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Localization of Herpetic Viruses Patterns in Sinonasal and Nasopharyngeal Malignant and Benign Tumors

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Abstract

Background: Sinonasal and Nasopharyngeal carcinomas are related to many environmental and genetic predisposing factors. Epstein Barr viruses are well known to induce tumorigenesis in proliferative epithelial tissues. HCMV plays an "oncomodulatory" role in the neoplastic process and can induce cellular responses that would provide a growth advantage for neoplastic cells.

Objectives: This study designed to determine the herpetic viruses' patterns (punctuated, diffused and mixed) in tissues from Nasopharyngeal and sinonasal lesions.

Methods: The study included a total number of (183) formalin fixed, paraffin-embedded tissue blocks including 35 inflammatory nasal polyps (INP), 35 sinonasal papillomas (SNP), 65 nasopharyngeal carcinomas (NPC), 18 sinonasal carcinomas (SNC) as well as 30 nasal healthy tissues as control group. After histopathological confirmatory re-examination, a Chromogenic in Situ Hybridization technique (CISH) for Epstein Barr virus encoded RNA (EBV-EBERs) and Human cytomegalovirus phosphoproteins 65 (HCMV-PP65) localization were performed.

Results: The mean (20.43) of EBV punctuators among tissues with malignant nasopharyngeal carcinoma was higher than the mean of punctuators (17.00) in sinonasal carcinoma group, whereas the mean of diffused form (71.89) in malignant sinonasal carcinoma tissues was higher than the mean (66.11) in nasopharyngeal carcinoma tissues. On the other hand, the mean of punctuated pattern in malignant sinonasal carcinoma tissues infected with HCMV was higher (21.56) than the means (16.94) of this pattern in nasopharyngeal carcinoma tissues, as well as the mean of diffused pattern in tissues with sinonasal carcinoma was higher (45.11) than its mean in HCMV infected nasopharyngeal carcinoma tissues (34.881).

Conclusion: The present findings indicate the impact of both punctuated and diffused patterns in sinonasal and nasopharyngeal pathogenesis and carcinogenesis.

Keywords: NPC; SNC; EBV-EBERS; HCMV-PP65; CISH

Introduction

Nasopharyngeal cancer is a highly malignant tumor, the primary tumor mass can extend within the nasopharynx and/or extend to the base of the skull, palate, nasal cavity or oropharynx and distant metastases can arise in bone, lung, mediastinum and liver [1,2]. Sinonasal carcinoma could arise from various tissues within the nasal cavity, including lymphoreticular, epithelial and nonepithelial tumors [3]. Sinonasal Papillomas are a rare disease which have an annual incidence of 0.6/105 in defined geographical regions. The investigations of these tumors have shaded the light on a viral agent as an etiological candidate, such as EBV [4]. In addition, an outdoor and industrial occupations are the major risk factors for sinonasal Papillomas development [5]. Inflammatory Polyps lesions are affecting 1-4% of all population [6]. Where they arise as an outgrowth from the mucosal membrane of nasal cavity and paranasal sinuses as a result of an allergic or chronic inflammation [7].

Epstein Barr virus (EBV) also has been shown to be associated to many human cancers, such as nasopharyngeal carcinoma, burkitt's lymphoma and Non-Hodgkin lymphoma etc. [8,9]. The Epstein Barr virus Early Repeats (EBER1 and EBER2), have 167

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and 172 nucleotides long in consequence with 54% sequence homology between them. EBERs were used for the recognition of EBV infected cells in tissues by CISH and are a reliable marker indicating the presence of EBV. EBER-1 interacts with interferon-inducible protein kinase R (PKR), inhibiting its activation and protecting infected cells from IFN induced apoptosis and induce the expression of growth factors that promote cell survival [10,11].

Human cytomegalovirus (HCMV) has an implications in the etiology of several human cancers; including cervical carcinoma, prostatic adenocarcinomas and colonic and brain cancers [12]. HCMV infection might have role in building up the tumor cells through protection of certain tumor cells from apoptosis and modulating angiogenesis [13]. The phosphoprotein 65 (Pp65) was used as a reliable marker indicating the presence of HCMV and the recognition of HCMV infected cells in tissues by CISH technique.

Materials and Methods

This study enrolled a total number of one hundred fifty-three (153), formalin-fixed, paraffin-embedded blocks from nasopharyngeal and sinonasal tissues. They were collected from archives of histopathological laboratories of several hospitals in Baghdad. Some of these tissues block were related to the past few years (2015, 2016, 2017 and 2018) and are including 18 sinonasal carcinoma, 65 nasopharyngeal carcinomas, 35 sinonasal inverted Papillomas and 35 nasal inflammatory polyps in addition to matched 30 healthy sinonasal tissue blocks obtained from patients subjected to nasal bridge reconstruction operation as a control group.

