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Expressional Proteins of p53 and p16 Genes in Human Papillomavirus16/18 - Associated Sinonasal and Nasopharyngeal Lesions: An Immunohistochemical Study

***Iman Alwan Hussien[1], Dr. Saad Hasan Mohammed Ali[2], Talib Abdullah Hussein[1], Dr. Mohammed Sobhi Kamal[3]**

[1]Department of Biology, College of Science for women, University of Baghdad, Baghdad, Iraq.
[2]Communicable Diseases Research Unit, Baghdad Medical College, University of Baghdad, Baghdad, Iraq
[3]Department of pathology, Ghazi Al-Hariri Teaching Hospital, Ministry of Health, Baghdad, Iraq.

Abstract : Background: High-risk HPV may play significant roles in cell malignancy through disregulation of cell cycle proteins expression via activity of two viral oncoproteins E6, E7.
Objectives: The present study was designed to determine the rates of p16 and p53 expression in relation to high- risk (HPV16/18) genotypes infections in of sinonasal and nasopharyngeal lesions.
Methods: This retrospective study included total 125 formalin -fixed paraffin-embedded tissue blocks including 35 nasal polyps(NP), 35 sinonasal papillomas(SNP), 29 nasopharyngeal carcinomas (NPC),6 sinonasal carcinomas(SNC) and 20 nasal apparently healthy tissues. After histopathological confirmation, a Chromogenic In Situ Hybridization technique (CISH) was done for HPV16/18 DNA localization and for P16 and p53 expression immunohistochemistry technique (IHC) were performed.
Results: The percentages of DNA detection of HPV16/18 genotypes in carcinoma group and sinonasal papilloma were 31.4 % and 25.7%, respectively, while in nasal polyps was detected in 11.4 % .these results have revealed non-significant differences ($P>0.05$). P53-IHC positivity in carcinoma group and sinonasalpapillomas was noticed in 68.6 % and 34.3%, respectively, in nasal polyps was 28.6% which showed highly significant differences at ($P<0.01$). P16-IHC positivity in carcinoma group and sinonasalpapillomas was 51.4 % and 60.0% respectively, whereas in nasal polyps was 37.1% and the statistical analysis shows significant differences among them at ($P<0.05$). Positive correlation between HPV16/18 and p16 among carcinoma group and nasal polyps group.
Conclusions: the present results indicate a possible association between infection with high oncogenic HPV types and carcinomas group as well as sinonasal papilloma group pathogenesis and tumorgenesis. The P53, as well as p16 expression, were increased significantly in sinonasal and nasopharyngeal carcinomas presenting for their addition impact in sinonasal and nasopharyngeal pathogenesis and carcinogenesis. The p16 is a good marker for detection oncogenic HPV in sinonasal and nasopharyngeal carcinoma group.
Key word : High- riskHPV-16/18, P53, P16, Sinonasal and Nasopharyngeal lesions.

Introduction:

Human papillomavirus (HPV) is a member of Papillomaviridae where their infections are related to the genesis of various benign and malignant human neoplasias. Depending upon their oncogenic potential, HPVs were classified into two types high- risk (HR) and low -risk (LR) (1) Human infection with high-risk HPV types 16 and 18 seem to be causally related to the development of most squamous cell carcinoma. (2). High-risk types of human papillomavirus (HPV) are now well established as major etiologic factors of head and neck cancer (3). , The P53 gene, is believed to serve as a gatekeeper against carcinogenesis where under normal circumstances, the function of p53 protein is to prevent the propagation of genetically damaged cell. (4). Protecting cells from malignant transformation involved in cell-cycle control and apoptosis. Mutations in p53 gene are observed in about 50% of primary tumors. (5,6).The ability of the high -risk HPV to induce cell carcinogenesis as recognized to be due to inactivation of tumor suppressor genes p53 and RB by early expressing of oncoproteins E6 and E7 respectively (7).The cell cycle is also controlled by a family of cyclins, cyclin-dependent kinase (CDK) and their inhibitors (CDKI) like p16, p21 through activating and inactivating phosphorylation events. The absence of p16 is often a critical event in tumor progression,where at least deletion of one copy is sufficient in the premalignant lesions for giving a selective advantage to cell tumorgenesis (8). The loss of p16 (INK4a) due to homologous deletion, point mutation or methylation has also been widely observed in some malignant tumors such as esophagus, lung and head and neck squamousHNSCC (9,10,11). Several studies have evaluated the relationship between the tumor suppressor protein p16 and HPV, and have proposed the over-expression of p16 reflect the biologically active HPV infection giving the functional inactivation of pRb (by HPV oncoprotein E7) induced p16 upregulation (12,13). The present study is the first study in Iraq which explains the relationship between the high -risk HPV and the cell cycle proteins(p53 and p16) disregulation in sinonasal and nasopharyngeal lesions.

