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Relationship between Cytokeratins expression and Human Herpes Viruses (EBV, CMV, HSV) infections in Nasopharyngeal Carcinoma

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Abstract: ***Background:*** *Cytokeratins(CK) are valuable for management of carcinomas, since they can be expression even in advanced stages of the disease; and as Epstein Barr Virus (EBV), Cytomegalovirus (CMV) and Herpes Simplex Virus (HSV) have been linked to etiology of Nasopharyngeal Carcinoma (NPC), the aim of this study was to find out the relationship between CK and these viruses.* ***Methodology:*** *In this study 150 patients with (NPC) were investigated retrospectively.CKexpression was demonstrated by immunohistochemistry using pan CK antibodies. EBV, CMV and HSV were identified by polymerase chain Reaction (PCR). Of the 150 samples, 144/150(96%) were CK positive and the remaining 6/150(4%) were CK negative (internal control).****Results:*** *CK and EBV correlation was identified in 92/1144(64%) with P = 0.003. CK and CMV correlation was identified in 53/144(37%) with P = 0.05. CK and HSV correlation was identified in 18/144(12.5%) with P = 0.4.* ***Conclusion:*** *In NPC, there is significant correlation between pan CK expression and EBV and CMV but not HSV.*

Keywords: Cytokeratin, EBV, CMV, HSV, Nasopharyngeal Carcinoma

1. Introduction

Nasopharynx carcinoma (NPC) is the most common cancer originating in mucosal lining epithelium of the nasopharynx. World Health Organization classifies nasopharyngeal carcinoma in three types. Type I (squamous cell carcinoma), Type II (keratinizing undifferentiated carcinoma) and Type III (nonkeratinizing undifferentiated carcinoma)(Paul, et al. 2010). Nasopharyngeal carcinoma is a distinct subtype of head and neck cancer, with significant differences in epidemiological features when compared with squamous cell carcinomas in other head and neck parts. NPC differs significantly from other cancers of the head and neck in its occurrence, causes, clinical behavior, and treatment (Hildesheim and Wang, 2012; IARC, 2014).

NPC has a distinct racial and geographical distribution and a multi-factorial etiology. Globally, there were approximately 65,000 new cases and 38,000 deaths in the year 2000. Although it is rare in most parts of the world, there are certain populations for which the incidence is considerably higher, notably native and foreign-born Chinese, Southeast Asians (e.g. in Thailand, Philippines, and Vietnam), North Africans (e.g. in Algeria and Morocco), as well as native peoples of the Arctic region (e.g. in Canada and Alaska). Within these populations, there is a remarkable heterogeneity among ethnic lines. The highest incidence of NPC has long been observed in Hong Kong, where 1 in 40 men develop NPC before the age of 75 years (Chan, et al. 2014; Kuang-Rong, 2014).

Many factors have been incriminated as etiological factors for occurrence of NPC: viral, environmental influences, heredity (Zhang and Zhang, 1999), genetic susceptibility, consumption of food (in particular salted fish) (Yu, 1986) containing carcinogenic volatile nitrosamines (Chang and Adami, 2006).

EBV is herpes virus that infects 90% of humans by adulthood, and it is a major risk for various cancers, including NPC, gastric cancer, Burkitt lymphoma, non-Hodgkin lymphoma (NHL), and Hodgkin lymphoma (Raghupath, et al. 2014). It is well documented that EBV is most frequent causal agent of NPC and is most likely to be involved in the multi-step and multi-factorial development of NPC (Ng et al. 2006). CMV is herpes virus that is found throughout all geographic locations and socioeconomic groups, and infects between 50% and 90% of adults worldwide (Mocarski, et al. 2013). However, the relationship between human CMV and NPC has been previously reported (Lin, et al. 1994).HSV is herpes virus that is infects between 30% and 40% of people worldwide (Chayavichitsilp, et al. 2009). However, there is a lack in studies in the relationship between HSV infection and NPC.

Cytokeratins are proteins of keratin-containing intermediate filaments found in the intracytoplasmiccytoskeleton of epithelial tissue.Cytokeratins are usually found in pairs: Basic or neutral cytokeratins include; CK1, CK2, CK3, CK4, CK5, CK6, CK7, and CK8. Acidic cytokeratins are; CK9, CK10, CK12, CK13, CK14, CK16, CK17, CK18, CK19 and CK20 (Schweizer, et al. 2006). Several studies have indicated the role of different types of CK in the subsequent management of NPC (Wei, et al. 2014; Li, et al. 2009). High levels of CK19-2G2 fragment expressed in tissue and serum are present in patients with nasopharyngeal carcinoma. The serum level of CK19-2G2 is helpful in the diagnosis of nasopharyngeal carcinoma and, the

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combination of serum CK19-2G2 and EB-VCA IgA improves the detection sensitivity (Lei, et al. 2012). Therefore, the aim of this study was to assess the role of CK expression and presence of these herpes viruses as possible etiological agents in NPC.

