before commencing with IHC staining protocol. Slides were then incubated sequentially with diluted primary antibodies for 1hour at 37°C, then secondary antibody was applied for 10 minutes at room temperature followed by incubation with Streptavidine-HRP for 10 minutes at 37°C. Diaminobenzidine hydrochloride (DAB) was used as the chromogen to visualize peroxidase activity. Sections were counter stained with Mayer's hematoxylin for 30 seconds, dehydrated and mounted (Khashman, 2017).

Statistical analysis

Statistical analysis was done using Social Science Statistic (http://www.socscistatistics.com) and Excel application. Fisher exact test was used to find out the relation between HPV16 and Twist2 expression with the cervical cancer and normal groups. P value (<0.05) was considered statistically significant.

RESULTS

The mean age of the patients with cervical cancer was 54 years (Fig. 1) in which the patients with age above 50 years comprises 57%, while those with age less than 50 years represents 43% of the studied sample.

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

Table 1 : The immunohistochemical expression of CMVpp65 in the studied groups.

IHC expression	Cervical cancer	Apparently healthy cervical tissues	Total	P-value
CMV pp65 Positive	**23**	**1**	**24**	**0.0003***
CMV pp65 Negative	**7**	**9**	**16**	
Total	**30**	**10**	**40**	

*The result is significant at p<0.05

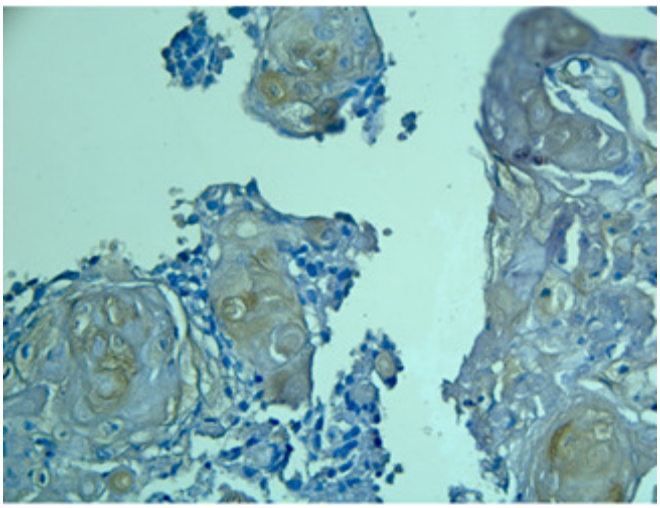

Fig. 2 : Immunohistochemstry expression of HPV16 in Cervical cancer tissues. The DAB produced (Brown) signals while the counter staining by Harris Hematoxylin produced (purple)

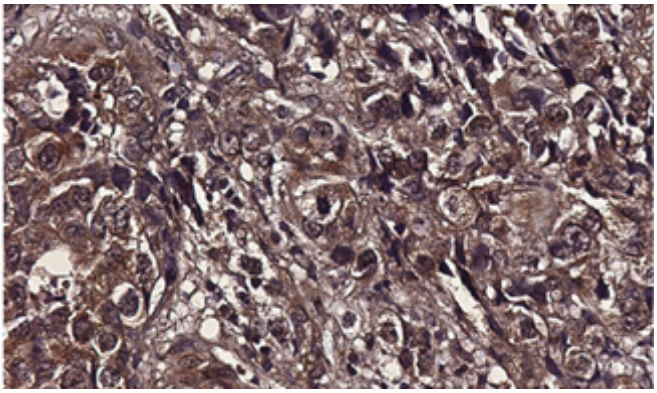

Fig. 3 : Positive immunohistochemical expression of Human Cytomegalovirus pp65 in Cervical cancer. The DAB produced (Brown) signals while the counter staining by Harris Hematoxylin produced (purple).

The current study HPV16 positive cervical cancer tissues where chosen to assess the HCMV infection in the studied groups using anti CMVpp65. The result revealed that there is a significant association in the cervical cancer tissues when compared with normal cervical tissues in which 23 out of 30 cases showed immunohistochemical positive reaction, while only one case of apparently healthy group showed positive result as shown in Table 1 and Figs. 2 and 3.

DISCUSSION

There was a lot of attention has been focused to find conclusive findings about role of HCMV as a potential causative agent in the development of cervical cancer (Thompson *et al*, 1994).

Although, there was a limitation in CMV detection in cytological samples of cervical smears (Elgert *et al*, 2018; Huang and Naylor, 1993; Gideon and Zaharopoulos, 1991).

It has reported since 1960s that Cytomegalovirus was able to produce oncogenic proteins and interact the cell cycle to induce cellular transformation and these findings increase the possibility of its involvement in the development of different cancers including the cervical cancer (Marinho *et al*, 2013; Marinho and Sousa, 2013).

There is increasing evidences of the involvement of HCMV in the development of cervical cancer (Raju, 2015). In Iraq, different Iraqi researchers confirmed the involvement of HCMV in the development of different types of tumors like colorectal adenocarcinoma, Glioma, OSCC and Breast carcinoma (Lazim and Kadhim, 2018).

In this context ,in the present study 76.6% (23 out of 30) showed positive expression for CMV P65 protein (table 1) which is the most abundant tegument protein and the major constituent of extracellular virus particles (Tomtishen, 2012) (Fig. 3).

This high percentage may be due to the false positive reaction immunereaction. To rule out this possibility of nonspecific detection in tumor cells, we performed IHC on negative control using cervical tissues and omitting the primary antibody which showed negative reaction with CMV pp65 antigen. These findings coincides with many researchers who found that despite the HPV was confirmed as the main cause of cervical cancer and they concluded that its rational to consider the human herpesviruses (HHVs) on of the causative agents of initiating HPV carcinogenesis cervical carcinoma (zur Hausen *et al*, 1984; Salcedo *et al*, 2008; Smith *et al*, 2002).

The ability of the virus to transform cells *in vitro* give rise to its ability to its possible ability to induce cancer *in vivo* (Alsamarai, 2018).

One of the explication of the present results that the persistent infection with the CMV results in chronic cervicitis and predispose the acquisition of HPV due to the immunosuppression in cervix which mediates HPV carcinogenesis while the other explication based on the synchronous infection which leads to the transactivation of the HPV genes due to the immediate and early gene products of CMV (Chan *et al*, 2001; Grce *et al*, 2004; Marinho and Sousa, 2013).

In conclusion, the result of the present study suggest the possible role of the CMV in the pathogenesis of the cervical cancer which suggests additional investigation for the CMV infection in parallel with the screening of HPV infection in the cervical carcinoma. Additional studies with a large samples are required to find exact role of the virus and pathogenesis.

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