In situ Molecular Hybridization of Kaposi's Sarcoma Associated Virus (Human Herpes Virus 8) in Nasopharyngeal Carcinoma Tissues

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Abstract

Background: Viral, dietary and genetic factors are implicated in the etiology ofnasopharyngeal cancer, a rare type of head and neck cancers. Unlike other viruses, HHV-8 encodes several human cytokines homologues and regulatory genes that play important roles in the viral pathogenesis.

Objective: To analyze the rates of HHV-8 infection in tissues obtained from a group of patients with nasopharyngeal carcinoma and inflammatory nasal polyps (INP) .

Patients and Method: One hundred- thirty formalin-fixed, paraffin- embedded nasopharyngeal carcinoma and nasal inflammatory polyps tissues enrolled in this study; 65 nasopharyngeal tissue biopsies from nasopharyngeal carcinoma; 35 tissue biopsies from nasal inflammatory polyps and 30 nasopharyngeal tissues with unremarkable pathological changes, as apparently healthy tissuecontrol. Detection of HHV-8 was done by chromogenic in situ hybridization (CISH) technique detection system.

Results: In nasopharyngeal carcinoma tissues, the HHV-8- DNA positive CISH reactions were detected in 23.1% while in nasal inflammatory polyps tissues HHV-8-positive CISH reactions were found in 8.6% of the examined tissues. The correlation between HHV-8 and NPC & INP was highly significant (P= 0.001).

Conclusion: Significant HHV-8 detection in nasopharyngeal carcinoma & inflammatory nasal polypstissues could point for their possible role in either pathogenesis or carcinogenesis of both these lesions.

Keywords: *HHV-8; Nasopharyngeal carcinoma; Inflammatory nasal polyps; CISH.*

Introduction

Nasopharyngeal carcinoma (NPC) is a common aggressive and highly malignant tumor, arising from nasopharyngeal mucosa, and locally extending to the base of the skull, palate, nasal cavity or oropharynx. High incidence of cervical lymph nodes as well as distant metastases was reported [(1).] NPC are more vastly common seen in certain regions of East Asia and Africa than other regions. Viral, dietary as well as genetic factors were implicated in its etiology [(2).]

Epithelialhead and neck malignancies demonstrate a relationship to oncogenic viruses including Human Papilloma virus, Epstein-Barr virus or Merkel cell polyoma virus, where oropharynx and respiratory tract were noticed as a common sites for the persistence and transmission of these oncogenic viruses [(3,4).]

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Kaposi's sarcoma, a low-grade angioproliferative malignant neoplasm, is associated with human herpes virus – 8 infection,, also known as the KS-associated herpes virus (KSHV)[5].

HHV-8 virions have morphological and structural features typical of other herpes viruses, including 3 proteins encoded by ORF 25,26, and 62, however, a fourth protein, (encoded by ORF 65) lacks significant similarity to other herpetic viral counterparts[6]. HHV-8 genome possesses approximately highly conserved 26 core genes, responsible for viral regulation, replication, and maturation [7]. HHV-8 has at least 12 other human host gene homologs, not shared by other human herpes viruses, implicated in oncogenesis[8]. In addition, a variety of gene products are encoded in HHV-8 for transformation, proliferation, cell signaling, antiapoptosis and angiogenesis, and immune modulation and immune evasion which may be involved in oncogenesis promotion and viral persistence[9].

Up to our best knowledge, the present study, represents the first in Iraq to analyze the rate of HHV-8 infection and to highlight a possible associative role of this virus in tissues obtained from a group of Iraqi patients with nasopharyngeal carcinoma (NPC) and inflammatory nasal polyps (INP).

Material and Method:

The detection of HHV-8 was performed on 4µm paraffin embedded tissue sections by chromogenic in situ hybridization (CISH) kit (purchased from ZytoVision GmbH. Fischkai, Bremerhaven. Germany). using digoxigenin-labeled oligo-nucleotides probe that targets HHV-8 DNA .

A probe complementary to a sequence of *Human Herpes Virus -8 gene DNA* was :*Amount; 8.9 OD, 271 µg, 39.5 nmol; Length : 20-mer; GC content : 50 % Concentration (volume 1ml): 39.5 pmol/µl; Molecular weight : 6849 g/mol; Modification : 5' Digoxigenin; Scale : 0.2 µmol; DMT : HPLC - Sequences of HHV8 Probe: (5'-ATG CAG CTA CAA CTT CGG AG-3')A G C T; 6 5 5 4, respectively.*

This study utilized SPSS program (version-21) for the statistical analysis, where Chi-Square test (χ2), Odd ratio and Spearman's rho have been used to evaluate the differences between variables.

Results

I. Distribution of patients with nasopharyngeal lesions according to their Age: The nasopharyngeal cancer patients in this study were related to the age range from 18 -77 years and the mean age of those patients was (42.85 ± 14.89) years. Whereas the mean age of patients with inflammatory nasal polyps was (28.22 ± 17.76) years and their age ranged from 7 – 67 years and the mean age of apparently healthy individuals was (43.25 ± 6.99) years and their age ranged from 32- 64 years and as shown in table (1) .