Materials and Methods

The study was designed as a retrospective one. Totally, 125 formalin- fixed paraffin-embedded tissue samples (81 male, 44 female)with age ranging from 1to 82 years were collected from archives of histopathological laboratories of several hospitals in Baghdad (Ghazi Al-Hariri Teaching Hospital, Al-kindy Teaching Hospital, Al-Yarmouk Teaching Hospital,) during the period extended from February to November 2015 and were related to the past four years(2012 ,2013 ,2014 and 2015). The collected samples include 6 sinonasalcarcinomas(SNC), 29 nasopharyngeal carcinomas (NPC), 35 sinonasalpapillomas(SNP). 35 inflammatory nasal polyps(INP) and 20 normal sinonasal tissues as apparently healthy control. All clinical data were taken from the accompanied histopathological reports with those collected archival tissues blocks. A confirmatory re-examination of each block was done by consultant histopathologist.

The study was carried out in the Research Laboratories at Communicable Diseases Research Unit/ Baghdad Medical College. The paraffin blocks are sectioned serially at 4 µm thickness and stuck on charged slides. Chromogenic In Situ Hybridization technique (CISH) and (ZytoVision /Germany) kits were used for detection HPVs DNA in tissue sections. CISH assays were done according to the manufacturer company protocols.

The ZytoFast Plus Implementation kit HRP-AEC: T-1062-40 and Digoxigenin – labeled DNA probe (ZyoVision GmbH, Bremerhaven / Germany: T-1056-400) were used for detection HPV 16/18 genotypes the signals were detected as bright red discoloration at the sites of complementary sequences and counter stained with nuclear blue solution. The positive reactions were performed by adding specific gene probe to reagents, while the negative control was contained all reagents except the diluted probe. Quantification in situ hybridization signal was assessed under light microscopy at X1000.Scoring and intensity of signals were based on the number and the strength of positive signals, respectively. The positive signals were counted in ten fields of 100 cells for each tissue section and the average of positive signals was scored according to one of the following score categories 1-25%: (1), 26-50 %:(2) and> 50%: (3). A scale of 0-3 was used for recording the relative intensity where 0 corresponding to no detectable signal and 1, 2, 3 refer to low, moderate, and high signal intensity, respectively(14).Immunohistochemical (IHC) assays were used to evaluate p53 and p16 genes expression in sinonasal and nasopharyngeal lesions by using (ab 80436 – Expose mouse and rabbit specific HRP/DAB detection IHC Kit, Abcam/England) for detecting anti-p53 antibody [Mouse monoclonal Anti-p53

antibody ,ab26, abcam/ England)and anti -p16 antibody[Rabbit monoclonal Anti- P16 ARC antibody ab51243 abcam/ England] and assays were done according to the manufacturer company procedures .

