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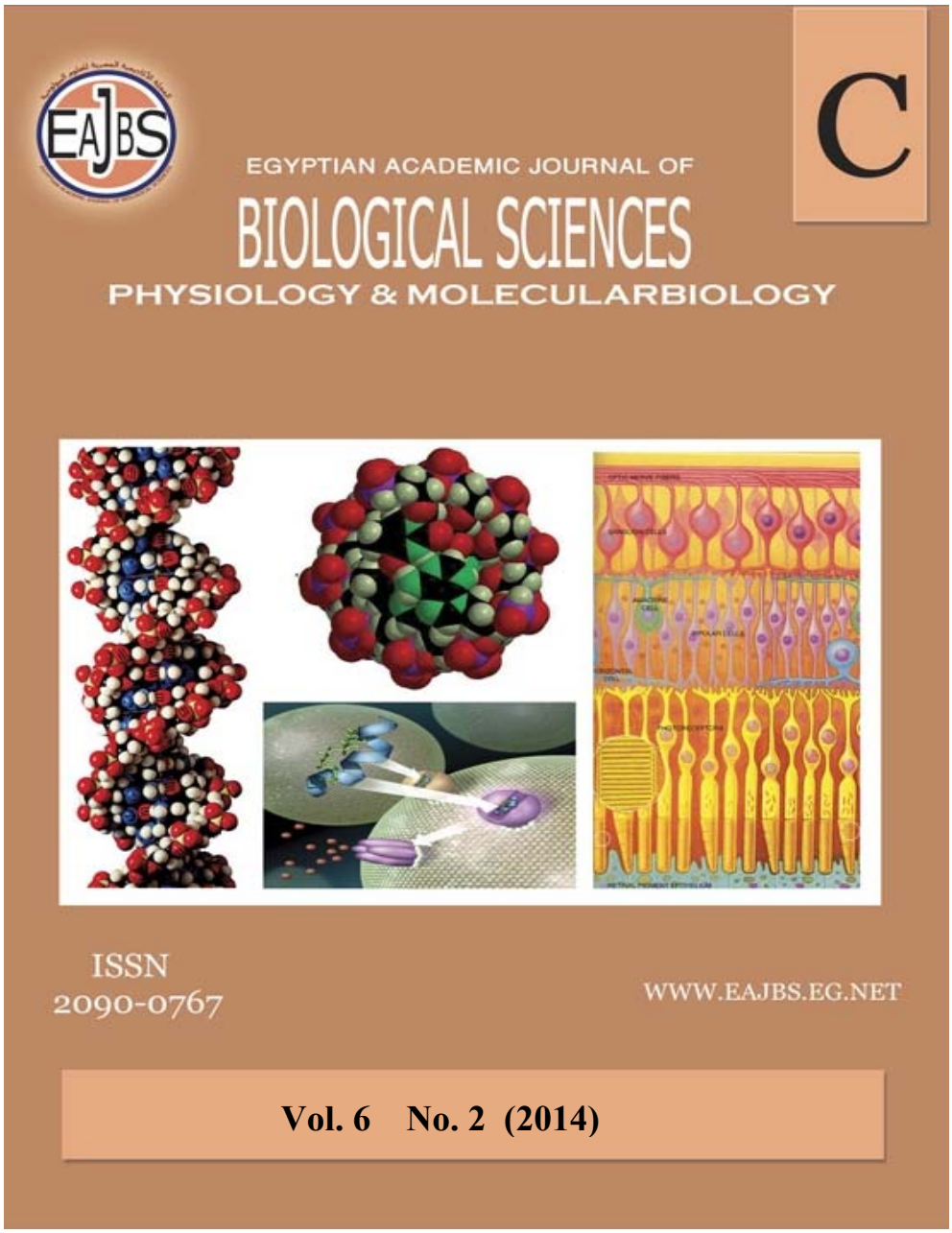

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Relationship between Epithelial MembraneProtein2 expression and Epstein Barr Virus, Cytomegalovirus and Herpes Simplex Virus infections in Nasopharyngeal Carcinoma.

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ABSTRACT

Background: Lack of expression of Epithelial Membrane Protein-2(EMP2) in Nasopharyngeal carcinoma (NPC) is associated with adverse prognosticators and might confer tumor aggressiveness through hampering its interaction with specific membrane protein; and as Human Herpes Viruses linked to etiology of NPC, the aim of this study was to find out the relationship between EME2and these viruses.

