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The Influence of Togetherness of Herpetic Virus infections on Cell Cycle Regulatory Gene Expressions in Sinonasal Malignancy

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ABSTRACT

The Epstein-Barr virus (EBV) is categorized as a human gamma-1 herpes-virus. The EBV infection can increase the risk of developing certain rare cancers such as sinonasal carcinomas (SNC). That due to the powerful ability of EBV to cause mutations in the cells and make cancerous changes in the tissues. The human cytomegalovirus (HCMV), mainly in tumor tissues, can target different types of cells. In addition, it can cause all hallmarks of cancerous changes in the tissues which is considered as a defining feature of these pathogens. The HCMV strictly exhibits both of cellular tumor-promoting and immune-evasive strategies as well. However, the p27(Kip1) protein, might contribute in many different signal transduction pathways and modulate the intermediate oncogenic roles in cancer. The p27(Kip1) protein combines with cyclin E-CDK2, cyclin A-CDK2, and cyclin D1-CDK4 forming complexes, resulting in preventing their activation. Mitogenic signals that cause reduction of p27 expression allow activation and progression of CDK/cyclin in the cell cycle. The p73 is a protein related to the p53 tumor protein; it considered as tumor suppressor that is significantly involved in the regulation of cell cycle and induction of apoptosis. This study aim at investigating the effect of both the HCMV and EBV infections on the gene expression of both p 27 and p73 in sinonasal carcinomas (SNC). The study involved eighteen (18) formalin-fixed, paraffin-embedded tissue blocks of sinonasal carcinomas (SNC). The verificatory re-examination for histopathological samples was done. The EBV-EBERs and HCMV-PP65 DNA localization detected by a Chromogenic In Situ Hybridization technique (CISH), while p27 and p73 gene product detected by immunohistochemistry technique (IHC). From the recent data, it is clear that the existence of those two viruses in SNC were at the highest range in poor different grades (45.5%). On the contrary, the co-expression rate of p27 and p73 genes were at elevated in poorly differentiated SNC tissues (73.5%).

Keywords: SNC; EBV-EBERS; HCMV-PP65; CISH; IHC.

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INTRODUCTION

Sinonasal carcinoma (SNC) possibly arise from different tissues within the nasal cavity [1,2]. Previous study showed that the percentage of death of EBV-attributable malignancies is increased by 13 % over a period of 20 years[2]. There are many etiological factors for it include, Epstein-Barr virus (EBV), genetic susceptibility and consumption of food with possible carcinogens–volatile nitrosamines [3,4].

Human cytomegalovirus belongs to the family of *Herpesviridae*, is a unique double-stranded DNA genome with 236 kbp in size and enclosed by capsomers. Recently, the role of herpesviridae in pathogenesis of cancer with a very restricted host range has been investigated. The (HCMV) could favor the progression and the spread of the tumor, and on comodulation [5]. The p27 usually stop of cell division via blocking the cells from entering the DNA replication-phase; (it is found in cells and tissues throughout the body) [6]. Within cells, p27 is located primarily in the nucleus, it plays a serious role in controlling cell growth and division[7]. When p27 is (sequestered) in the cytoplasm instead of being transported into the nucleus, the protein is unavailable to block cell cycle progression [8].

The tumor suppressor gene p73 is contribute in the apoptosis in response to DNA damage. It have many isoforms some of it containing the (pro-apoptotic) domain, while the others lacking that domain therefore it have anti-apoptotic function. Similarly to p53, following DNA damage, p73 is stabilized and activated in attempt to DNA repair and, if needed, eventually apoptosis through controlling on the expression of target genes that are involved in the regulation of cycle arrest and apoptosis [9] . The p73 gene is frequently mutated in diverse cell lines of human cancer. The deletion or silencing of gene p73 in pancreatic carcinomas, nasopharyngeal carcinoma, neuroblastoma, breast and hepatocellular carcinomas suggesting its potential role as tumor suppressor gene [10].

MATERIALS AND METHODS

Experimental Design

This study includes eighteen selected formalin-fixed, paraffin-embedded blocks from sinonasal tissues. They were collected from archives of histopathological laboratories of several hospitals in Baghdad (Ghazi Al-

Hariri Teaching Hospital, Al-kindy Teaching Hospital, Al-Yarmok Teaching Hospital). Some of these tissues block were associated with the prior 3 years 2017, 2018 and 2019. The diagnoses were based on their accompanied pathological reports of the corresponding patients. The age of these individuals patients were ranged between (42-80) years (7 males and 11 females).