Table (1): Distribution of study groups according to their age.

Studied groups (Age/ Year)	N	Mean	Std. Deviation	Std. Error	Range		ANOVA test (P-value)
					Mini.	Maxi.	
Apparently healthy individuals Control	30	43.25	6.99	1.44	32	64	P=0.006 sign. (P<0.01)
Inflammatory nasal polyp(INP)	35	28.22	17.76	2.80	7	67	
Nasopharyngeal carcinoma (NPC)	65	42.85	14.89	1.94	18	77	
Total	**130**						

II. Histological grading of Nasopharyngeal carcinoma (NPC): The grading of carcinoma group in the present study revealed that well differentiated (keratinizing) carcinomas constituting 10(15.4%) tissues of NPC group, while 2(3.1%) tissues of NPC have moderately differentiated grade. The poorly differentiated non- keratinizing grade was observed in 4(6.2%) tissues while undifferentiated carcinomas grade was observed in 49(75.4%) (Table 2). The statistical analysis of grading distribution of NPC revealed highly significant differences at (P<0.01).

Table (2): Nasopharyngeal carcinoma according to their grades.

Grades	No.	%	Comparison of significant	
			P-value	Sig.
Well differentiated	10	15.4	0.283	P=0.003 Highly Sign. (P<0.01)
Moderately differentiated	2	3.1		
Poorly differentiated	4	6.2		
Undifferentiated	49	75.4		
Total	65	100		

III. Human Herpes Virus -8 -CISH expression in patients with nasopharyngeal & inflammatory nasal polyps:

Positive HHV-8 DNA- CISH signal scoring: Fifteen out of sixty five (23.1%) nasopharyngeal tissue biopsies with NPC showed positive CISH reactions for HHV-8-DNA (Figure 1). The Inflammatory nasal polyp (INP) tumors group revealed 8.6% positive signals which represented 3 out of 35 tissues in this group. None of control tissues group presented positive signals for HHV-8-CISH test. However, in comparison to the percentage of HHV-8 -DNA in healthy control group as well as in the group of INP, the differences between the percentages of HHV-8-DNA in tissues of patients with nasopharyngeal cancers and each of these groups are statistically very highly significant (P value = < 0.0001) and as revealed in (Table 3).

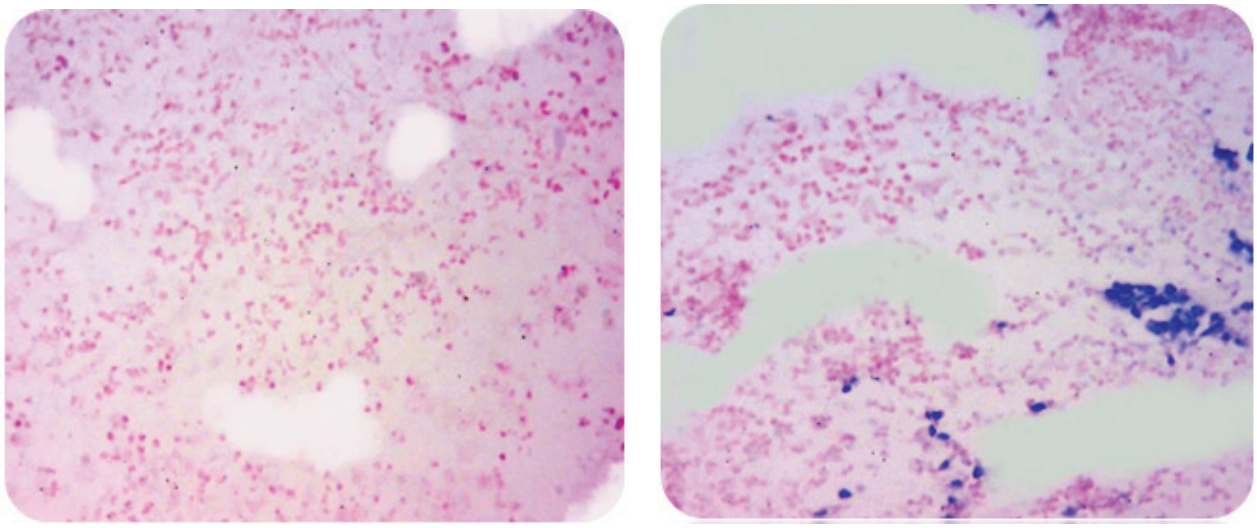
Table (3): Distribution of signal scores of HHV-8-DNA-CISH reactions.