Methodology: In this study patients with NPC were investigated retrospectively. EMP2 expression was demonstrated by immunohistochemistry using n EMP2 antibody. EBV, CMV and HSV were identified by polymerase chain Reaction (PCR).

Results: loss of EMP2 (negative) was identified in 10/92 (10.9%), P < 0.04; 10/53 (18.9%), P <0.05 and 4/18(22.2%), P<0.001 of EBV, CMV and HSV, respectively.

Conclusion: In NPC, there is significant correlation between loss of expression of EMP2 and human herpes virtues (EBV, CMV and HSV).

INTRODUCTION

Nasopharyngeal carcinoma (NPC) is a distinctive head and neck cancer, which is commonly among Chinese and is more frequently associated with the Epstein Barr virus (EBV) (Wei, *et al.*2010). NPC was previously called lymphoepithelioma, as the malignant epithelial cells of the nasopharynx frequently intermingled with lymphoid cells in the nasopharynx, giving rise to the term lymphoepithelioma (Godtfredsen 1944). Latter on studies however have determined that these tumor cells are of squamous origin and the undifferentiated carcinoma is a form of squamous cell carcinoma with minimal differentiation (Prasad 1974). The prognosis of patients with NPC depends on the stage of the disease at diagnosis. Unfortunately, at diagnosis, 70% of patients have locally advanced, nonmetastatic stage III or IV disease (Mackie, *et al.* 2000; Afqir, *et al.* 2009).

EBV infection is strictly linked to undifferentiated nasopharyngeal carcinoma (UNPC), strongly implicating a role for EBV in NPC pathogenesis. EBV infection in epithelial cells could be achieved via the interaction of glycoproteins on the viral envelope with surface integrin on epithelial cells, which might trigger membrane fusion to internalize EBV into cells. EBV infection is rarely detected in normal nasopharyngeal epithelial tissues (Tsang, *et al.* 2014).

Some studies have indicated that EBV, and HCMV may be present in certain NPC cell lines and in only a small fraction of each positive cell line, and that a cell line may contain these viruses concurrently. EBV is present not only in a fraction of tumor cells, but also in some lymphocytes and glandular epithelia in biopsy specimens. It is present as an episomal form but not as an integrated form in the infected cells, suggesting that the carcinogenesis of non-EBV containing NPC tumor cells is not related to EBV infection (Lin,*et al.* 1994).

Human epithelial membrane protein-2 gene (*EMP2*), mapped to chromosome 16, is well-maintained across vertebrates (Wang,*et al*. 2001). EMP2 was detected as a novel member within four-transmembrane (tetraspan) superfamily (Berditchevskiand Odintsova 1999). Practically, the best known tetraspan proteins are connexins, which form the major structural element of gap junctions. Connexins play vital parts in the regulation of cell growth and differentiation. Cancer cells generally have down regulated levels of gap junctions. Moreover, numerous lines of evidence suggest that loss of gap junctional intercellular communication is an important step in carcinogenesis. Re-expression of connexins in cancer cells causes normalization of cell growth control and reduced tumor growth (Kandouzand Batist 2010).

Therefore, our aim in this study was to assess the association between loss of EMP2 expression and the presence of herpes viruses (EBV, CMV and HSV), particularly, the overexpression of latent membrane protein 1, an important oncoprotein of EBV.

MATERIALS AND METHODS

In this study 112 tissue blocks that were previously diagnosed as having NPC and their related data were retrieved form Histopathology Laboratories in Khartoum State, Sudan. Three micron tissue sections were obtained from each sample and subsequently immune-stained using EMP2 antibodies adopting ^{En}vision method. Also small tissue section was obtained for DNA extraction and subsequently screened for the presence of viruses (EBV or CMV or HSV) using conventional PCR.

Data analysis: Data management was done using Statistical Package for Social Sciences (SPSS version 16). SPSS was used for analysis and to perform Pearson Chi-square test for statistical significance (P value). The 95% confidence level and confidence intervals were used and $P < 0.05$ was considered statistically significant.