Preparation of Tissue and Data collection

Histopathological sections stained by hematoxylin and eosin (H&E) staining for final definitive diagnosis.

The detection of EBV and HCMV by Chromogenic *in situ* hybridization (CISH) reactions kit purchased from ZytoVision GmbH. (Germany; Cat. Numbers:T-1061-40) was performed on 5µm paraffin embedded tissue sections by using (digoxigenin-labeled oligo-nucleotides probe) that targeting EBV-EBERs and HCMV-pp65 DNA (Cat. Numbers: T-1114-400 and T-1113-400, respectively).

The CISH reactions method for viral detection performed Under the production company's guidance. Immunohistochemical (IHC) tests were used to evaluate p73 and p72 expressions in sinonasal tissues by using Monoclonal Rabbit Anti-P73 and Anti-p72 antibodies (Cat. Number: ab40658 and ab32034) and (rabbit specific HRP/DAB detection IHC Kit, Abcam /England) that targeted nuclear specific proteins. The detection of CISH and IHC signals were assessed under light microscopy at (100X). Scoring and intensity of signals were done according to [11].

STATISTICAL ANALYSIS

In this study, (model-21) for statistical analysis, the SPSS software was used, where the Chi-Square test ($\chi 2$) was used to estimate the significances between variables.

RESULTS

Co-infection of CMV and EBV with Malignant Tumors (SNC):

The SNC grading association with co-infection of EBV and HCMV is clarified in Table (1). The findings of the study have shown that the existence of these two viruses in SNC was at the highest range in poorly layer at grade (45.5%) followed by moderate grade (36.4%) and last by undifferentiated grade (18.2%). On the other hand, among moderate differentiated group of SNC tissues, no positive blue nuclear signals for CMV-DNA alone was revealed, whereas among poor group (100%) revealed positive-HCMV results. Moreover, among moderate class of variance of SNC cases (80%) positive blue nuclear signs detect for EBV-EBERS, whereas among undifferentiated group (20%) revealed positive-EBV results alone.

Through this analysis, we can see that EBV co-infection and CMV was listed (63.7%) in sinonasal (SN) malignant tissues with advance stages (Table 1). Regarding carcinogenesis, In tissue cancers/oncogenesis, viral agents can play a role at an early or late stage or continually from early to late in such a process. In this study, the percentage of HCMV-related SNC regularly are high in the late stages of the disease consist of (100%). However, these findings revealed that the virus played a part in late sinonasal carcinogenesis multistep events. Moreover, and as a referring, Ali et al. noted that CMV may a late role will play in cervical adenocarcinoma. There is no doubt, these results might be related to the small size of sample enrolled and to singular variability elements related to the singular diversity in the visible verdict of pathologists that could preclude evident conclusions about the exact function of CMV in sinonasal cancer.

Moreover, HCMV infection may have been linked to the further development of SNC carcinomas and may have affected the oncogenesis in combination with co-factors in the viral infection.

It can be an inference that the high levels of HCMV in their evident correlation with the classify of SNC could refer to a molecular role for HCMV in the etiology of these cancers which could perhaps occur late, along with other significant oncogenic viruses.

More detailed researches and the use of broad aggregate numbers to discover their exact role in the oncogenesis of the SNC end.

Regarding EBV in SNC, the findings suggested that this virus played an early role in events and continued (although to a lesser effect) in the late events in SNC carcinogenesis in collaboration with Several other molecular variables were identified due to the highest percentage (80 percent) of the virus in moderately differentiated SNC.

The limited sample size scored in the examined groups has weakened the statistical capacity of this analysis to determine the influence of certain variables under consideration. Furthermore, the lack of accurate clinical evidence for these cancer patients (Where this study included only tissue samples) has also deprived the present study from reaching to a solid impression for the real role of those mixed viral infections in sinonasal carcinogenesis. These limitations have raised a suggestion to compel an integrated team-work study, at molecular and virological levels to elucidate the role of these factors in SNC. Moreover, the present study shows other agents play crucial role in SN carcinogenesis in the vast majority of populations in different countries.

The high incidence of EBV infections and their apparent association with SNC differentiation may point to the role of EBV in these cancers as a molecular attack that probably occurred early or late (respectively) with other important oncogenic viruses.

Nevertheless, the authors of the research feel that more studies are needed to explain the role of EBV in SNC and the pathogenesis of EBV in order to better understand the relation of EBV infection to SNC initiation and progression in clinical trials in the field of vaccine.