The diagnoses were based on accompanied pathological reports of the corresponding patients. The age of patients and controls (120 males, 63 female) ranged between (17-83) years. Histopathological sections were made for biopsies and stained by hematoxylin and eosin for final definitive diagnosis. The detection of EBV and HCMV by Chromogenic in situ hybridization (CISH) reactions kit (purchased from Zyto Vision GmbH. Fischkai, Bremerhaven. Germany) (Cat. Numbers: T-1061-40) was done on 5µm tissue sections by using digoxigenin-labeled oligo-nucleotides probe which targets EBV-EBERs-RNA and HCMV-pp65 DNA (Cat. Number: T-1114-400 and T-1113-400) (Zyto Visions/Germany).

The detailed methods for performing CISH reactions were conducted according to the instructions of the above manufacturing company. The CISH signal patterns were classified as punctuate, diffused and mixed. The punctuate pattern, is, a dot-like signals or tiny particles that are scattered in the nucleus, this pattern refers to the integrated viral genome, and the diffused pattern, appears as a large globular with homogeneous strong staining in the nucleus or cytoplasm, this pattern indicates an episomal viral genome, while the mixed patterns represent the integrated and episomal viral genomes which appear together in the same sample. The quantification of CISH signal patterns (punctuated and diffused) were assessed under light microscopy at (100X) to visualize where virus-associated dsDNA localized in cytoplasm or nucleus by screening ten field in tissue loaded on chargeable slide by using the mean percentage of viral location [14]. Scoring and intensity of signals were done according to [8].

Statistical analysis

In this study, SPSS program (version-21) was used for statistical analysis where ANOVA test and LSD were used to assess the significances between variables.

Results

Distribution of EBER-CISH Signals patterns among sinonasal and nasopharyngeal tissues

A-Punctuated pattern: As shown in (Table 1) and (Figure 1,2) of the punctuated patterns in the sinonasal and nasopharyngeal cancer tissues, the mean of punctuators among tissues with nasopharyngeal carcinoma was higher (20.43) than the mean of punctuators in sinonasal carcinoma group (17.00), and the mean of sinonasal papilloma and inflammatory nasal polyp groups (13.29) and (6.51), respectively. In comparison, the mean of punctuators pattern in healthy control tissues was (2.30). Accordingly, there were highly significant statistical differences (P<0.01) between different groups.

Table 1: Distribution of punctuated and diffused patterns of EBER-CISH patterns among Studied groups.

Studied groups (EBV)	N	Punctuated	Diffused
		M±SD	M±SD
AH Control	30	2.30±8.918	4.37±16.705
Inflammatory Nasal Polyp (INP)	35	6.51±11.500	50.66±45.608
Sinonasal Papilloma (SNP)	35	13.29±20.636	43.86±42.126
Sinonasal Carcinoma (SNC)	18	17.00±15.146	71.89±29.583
Nasopharyngeal Carcinoma (NPC)	65	20.43±19.997	66.11±32.141
Total	183		
ANOVA Test (P-Value)	P=0.00 Highly Sign (P<0.01)		P=0.00 Highly Sign (P<0.01)

Table 2: LSD Statistical Analysis of Punctuated EBER-CISH Signals.

EBERs-CISH Punctuated Patterns		LSD Test (P-value)
AH Control	Inflammatory nasal polyp (INP)	P=0.317
	Sino-nasal papilloma (SNP)	P=0.009
	Sino-nasal carcinoma (SNC)	P=0.004
	Nasopharyngeal Carcinoma (NPC)	P=0.00
Inflammatory Nasal polyp (INP)	Sino-nasal papilloma (SNP)	P=0.095
	Sino-nasal carcinoma (SNC)	P=0.034
	Nasopharyngeal Carcinoma (NPC)	P=0.00
Sino Nasal Papilloma (SNP)	Sino-nasal carcinoma (SNC)	P=0.449
	Nasopharyngeal Carcinoma (NPC)	P=0.045
Sino Nasal Carcinoma (SNC)	Nasopharyngeal Carcinoma (NPC)	P=0.447

As detailed in (Table 2) a highly significant statistical differences were noticed between SNP, NPC, SNC and healthy control group

and between NPC and INP groups. On the other hand, there were significant differences between SNC and INP and between NPC and SNP groups.