Ethical Consent: The study was approved by Faculty Research Board, Faculty of Medical Laboratory Science, Sudan University for Science and Technology. This in addition to the fact that, the authors followed the tenants of the Declaration of Helsinki.

RESULTS

In this study tissue specimens from patients with NPC were investigated for immune-expression of EMP2 and molecular identification of herpes viruses (EBV=92, CMV=53 and HSV=18) correlations. Of the 92 specimens with EBV infection, loss of EMP2 (negative) was identified in 10/92 (10.9%) and the remaining 82/92(89.1%) were positive. Furthermore, 20 EMP2 negative were found EBV negative, accordingly, the 95% confidence level and Odd Ratio (OR) is 2.2439(0.9929 to 5.0713), $P< 0.05$.Of the 53 positive CMV loss of EMP2 (negative) was identified in 10/53 (18.9%) and the remaining 43/53(81.1%) were positive. Furthermore, 20 EMP2

negative were found CMV negative, consequently, the 95% confidence level and Odd Ratio (OR) is 2.4651(1.0441 to 5.8203), P< 0.04).

Of the 18 positive HSV loss of EMP2 (negative) was identified in 4/18 (22.2%) and the remaining 14/18(77.8%) were positive. Furthermore, 26 EMP2 negative were found HSV negative, consequently, the 95% confidence level and Odd Ratio (OR) is 8.3571(2.3627 to 29.5604), P< 0.001), as indicated in Table1, and Fig.1.

Table1. Distribution of the studied samples by EMP2 and Herpes Viruses (EBV, CMV and HSV).

Virus	EMP2		Total
	Positive	Negative	
EBV			
Positive	82	10	92
Negative	0	20	20
CMV			
Positive	43	10	53
Negative	0	20	20
HSV			
Positive	14	4	18
Negative	0	26	26

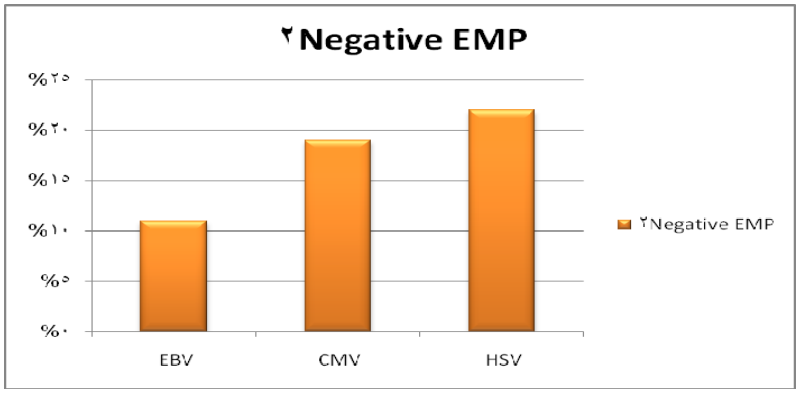

Fig.1: Description of loss of EMP2 by human herpes viruses.

DISCUSSION

NPC is the most common malignancy originating from the nasopharynx and its etiology is closely associated with viral infection particularly EBV. Management of NPC remains one of the prevalent clinical challenges. There have been breakthroughs in early detection, diagnosis, multi-modality treatment, and also disease monitoring for NPC. Therefore, in the present study, we tried to investigate some parts of the disease that link the etiology to biological interactions through disease pathogenesis.

In the current study, all three herpes viruses were significantly correlated with loss of EMP2. With the beginning of molecular targeted therapy and personalized medicine, novel therapies based on molecular targets of NPC have become the focus of research and progress over the last decade. What's more, as NPC is closely associated with the EBV infection, the role of tumor-associated viral antigens in NPC renders it an interesting nominee for cellular immunotherapy (Tsang,*et al.* 2014). The close association of EBV infection with NPC suggests that EBV infection is a crucial in the occurrence of this cancer. The difficulties encountered in transforming primary epithelial cells in experimental systems suggest that the role of EBV in epithelial malignancies is complex and multi-factorial in nature. Genetic alterations in the premalignant epithelium may support the establishment of latent EBV infection, which is believed to be an initiation event. Oncogenic properties have been reported in multiple EBV latent genes. However,

improved proliferation may not be the crucial role of EBV infection in epithelial malignancies. Genetic alterations in host cells as well as inflammatory stroma could modulate expression of EBV gene expression and alter the growth properties of infected premalignant epithelial cells encouraging their selection during carcinogenesis (Tsao, *et al*. 2014).