The positive result appears as dark brown precipitate in nucleus or cytoplasm of p53 and p16, positive cells on tissue sections. Signals were evaluated under light microscopy at X1000 and the scoring of positive results were done as follow: No positive cells (0), 1-25% positive cells (+), 26-50% positive cells (++) and 51-100% positive cells (+++)(14). The intensity of IHC staining was based on theintensity of positive cell staining also a scale of 0-3 was used to determine the relative intensity as in the following: IHC negative reaction: 0,low intensity: (1),moderate intensity: (2) andhigh intensity: (3).In this study Chi-Square test (χ^2), Odd ratio andSpearman's rho were used to assess the significances between variables and the statistical analysis was done by SPSS program (version-18).

Results:

Detecting of High- Risk HPV16/18 DNA:

The positive signals for theHPV16/18-DNA-CISH test in sinonasal and nasopharyngeal cancers were observed in (31.4%:11).Insinonasal papilloma group, the tissues with positive signals were (25.7%:9) and those inflammatory nasal polyps tissues the positive signals were (11.4%:4). While in theapparently healthy nasal control group, the positive signals were (5%:1).Statistically, there are non- significant differences (P>0.05) among the studied groups. The results have revealedthat SNC-NPC and SNP have odds ratio value of 8.708 (770.8%) and 6.577(557.7%), respectively, while INP has anodds ratio of 2.452(145.2%) (Table.1 and 2).

Table (1): **The HPV16/18 DNA-CISH signal results of the studied groups according to their scores.**

HPV16/18- CISH signal (scores)		Studied groups				Pearson Chi-Square (P-value)
		Healthy Control	Inflammatory Nasal polyp	Sino-nasal Papilloma	Sino-nasal &naso-pharyngeal Carcinoma	
No stain	N	19	31	26	24	P=0.246 Non Sign. (P>0.05)
	%	95.0%	88.5%	74.3%	68.6%	
+	N	0	1	4	4	
	%	0.0%	2.9%	11.4%	11.4%	
++	N	0	1	2	5	
	%	0.0%	2.9%	5.7%	14.3%	
+++	N	1	2	3	2	
	%	5.0%	5.7%	8.6%	5.7%	
Total	N	20	35	35	35	
	%	100.0%	100.0%	100.0%	100.0%	
Odds ratio			2.452	6.577	8.708	

Non- significant differences (P>0.05)

Table(2): The HPV16/18DNA-CISH signal intensities results among the studiedgroups.

HPV16 /18-CISH signal(intensity)		Studied groups				Pearson Chi-Square (P-value)
		Healthy Control	Inflammatory Nasal polyp	Sino-nasal Papilloma	Sino-nasal &naso-pharyngeal Carcinoma	
NO stain	N	19	31	26	24	P=0.199 Non Sign. (P>0.05)
	%	95.0%	88.6%	74.3%	68.6%	
Weak	N	0	1	1	1	
	%	0.0%	2.9%	2.9%	2.9%	
Moderate	N	1	2	1	4	
	%	5.0%	5.7%	2.9%	11.4%	
Strong	N	0	1	7	6	
	%	0.0%	2.9%	20.0%	17.1%	
Total	N	20	35	35	35	
	%	100.0%	100.0%	100.0%	100.0%	
Odds ratio			2.452	6.577	8.708	

Non- significant differences (P>0.05)

Distribution of P53- Protein among Studied Groups:

In sinonasal and nasopharyngeal cancers group the positive P53-IHC signals were detected in (68.6%:24), in sinonasal papilloma group signals were observed in (34.3%:12) and in those inflammatory nasal polyp positive signals were in (28.9%:10), while in apparently healthy tissues (30%:6). Statistically, there are significant scoring differences (P<0.05) and highly significant intensity differences among studied groups. The results have revealed that SNC-NPC has anodds ratio of 5.091 (409.1%), while SNP and INP have anodds ratio of 1.217 (21.7%) and 0.933(-6.7%), respectively.(Table.3 and.4)**.**

Table.(3): Immunohistochemical signal scoring results ofP53 expression among the studied groups