2. Materials and Methods

In this study 150 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 pan cytokeratin 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.

3. Results

In this study 144 tissue samples from patients with NPC were studied for immune-expression of CKand molecular identification of EBV, CMV and HSV correlations. All of the 144 samples were Pan CK positive. Six additional samples (CK negative) were added as internal control. EBV was detected in 92/144 (64%) of CK positive samples, consequently, the 95% confidence level and Odd Ratio (OR) is 22.9 (1.2649 to 414.7633), P< 0.003). No EBV was found in the samples negative by CK. CMV was identified in 53/144(37%) of CK positive samples consequently, the 95% confidence level and Odd Ratio (OR) is 7.6 (1.2649 to 414.7633), P< 0.003). No CMV was found in the samples negative by CK. HSV was detected in 18/144 (12.5%) of CK positive samples (P< 0.4). No EBV was found in the samples negative by CK, as indicated in Table1, Fig1 and 2.

Table 1: Distribution of the studied samples by CK and Herpes Viruses(EBV, CMV and HSV).

Virus	CK		Total
	Positive	Negative	
EBV			
Positive	92	0	92
Negative	52	6	58
CMV			
Positive	53	0	53
Negative	91	6	97
HSV			
Positive	18	0	18
Negative	126	6	132

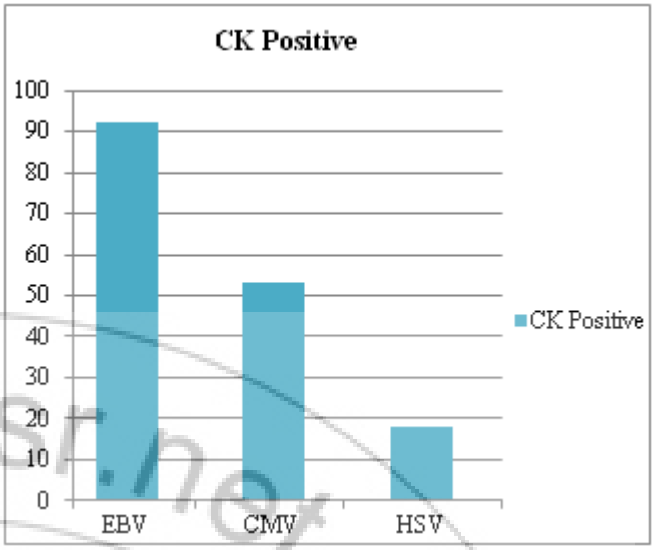

Figure 1: Description of positive immune-expression of CK by presence of EBV, CMV and HSV

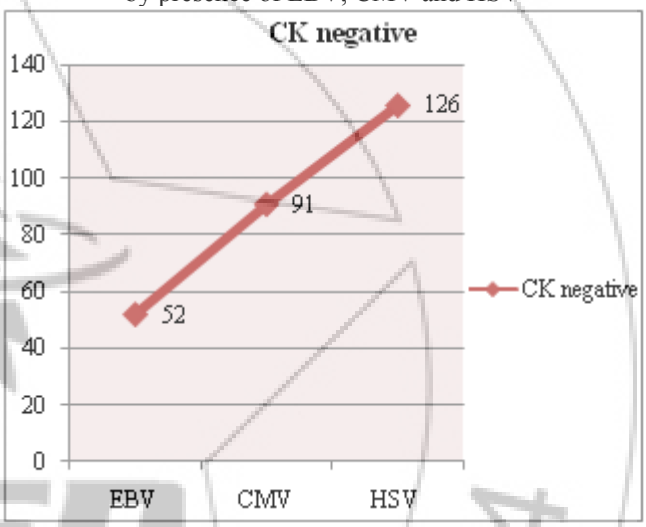

Figure 2: Description of negative immune-expression of CK by presence of EBV, CMV and HSV

4. Discussion

Although, NPC is endemic in china with the incidence up to 25 per 100 000, but it is rare in Europe and the USA with an incidence of 0.5–2 per 100 000 (Ferlay, et al. 2004). These variations strongly indicate the role of etiological factors interacting with genetic predisposition and other environmental factors.