In regards to EBV and CMV with grading in research, the grade of SNC co-infected with CMV and EBV, the viruses constituted (63.7%) in the advanced stages of differentiation, that means these viruses collaborated with each other to play a vital role in late events of SNC carcinogenesis in association with other genetic or environmental factors assisted in this process.

p27 and p73 Gene Coexistence Products in Malignant Tumors:

Table (2) shows the presence of p27 and/or p73 and their co-presence or co-absence in SNC tissues. In regards to SNC, the highest co-presence of p27 and p73 was in a poorly differentiated group (41.7%) followed by moderately differentiated tissues (33.3%) and finally undifferentiated tissues (25%). while, p73 expressions alone in moderate and poor groups were (80% and 20%), respectively.

The present investigation shows that the p27 protein not expressed alone in all Sino nasal carcinoma tissues.

However, according to the results of grading in SNC groups there are non-significant differences.

Regarding the coexistence of P73 and P27, the SNC tumorigenesis plays a late role as they accounted for (66,7%) at later stages of this cancer..

On the other hand, positive-p73 marker in association with SNC grades are pointing to the effect of p73 which was mainly as an early event and continued (although to a lesser effect) as a late event in Sino nasal carcinogenesis along with the role of other viruses as well as many other factors in this process. This analysis is supported by the findings of another corresponding study by Ali et al., (2017) who found the grade of BRCA1-related breast cancers consistently elevated with grade 3.

DISCUSSION

Little information is available regarding the expression of thes regulatory molecules in sinonasal carcinoma in relation tumor grading. A large body of evidence have showed that th strong expression of p27 in malignant neoplasms [12,13]. addition, the present study has documented that there a accumulation of p27 in malignant tumor, which are consiste with the data obtained from numerous studies c hepatocellular carcinoma and squamous cell carcinoma [14,] The elevation in the levels of these proteins may a synergistically with the activities of viral oncoproteins, name pp65 and EBERs.

The authors have demonstrated that the alteration of p2 expression correlates with the increase of cell proliferation sinonasal tumors. Although the results of present study hav reported overexpression of p27KIP1 in sinonasal malignar tumor tissues, it is haplo-insufficient tumor suppress (unusual) as suggested by previous study [16]. Generally, cancer, p27 is often inactivated via mislocalization, whic makes cancer cell undergo rapid division and uncontrolle proliferation. The p27 may be inactivated upon binding to th viruses where it interacts physically with p27 which preven its association with cyclin A/E-Cdk2 complexes (which revealed to contribute to the increase of cell proliferation ar tumor formation) [17].

Another possibility for that is the effect of EBV and HCMV sequestration of this protein (i.e., p27 protein) , that ma prevent its role in inhibiting the cell cycle, although the positiv percentage of these viruses is very high in both benign ar carcinoma groups[18]. Also the oncogenesis process may excee the repairing pathways. Herein the defective p27 protien mig be due to its mutation and/or haploinsufficiency resulting not accomplish the reformative role responsible for it. Th resultant repairing processes were insufficient to reverse th oncogenic role of such viruses besides the other oncogen factors. The p73 functional homology of p53, Yamashita *et a* (2015) and AL-Ddoory, (2017) found high percentages of p5 expression in SNC which (62.5%) [19,20]. The present resul detect mutant forms and wild type of human p73 since th monoclonal antibody used in the p73 detection has the ability react with both types. The findings of the present study refle increase of mutant p73 expression, and decrease copies of wi type p73 in malignant cells as a part of the malignant process.

Mutation of both p73 alleles often results in overexpression nonfunctional forms of p73 in an attempt to compensate for th loss of the function of p73 [21]. However, it is well accepted no that the loss of heterozygosity of the wild type p73 is ver important for the defective p73 activity and this could b through deletion of wild type p73 or replaced by a mutate or [22,23]. Herein, we recommended to study the effect of p7 isoforms to elucidate their ratios as well as their effect on SN and NPC tumor progression. Regarding oncogenic vir transformation or oncogenesis, the impact of EBV on p73 has been poorly investigated, although initial findings indicated that, the similarly of p53 and p73 could be targeted by EBV. Which leads to developing multiple mechanisms that may alter its function. In addition, the variability in the expression pattern of the latent genes in EBV-positive cancer cells has a powerful ability to target the p53/p73 pathway more than the ability of one viral protein [23,24].