P53- IHC signal (scores)		Studied groups				Pearson Chi-Square (P-value)
		Healthy control	Inflammatory Nasal polyp	Sino-nasal Papilloma	Sino-nasal &naso-pharyngeal Carcinoma	
Negative	N	14	25	23	11	P=0.034 Sign. (P<0.05)
	%	70.0%	71.4%	65.7%	31.4%	
+	N	3	4	5	11	
	%	15.0%	11.4%	14.3%	31.4%	
++	N	3	5	4	7	
	%	15.0%	14.3%	11.4%	20.0%	
+++	N	0	1	3	6	
	%	0.0%	2.9%	8.6%	17.1%	
Total	N	20	35	35	35	
	%	100.0%	100.0%	100.0%	100.0%	
Odds ratio			0.933	1.217	5.091	

Significant differences (P<0.05)

Table.(4):Immunohistochemical signal intensity results of P53 expression among the studied groups.

P53-IHCsignal (intensity)		Studied groups				Pearson Chi-Square (P-value)
		Healthy Control	Inflammatory Nasal polyp	Sino-nasal Papilloma	Sino-nasal &naso-pharyngeal Carcinoma	
No stain	N	14	25	23	11	P=0.00 Highly Sign. (P<0.01)
	%	70.0%	71.4%	65.7%	31.4%	
Weak	N	4	1	2	2	
	%	20.0%	2.9%	5.7%	5.7%	
Moderate	N	2	3	4	2	
	%	10.0%	8.6%	11.4%	5.7%	
Strong	N	0	6	6	20	
	%	0.0%	17.1%	17.1%	57.1%	
Total	N	20	35	35	35	
	%	100.0%	100.0%	100.0%	100.0%	
Odds ratio			0.933	1.217	5.091	

Highly significant differences (P<0.01)

Distribution of P16- Protein among Studied Groups:

The positive P16-IHC signals in sinonasal and nasopharyngeal cancers group were detected in (51.4%:18), in thesinonasal papilloma group were (60%:21), in inflammatory nasal polyp the positive signals were (37.1%:13), and in apparently healthy tissues (40%:8). Statistically, there are significant scoring differences (P<0.05) and non-significant intensity differences (**(**P>0.05**)** among studied groups.the results have revealed that SNP and SNC-NPC have anodds ratio of 2.251(125.1%) and 1.588 (58.8%), respectively, while INP has anodds ratio of 0.886 (-11.4%).(Table.5 and6).

Table(5): Immunohistochemical signal scoring results ofP16 expression among the studied groups.

P16-IHCsignal (scores)		Studied groups				Pearson Chi-Square (P-value)
		Healthy control	Inflammatory Nasal polyp	Sino-nasal Papilloma	Sino-nasal &naso-pharyngeal Carcinoma	
Negative	N	12	22	14	17	P=0.475 Non Sign. (P>0.05)
	%	60.0%	62.9%	40.0%	48.6%	
+	N	2	5	6	2	
	%	10.0%	14.3%	17.1%	5.7%	
++	N	3	5	11	10	
	%	15.0%	14.3%	31.4%	28.6%	
+++	N	3	3	4	6	
	%	15.0%	8.6%	11.4%	17.1%	
Total	N	20	35	35	35	
	%	100.0%	100.0%	100.0%	100.0%	
Odds ratio			0.886	2.251	1.588	

Non- significant differences (P>0.05)

Table(6):Immunohistochemical signal intensity results of P16 expression among the studied groups.

P16 IHC-signal (intensity)		Studied groups				Pearson Chi-Square (P-value)
		Healthy control l	Inflammatory nasal polyp	Sino-nasal papilloma	Sino-nasal & nasopharyngeal carcinoma	
No stain	N	12	22	14	17	P=0.012 Sign. (P<0.05)
	%	60.0%	62.9%	40.0%	48.6%	
Weak	N	7	5	3	3	
	%	35.0%	14.3%	8.6%	8.6%	
Moderate	N	0	3	10	9	
	%	0.0%	8.6%	28.6%	25.7%	
Strong	N	1	5	8	6	
	%	5.0%	14.3%	22.9%	17.1%	
Total	N	20	35	35	35	
	%	100.0%	100.0%	100.0%	100.0%	
Odds ratio			0.886	2.251	1.588	