B-Diffused pattern: (Table 1) and (Figure 1,2) showed a diffused pattern in sinonasal and nasopharyngeal cancer groups, the mean of diffused form in malignant sinonasal carcinoma tissues was higher (71.89) than the mean in nasopharyngeal carcinoma tissues (66.11) and both were higher than the mean in the benign tumors in sinonasal papilloma and inflammatory nasal polyp groups (43.86 and 50.66), respectively. Whereas, the mean of diffused pattern in healthy control tissues was (4.37). Statistically there were a highly significant differences (P<0.01) between different groups.

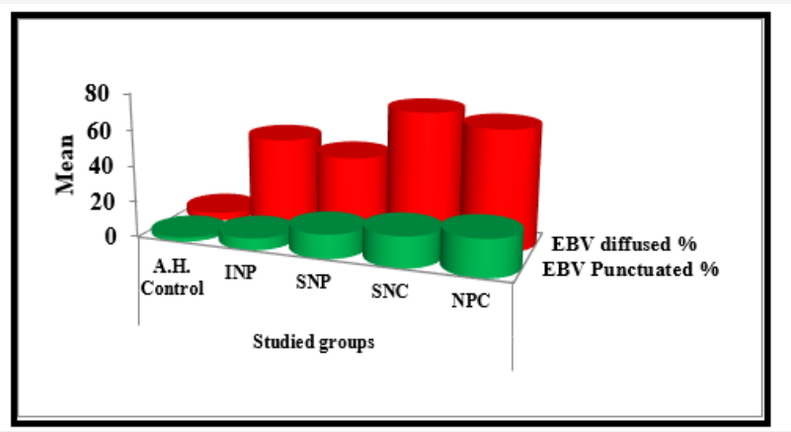

Figure 1: Distribution of EBER Punctuated and diffused forms.

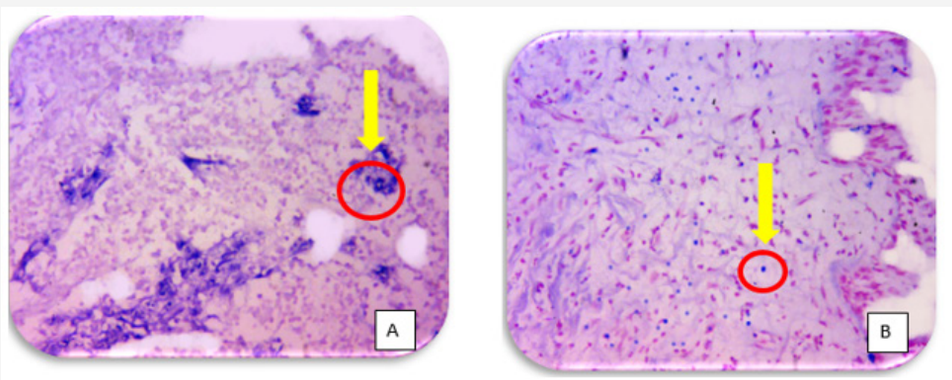

Figure 2: Microscopic appearance of EBERS-CISH signals in nasopharyngeal and sinonasal carcinoma. A. Diffused pattern. B. Punctuated pattern of EBV.

Table 3: LSD Statistical Analysis of Diffused Patterns of EBER-CISH Signals.

EBERs/CISH Diffused Reaction Patterns		LSD Test (P-value)
AH Control	Inflammatory nasal polyp (INP)	P=0.00
	Sino-nasal papilloma (SNP)	P=0.00
	Sino-nasal carcinoma (SNC)	P=0.00
	Nasopharyngeal Carcinoma (NPC)	P=0.00
Inflammatory Nasal Polyp (INP)	Sino-nasal papilloma (SNP)	P=0.095
	Sino-nasal papilloma (SNP)	P=0.421
	Sino-nasal carcinoma (SNC)	P=0.039
	Nasopharyngeal Carcinoma (NPC)	P=0.038
Sino Nasal Papilloma (SNP)	Sino-nasal carcinoma (SNC)	P=0.007
	Nasopharyngeal Carcinoma (NPC)	P=0.003
Sino Nasal Carcinoma (SNC)	Nasopharyngeal Carcinoma (NPC)	P=0.538

In detail there were a highly significant differences between SNP, INP, NPC, SNC and healthy control group and highly significant differences between each of (NPC and SNC) when compared to SNP group. Furthermore, significant differences between each of (NPC and SNC) and INP group. While there were no significant differences neither between SNC and NPC nor between SNP and INP groups (Table 3).