In the present study we evaluated the relationship between cytokeratins as factors that are involved in the management of PNC and Herpes viruses as a major causes involved in the development of PNC. Several studies have shown that many cytokeratins are potential biomarker for the differentiation and prognosis of NPC, and its dysregulation might play an important role in the pathogenesis of NPC (Li, et al. 2009; Lei, et al. 2012; Wei, et al. 2014). Since, we have applied a pan cytokeratin in this study, spectrum of cytokeratins have been expected to be expressed.

However, the relationship between Cytokeratin expression and Human herpes viruses (EBV or CMV) was found to be statistically significant. To the best of our knowledge there is

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no study correlated the relationship between these viruses and cytokeratins in NPC.

Cytokeratins, are useful protein markers which are related to epithelium tissues and their related tumors. There are more than 20 different cytokeratins, of which cytokeratins 8, 18, and 19 are the most frequently identified in simple epithelial cells. Upon release from proliferating or apoptotic cells, cytokeratins provide useful markers for epithelial malignancies, specifically reflecting current cell activity (Vivian, et al. 2004). The expression of cytokeratins varies with epithelial cell type, degree of differentiation, and development of the tissue. Through the transformation of normal cells into malignant cells, the cytokeratin patterns are typically maintained, and this property has enabled cytokeratins to be applied as tumor markers (Chu, 2002). The processes that cause the release of soluble cytokeratin fragments have not yet been completely elucidated but appear to involve multiple pathways including proteolytic degradation of cytokeratins in dying cells, abnormal mitosis, spillover of monomeric cytokeratin polypeptides from proliferating cells, apoptosis, and/or neovascularization(Vivian, et al. 2004).

EBV is found to be associated with 100% of poorly or undifferentiated NPC, a tumor of epithelial origin. The latent membrane protein-1 (LMP1) of EBV may play a causal role in the development of this disease (Curran, et al. 2001). Expression of LMP1 in the epidermis of transgenic PyLMP1 mice induces hyperplasia, an early step in the carcinogenic process (Wilson et al. 1990). Furthermore, in cultured carcinoma cell lines, heterologous expression of LMP1 leads to low serum requirements, loss of anchorage dependence, increased invasive capacity, and, in some cases, inhibition of terminal differentiation (Nicholson et al. 1997). Furthermore, growth characteristics of NPC tumors have been correlated with LMP1 expression levels. Detectable LMP1 protein is linked with the expression of EGFR and Ki67 in NPC biopsies (Zheng, et al. 1994) and LMP1-positive NPC tumors appear to grow faster and more expansively than LMP1-negative NPC tumors (Hu, et al. 1995, Temple, et al. 2014)). These complex processes in the EBV carcinogenesity and cytokeratins development may express the correlation between these factors. However, one of limitations in this study is the use of pan cytokeratins which prevent the chance of identification of different cytokeratin types.

Human cytomegalovirus (HCMV), a widely-spread β-herpesvirus, is a major cause of birth defects. CMV a large DNA virus, blocks host DNA synthesis and deregulates cell cycle progression (Qian, et al. 2010).A major strategy employed by many DNA viruses to replicate their genomes is to promote host cell entry into S-phase in order to utilize the cellular resources needed for viral DNA synthesis. The CMV UL97 protein has cyclin-dependent kinase (CDK) activity, allowing the virus to inactivate Rb-family proteins and activate transcription of S-phase genes (Kamil, et al. 2009). How far these interactions between viral genome and epithelial host cells can indicate intracellular process that involved in the production of cytokeratins require further investigations.

Although, HSV showed no correlation with cytokeratins, but still there are some positive samples in the present study. The HSV virion carries its own specific transcription initiation factor (alpha-TIF), which functions together with other components of the cellular transcriptase complex to mediate virus-specific immediate early (IE) transcription. The virus-coded IE proteins are the transactivator and regulatory elements modulating early transcription and subsequent translation of nonstructural virus-coded proteins needed mainly for viral DNA synthesis and for the supply of corresponding nucleoside components (Rajcáni andDurmanová, 2000). Skin keratinocytes represent a primary entry site for herpes simplex virus type 1 (HSV-1) in vivo. The cellular proteins nectin-1 and HVEM act as efficient receptors for both serotypes of HSV and are sufficient for disease development mediated by HSV-2 in mice. How HSV-1 enters skin, and whether both nectin-1 and HVEM are involved, is not known (Petermann, et al. 2014).

Future prospect: Further studies on the exact relationship between Human Herpes viruses and cytokeratin expression is needed, which may help in patients management. Study of the correlations between herpes viruses and different cytokeratins types is required.

In conclusion: There is strong correlation between Herpes viruses EBV and CMV) and cytokeratin expression in NPC. Knowledge of the exact interaction between cytokeratins and these viruses may stimulate new ideas that help in prognosis, treatment and overall management of patients with NPC.

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