Significant differences (P<0.05)

Correlation of HPV16/18,withp16, in Patients with Sinonasal and Nasopharyngeal Lesions :

There are strong positive relationships and significant differences at (p <0.05) between HPV16/18 scores with P 16 scoring {r = 0.335, P = 0.049, (p <0.05)} in sinonasal and nasopharyngeal carcinoma group. In nasal polyps there are strong positive relationships with highly significant differences in correlation between HPV16/18 scores with P16scoring {r = 0.473, P = 0.004, (p<0.01)}.while no correlation between HPV16/18 scores with P16scoring in sinonasalpapillomas .(Table. 7)

Correlation of HPV16/18, with p53, in Patients with Sinonasal and Nasopharyngeal Lesions.

No relationships and non- Significant differences at (P > 0.05) in the correlations of HPV16/18, with p53among the studied groups.(Table.7).

Table(7): Spearman's rho statistical testing to evaluate studied molecular markers scoring in relation with HPV infections in sinonasal –nasopharyngeal lesions.

Spearman's rho (Scoring)		HPV16/18		
		Nasal polyp	Sino-nasal Papilloma	Sino-nasal & nasopharyngeal Carcinoma
p53	r.	-.061	-.114	-.130
	P-value	.729	.515	.455
P16	r.	.473**	.272	.335*
	P-value	.004	.114	.049

***. The correlation is significant at the P < 0.05 level.**
****. The correlation is Highlysignificant at the P < 0.01 level.**

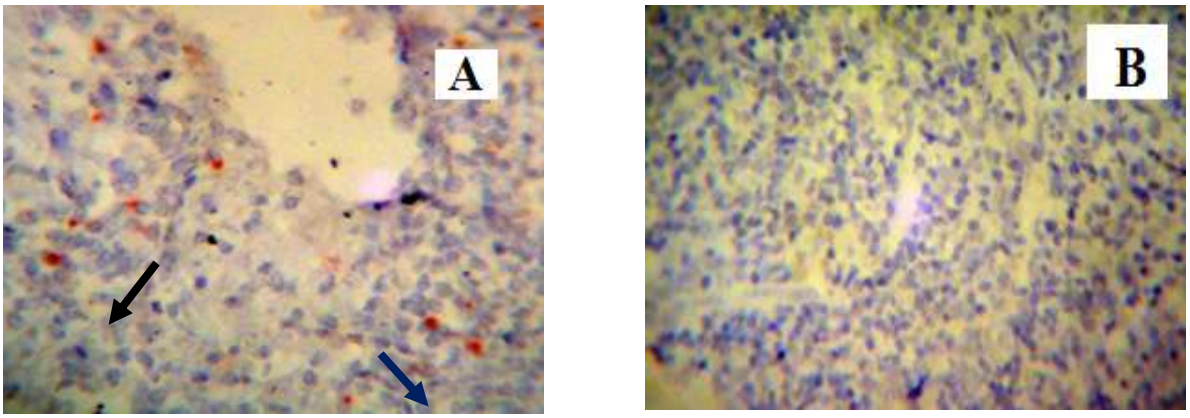

Figure (1): Microphotographs of HPV 16/18 DNA CISH-signals (x400):A-redsignal of HPV16/18 in nasophryngeal cancer tissue (integrated (punctuated) DNA black arrow and diffused signal blue arrow) .B- Nasophryngeal cancer tissue CISH- negative signal.