Patterns of HCMV-DNA distribution among studied sinonasal and nasopharyngeal tissues

A-Punctuated Pattern: (Table 4) and (Figure 3,4) show that the mean of punctuated pattern in malignant sinonasal carcinoma tissues was higher (21.56) than the means (16.94) of this pattern in nasopharyngeal carcinoma tissues and each of sinonasal papilloma and inflammatory nasal polyps (14.14 and 16.60) respectively, in comparison it was (1.80) in healthy control tissues. Statistically, highly significant differences (P<0.01) were recorded between different groups. Highly significant differences were recorded between the groups of SNPs, INP, NPC, SNC and the healthy control group, while there were no significant differences between all other groups and as it shown in (Table 5).

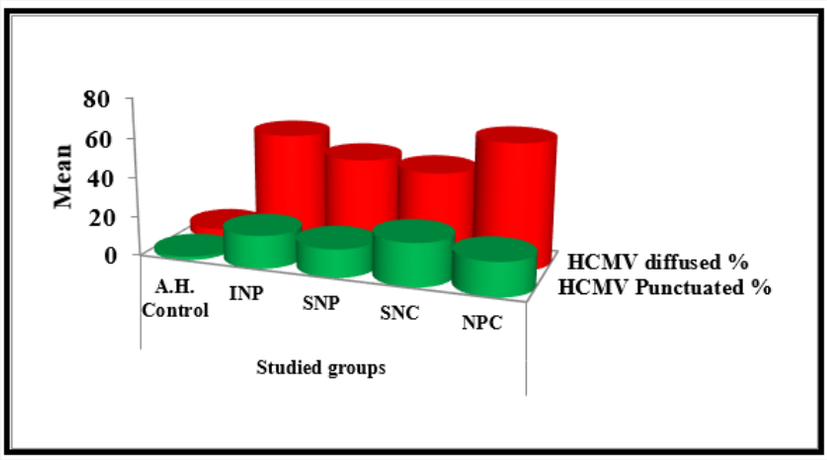

Figure 3: Distribution of Punctuated and diffused form of HCMV-DNA.

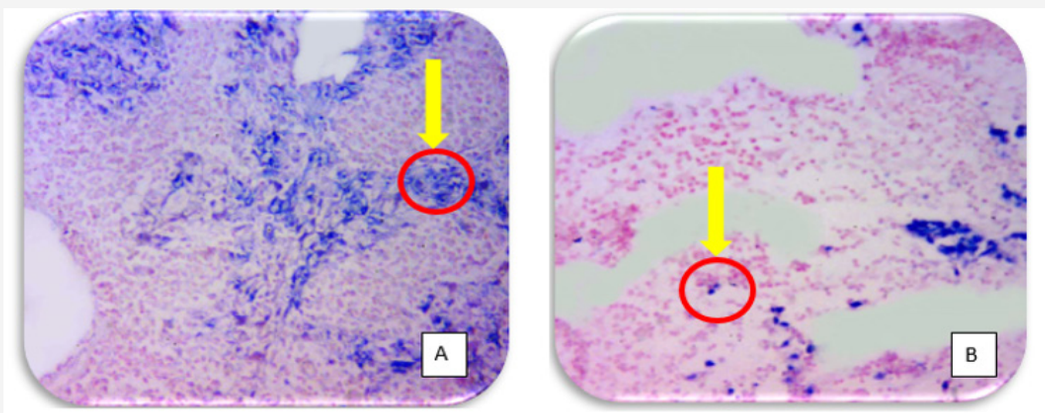

Figure 4: Microscopic appearance of HCMV-CISH signals in nasopharyngeal and sinonasal carcinoma. A. Diffused pattern. B. Punctuated pattern.

Table 4: Distribution of Punctuated and Diffused Pattern of HCMVpp65-DNA among the Studied Tissues.

Studied groups (HCMV)	N	Punctuated	Diffused
		M±SD	M±SD
AH Control	30	1.80 ±7.336	4.87±18.706
Inflammatory Nasal Polyp (INP)	35	16.60 ±19.577	57.86±38.476
Sinonasal Papilloma (SNP)	35	14.14±18.892	48.43±41.091
Sinonasal Carcinoma (SNC)	18	21.56±27.445	45.11±39.807
Nasopharyngeal Carcinoma (NPC)	65	16.94±16.728	34.881±4. 327
Total	183		
ANOVA Test (P-value)	P=0.00 Highly Sign (P<0.01)		P=0.00 Highly Sign (P<0.01)

Table 5: LSD Statistical Analysis of Diffused Patterns of EBER-CISH Signals.