HHV-8 scores		Studied groups			
		A.H. Control	Inflammatory nasal polyp (INP)	Nasopharyngeal carcinoma (NPC)	P-Value
Negative	N	30	32	50	P=0.004 Highly Sign. (P<0.01)
	%	100%	91.4%	76.9%	
Positive	N	0	3	15	
	%	0.00%	8.6%	23.1%	
+	N	0	2	8	
	%	0.00%	5.7%	12.3%	
++	N	0	1	5	
	%	0.00%	2.9%	7.7%	
+++	N	0	0	2	
	%	0.00%	0.00%	3.1%	
Total	N	30	35	65	
	%	100%	100%	100%	
Odds ratio			32.333	51	

II. Signal intensity of HHV-8 - CISH testing: The signal intensities of HHV-8-CISH signal detection in NPC tissues group illustrated the strong signal intensity in (15.4%) whereas (6.2%) and (1.5%) have weak, and moderate intensity, respectively. In Inflammatory nasal polyp lesions, (5.7%) have moderate intensity; while (2.9%) have weak intensity. Statistically highly significant differences were recorded between studied groups at (P<0.01) as detailed in (Table 4).

Table (4): Distribution of signal intensities of HHV-8 reactions.

HHV-8 intensities		Studied groups			
		A.H. Control	Inflammatory nasal polyp (INP)	Nasopharyngeal carcinoma (NPC)	P-Value
Negative	N	30	32	50	P=0.003 Highly Sign. (P<0.01)
	%	100%	91.4%	76.9%	
Positive	N	0	3	15	
	%	0.00%	8.6%	23.1%	
weak	N	0	1	10	
	%	0.00%	2.9%	15.4%	
Moderate	N	0	2	4	
	%	0.00%	5.7%	6.2%	
strong	N	0	0	1	
	%	0.00%	0.00%	1.5 %	
Total	N	30	35	65	
	%	100%	100%	100%	
Odds ratio			32.333	51	



A B

Figure (1): Microscopic appearance of HHV-8–CISH signals in nasopharyngeal carcinoma. Two patterns of blue –violet signals are observed at the site of complementary sequence in the cell nuclei of HHV-8-DNAA: Carcinoma with negative reaction (10X).B: Carcinoma with low score and moderate intensity reactions (10X).

IV. Correlations among the HHV-8 and the patient characteristics of nasopharyngeal carcinoma & Inflammatory nasal polyp:: A strong positive relationship (with highly significant correlation) was found between HHV-8 and grade in NPC & INP (r = 0.419, P = 0.006). In addition, a strong positive relationship (with highly significant correlation) was found between HHV-8 and site of NPC & INP (r = 0. 483; p= 0.003). However, there were no significant correlations among HHV-8 and age as well as gender (Table 5).

Table 5. Spearman's rho statistical testing of age, grade, HHV-8-CISH to evaluate the studied markers in in patients with NPC & INP.

Spearman's rho		Age groups (years)	Grade	HHV-8	Site	Gender
Grade	r	-0.146				.125
	P	0.352				-.034
Site	r	0.040	0.133			
	P	0.956	0.412			
HHV-8	r	0.172	0.419		0.483	
	P	0.376	0.006*		0.003*	
Gender				.723		
				-.351		

*Correlation is highly significant (P<0.01).

Discussion

In this study, the nasopharyngeal cancer patients have age range from 18 to77 years and their mean age was (42.85 ± 14.89) years while the mean age of patients with inflammatory nasal polyps was (28.22 ± 17.76) years and their age ranged from 7 – 67 years (Table 1) .The patients' age in the present results coincides with the results of many other studies: Oga*et al.*[10] and Parmar *et al.,* [11] who found the mean age of Nigerian patients with head and neck cancers, including nasopharyngeal cancers, was 43.3 years. Several other studies are also in agreement with the current results, where nasopharyngeal cancers increased with the advancing age with a peak age of 41-60 years and decreased above 60 years [12;13].

Also the current results could reflect that age is an important risk factor in nasopharyngeal tumorigenesis, where could be related by many risk factors that enhance appearance of malignant nasopharyngeal tumor in young age group in relation to the proceeding of age such as genetic predisposition,smoking and changes in life style (a highly caloric diet-rich in fat, refined carbohydrate, alcohol uptake)[14;15].

Fifteen out of sixty five (23.1%) nasopharyngeal tissue biopsies with NPC showed positive CISH reactions for HHV-8-DNA (Figure 1). The Inflammatory nasal polyp (INP) tumors group revealed 8.6% positive signals which represented 3 out of 35 tissues in this group. None of control tissues group presented positive signals for HHV-8-CISH test.

Ablashi*et al*.,[16] found that out of 42 NPC patients, only two of these patients demonstrated antibodies to HHV-8 .However, the complete lack of HHV-8 by PCR evaluation in [17] study led them to hypothesize that HHV-8 is an unlikely etiologic candidate. HHV-8 infection was more prevalent in countries where classic and endemic KS occured[18].

HHV-8 encodes a viral homolog of interleukin 6, that induces increased motility, cell-cell and cell-substrate dyshesion and epithelial-to-mesenchymal transformation in breast cancer cells [19-21].

The relationship between HHV-8 and NPC is based on the concept of identification of HHV-8 genome sequences in NPC tissues and immortalization of primary mammary epithelial cells by HHV-8. Although the presence of HHV-8 is not enough for the tumorigenic transformation, yet it is expected to become an early events along cumulative changes over the years that become the starting step, similar to what happened in Kaposi's carcinogenesis [22-25].

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