For the other herpes viruses (CMV and HSV), there is a lack in the literature, regarding their relation to NPC. Human cytomegalovirus (HCMV), a widely-spread β-herpesvirus, is a major cause of birth defects and opportunistic infections in HIV-1/AIDS patients. HCMV displays an intricate system-wide modulation of the human cell proteome. An impressive array of virus-host protein interactions occurs throughout the infection (Jean, *et al.* 2014). There are two types of herpes simplex virus, type 1 and type 2 (HSV-1 and HSV-2). HSV-2 is a common human pathogenic virus and is associated with sexually transmitted diseases. Acute HSV-1 infection generally involves gingiva stomatitis (Shen, *et al.* 2006). Several independent studies suggest that HSV-2 infections correlate with a higher than normal incidence of cervical cancer (Jones 1995).

Human epithelial membrane protein-2 gene (*EMP2*), mapped to chromosome 16, is highly conserved across vertebrates (Liehr, *et al*. 1999). High EMP2 expression was detected in ovarian cancer through triggering of caveolins/glycosylphosphatidyl inositol-linked proteins (Wadehra, *et al.* 2004)and was recognized as an early predictor of endometrial cancers with unfavorable consequence (Wadehra, *et al*. 2006).Loss of EMP2 expression confers an independent prognosticator in nasopharyngeal carcinoma, which might confer tumor aggressiveness through hindering its interaction with specific membrane protein(s) and hence the downstream signal transduction pathway(s) (Chen, *et al.* 2012).EBV oncoproteins may contribute to the highly metastatic phenotype of NPC (Raab-Traub2002), so clarifying their underlying mechanisms should shed light on therapeutic strategies to block malignant progression of this cancer. Latent membrane protein 2A (LMP2A) is an EBV oncoprotein generally expressed in NPC and other EBV-associated cancers (Knipe, *et al.* 2007). LMP2A is detected in around half of NPC tumor specimens at the protein level and in more than 95% of the tumors at the mRNA level (Heussinger, *et al*. 2004).

However, and to the best of our knowledge, no study has investigated the exact interaction(s) between EMP2 and Herpes viruses. So, this study is a stimulation for further research in this area.

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ARABIC SUMMARY

العلاقة بين وجود البروتين٢ الغشائي الطلائي وفيروسات (EBV, CMV, HSV) في سرطان البلعوم

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الخلفية: نقص الكاشف المناعي (بروتين الاغشية الطلائية) في سرطان الانف البلعومي له علاقة بالتنبؤ بالمرض من حيث وجوده او عدمه .واحتمالية منح المرض صفة العدوانية من خلال اعاقة تفاعله مع اغشية بروتينيه معينه وذلك كما يحدث في علاقة فيروسات الهربس بكونها مسببة لسرطان الانف البلعومي.الهدف في هذة الدراسة كان لايجاد العلاقة بين بروتين الاغشية الطلائية وهذة الفيروسات .

طريقة البحث :في هذا البحث كانت العينات مأخوذة بأثر رجعي للمرضى المصابين بسرطان الانف البلعومي وتم التعرف على كاشف بروتين الاغشية المناعية بواسطة كيمياء الانسجة المناعية لمستضدات بروتين الاغشية المناعية. فيروس الورم الحليمي ،والفيروس المضخم للخلايا، وفيروس الهيربس يتم اكتشافها بتفاعل البلمرة المتسلسل.

النتائج :تم تحديد النتيجة السلبية لبروتين الاغشيه الطلائيه كالتالي 10/92 (10.9%), P < 0.04; 10/53 (18.9%), P <0.05 and 4/18(22.2%), P<0.001 على التوالي للفيروسات التاليه ،فيروس الورم الحليمي ،فيروس مضخمات الغدد وفيروس الهربس.

الخلاصه : هناك علاقة وثيقه بين سرطان الانف البلعومي ونقص بروتين الاغشيه الطلائية وفيروسات الهربس (فيروس الورم الحليمي ،فيروس مضخمات الغدد وفيروس الهربس).