Studied Groups (HCMV Punctuated)		LSD Test (P-value)
AH Control	Inflammatory nasal polyp (INP)	P=0.001
	Sino-nasal papilloma (SNP)	P=0.006
	Sino-nasal carcinoma (SNC)	P=0.00
	Nasopharyngeal Carcinoma (NPC)	P=0.00
Nasal polyp (INP) Inflammatory	Sino-nasal papilloma (SNP)	P=0.568
	Sino-nasal carcinoma (SNC)	P=0.343
	Nasopharyngeal Carcinoma (NPC)	P=0.928
Sino Nasal Papilloma (SNP)	Sino-nasal carcinoma (SNC)	P=0.157
	Nasopharyngeal Carcinoma (NPC)	P=0.459
	Nasopharyngeal Carcinoma (NPC)	P=0.538
Sino Nasal Carcinoma (SNC)	Nasopharyngeal Carcinoma (NPC)	P=0.336

B-Diffused Pattern: The mean of diffused pattern in tissues with malignant sinonasal carcinoma was higher (45.11) than its mean in nasopharyngeal carcinoma tissues (34.881) and the mean in benign sinonasal papilloma (48.43), inflammatory nasal polyp (57.86), and the mean of this pattern in healthy control tissues (4.87). There were a highly significant differences (P<0.01) among these groups (Table 4) (Figure 3,4). These differences were highly significant between SNP, INP, NPC, SNC and healthy control groups, while there were no significant differences between the rest of groups (Table 6).

Table 6: LSD Statistical Analysis of Diffused Pattern of HCMV pp65-DNA-CISH Signals.

Studied groups (HCMV Diffused)		LSD Test (P-value)
AH Control	Inflammatory nasal polyp (INP)	P=0.00
	Sino-nasal papilloma (SNP)	P=0.00
	Sino-nasal carcinoma (SNC)	P=0.00
	Nasopharyngeal Carcinoma (NPC)	P=0.00
Inflammatory Nasal Polyp (INP)	Sino-nasal papilloma (SNP)	P=0.266
	Sino-nasal carcinoma (SNC)	P=0.216
	Nasopharyngeal Carcinoma (NPC)	P=0.484
Sino Nasal Papilloma (SNP)	Sino-nasal carcinoma (SNC)	P=0.747
	Nasopharyngeal Carcinoma (NPC)	P=0.051
Sino Nasal Papilloma (SNP)	Nasopharyngeal Carcinoma (NPC)	P=0.058

Discussion

Epstein Barr virus integration into host genome has been reported by [15-17]. Chromosomal integration of EBV genome represent a state of interaction between virus and host cells, this may reflect chromosomal instability that induce further genetic change in tumor cells and cause frequent DNA recombination which promote EBV integration into fragile DNA sites, that effect cell cycle genes such as tumor suppressor genes and cause accumulation of mutations that leads to transformational changes and induce oncogenesis as reported by [18]. Sugden and Dheekollu et al. [19,20] reported episomal EBV genome in NPC and some other tumors. The EBV episomal replicate independently of host cell genome and transfer to generations and in some instances, it recombines with the human genome to create one or more chromosomal integrations of varying structure with respect to viral and host breakpoints and cause accumulation of mutations and induce toumergenesis [21].

In regard to HCMV, both patterns were found in all of the examined tissue specimens, however the percentages of diffused pattern subsets of NP and SN tissues were higher than the subsets of these tissues which showed punctuated patterns. These findings indicate that this virus has established a persistent/latent state in all these benign and malignant tissues. In addition, this nuclear localization of such punctuated pattern of CISH found in the present study is an indication of viral genome integration in the cellular DNA, which is regarded as an irreversible as well as a critical step or process that associated in the transformation of human cellular DNA inevitably [22-24].

In a previous study by [25,26] also detected HCMV nucleic acids predominantly in the nuclear localization of the cancerous tissues. Moreover, nuclear integration HCMV-DNA of tumor tissues was regarded as an early event in the mutagenic process of their involvement associated with viral promotion of DNA damage and/or tumor initiation [27,28]. Furthermore, viral integration and insertional mutagenesis was found to be involved in tumor progression via the dysregulation of cellular processes, an effect of viral proteins at later stages modulating signaling pathways are involved in cell proliferation, inhibiting apoptosis, increasing rates of immortalization [29,30].

However, episomal genomic form observed in this study could have the chance to replicate as well as have the chance of integration, (although to a lesser extent) causing genomic instability and tumerigenesis [31,32]. One can concludes that these tissues were under the potential effects of being transformed, (in association with many other risk factors in the multistep development of such SN and NP carcinogenesis). Since it was revealed that the virus has either initiatory, co-factor or a promoter role in viral carcinogenesis [22].

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Conflict of Interest

No conflict of interest.

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