It was found that protein product of transformed genes (i.e. ,oncogenes) of many tumor viruses have the ability to abrogate the function of tumor suppressor gene proteins and there is no need for mutation in tumor suppressor genes where their products are normal but not to let function. Herein, we recommended a large scale study on different viruses in this research such as human papilloma virus (HPV), Kaposi sarcoma (KSHV) and hepatitis-C virus (HCV).

CONCLUSION

In this research we can infer that sinonasal carcinogenesis may involve co-infections of EBV and HCMV. According to this evidence, the hyper-expansion of both p27 and p73 that are most clearly seen in SNC (infected malignant tissue) might restrict the prominent regulatory functions of those proteins which is classically seen in virus–host interaction.

CONFLICTS OF INTEREST

The authors declare that there is no conflicts of interest.

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Tables:

Table 1: Co-association of CMV and EBV infections with the differentiation of SNC.

Studied groups	Diagnosis		Viral infections				Pearson Chi-Square (P-value)
			Negative	HCMV	EBV	HCMV & EBV	
Sinonasal carcinoma (SNC)	Moderately differentiated	N	0	0	4	4	P=0.336 Non Sign. (P>0.05)
		%	0%	0%	80%	36.4%	
	Poorly differentiated	N	1	1	0	5	
		%	100%	100%	0%	45.5%	
	Undifferentiated	N	0	0	1	2	
		%	0%	0%	20%	18.2%	
	Total	N	1	1	5	11	
		%	100%	100%	100%	100%	

Table 2: The p27 and p73 Co-presence markers in malignant tumors.

Studied groups	Diagnosis		P27 & P73				Pearson Chi-Square (P-value)
			Negative	P27	P73	P27 & P73	
Sinonasal carcinoma (SNC)	Moderately differentiated	N	0		4	4	P=0.273 Non Sign. (P>0.05)
		%	0%		80%	33.3%	
	Poorly differentiated	N	1		1	5	
		%	100%		20%	41.7%	
	Undifferentiated	N	0		0	3	
		%	0%		0%	25%	
	Total	N	1		5	12	
		%	100%		100%	100%	

Figures:

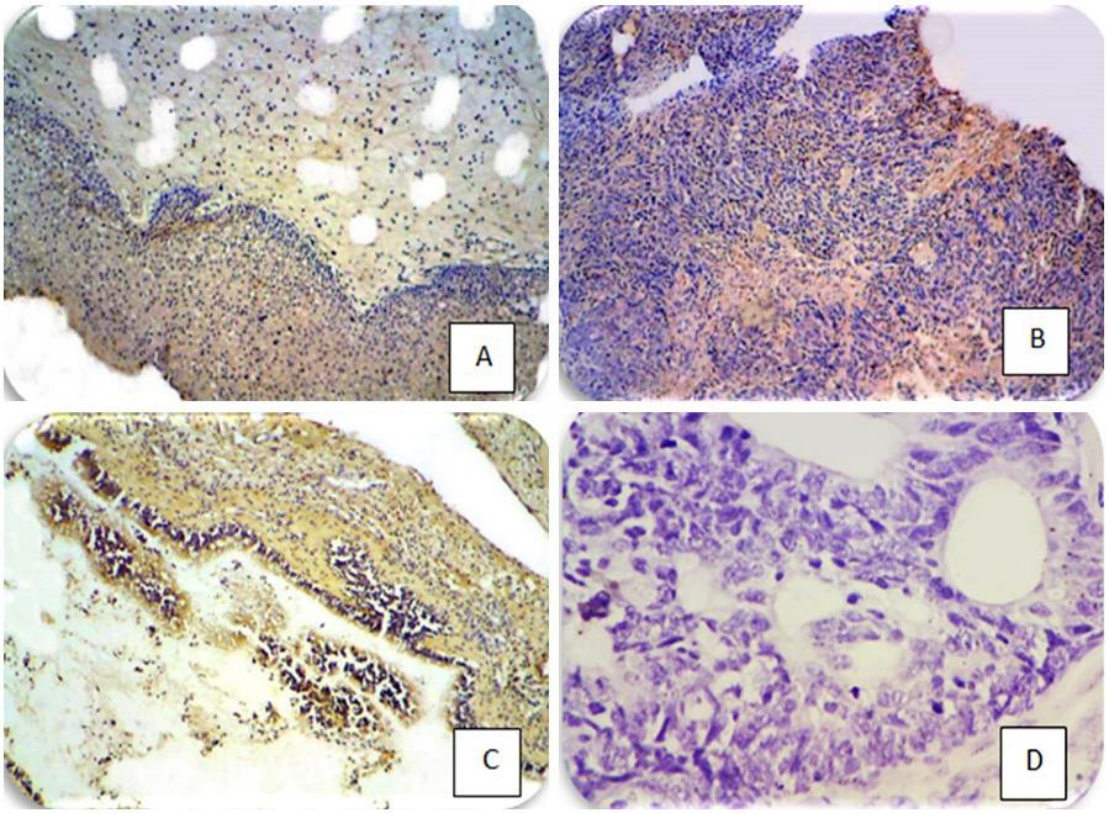

Fig. 1: IHC-p27 positive signals in Micrograph showed as a brown discoloration. A: Sino nasal tissue infected with HCMV. B: Sino nasal tissue infected with EBV. C: Sino nasal tissue co-infected with HCMV& EBV. D: Sino nasal with negative staining.

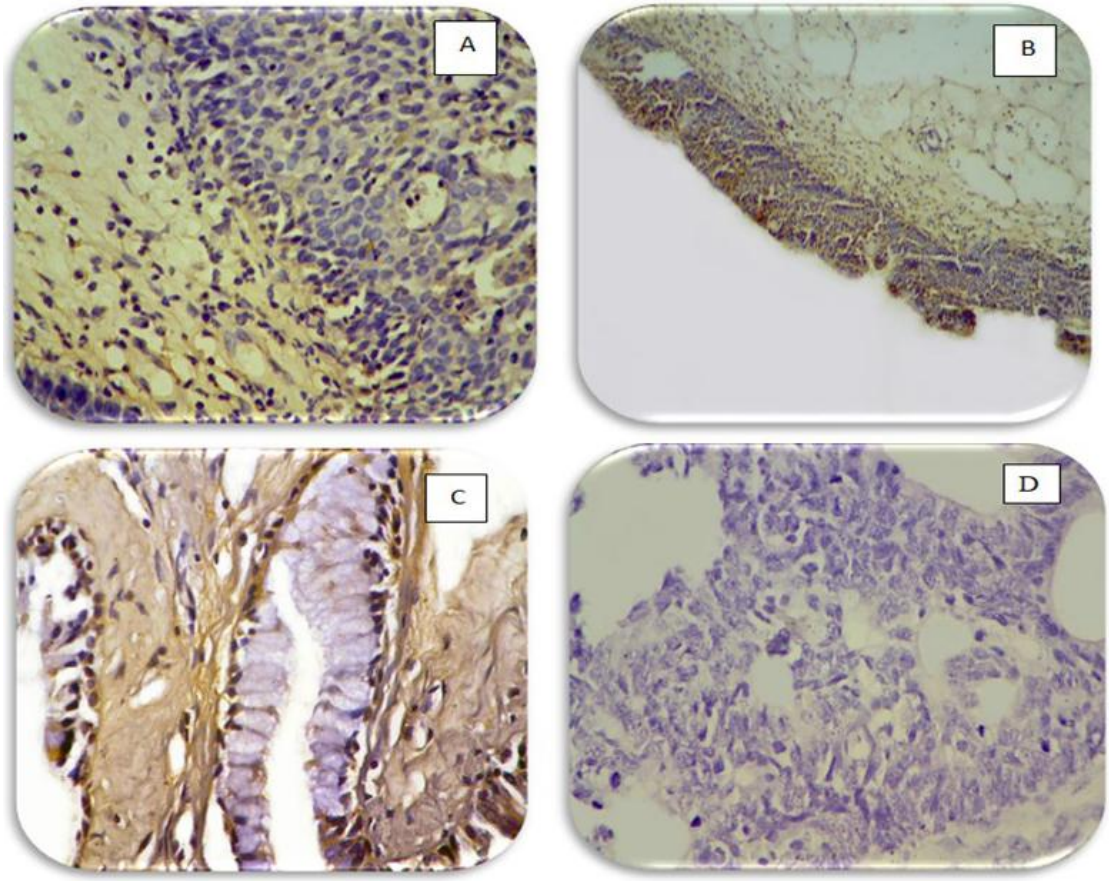

Fig. 2: Micrograph of IHC-p73 positive signals appeared as a brown discoloration. A: Sinonasal tissue infected with HCMV. B: Sinonasal tissue infected with EBV. C: Sinonasal tissue co-infected with HCMV&EBV. D: Sinonasal with negative staining.