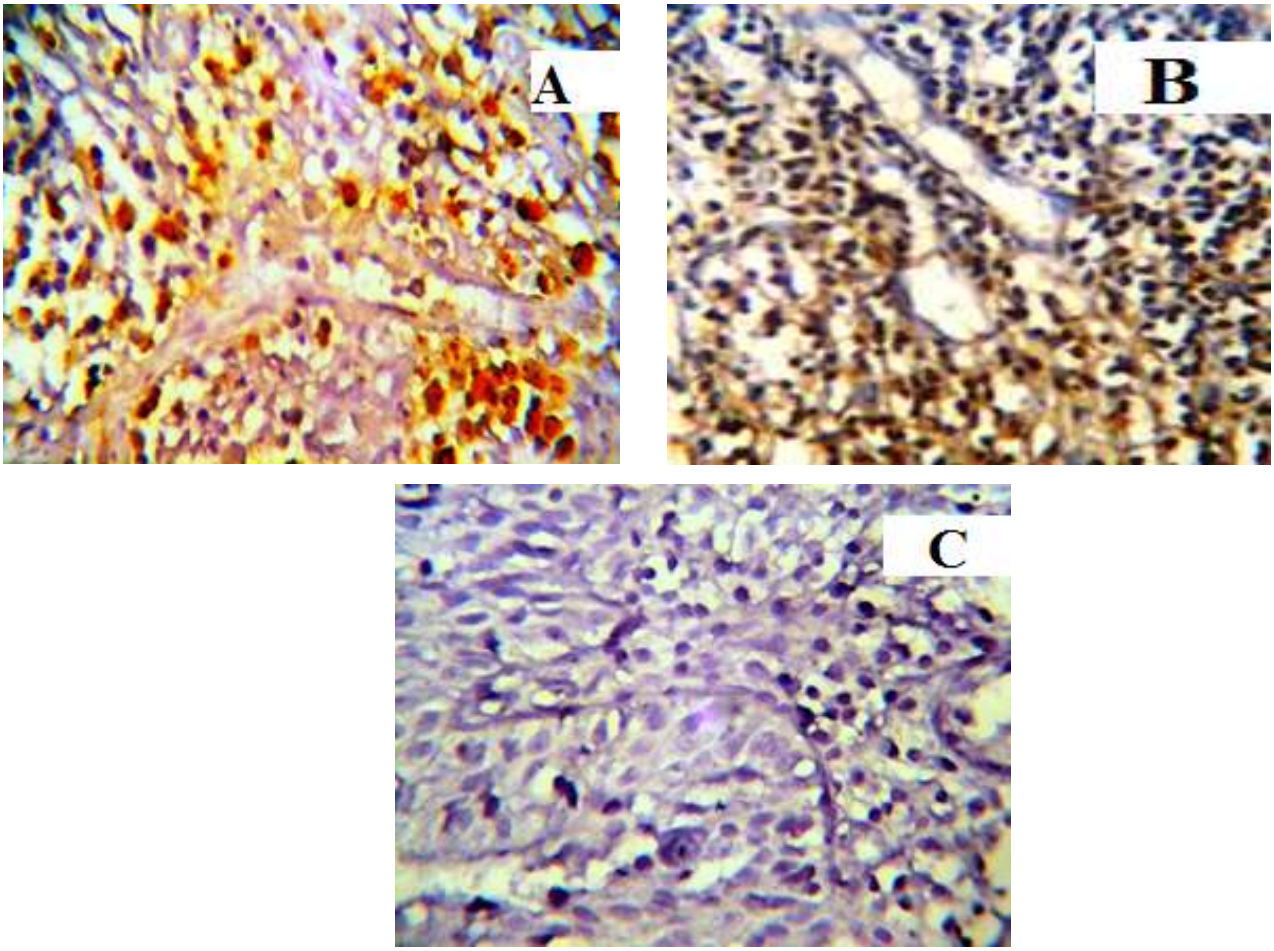

Figure (2): Microphotographs of IHC-signals (x400):A- brown –nuclear signals detected for p53-IHC in Nasopharyngeal cancer tissue. B- Brown –nuclear signals detected for p16-IHC inNasopharyngeal cancer tissue. C.Nasopharyngeal cancer tissue IHC- negative signal.

Discussion:

Head and neck cancers (NHCs) are close related to tobacco smoking, alcohol intake, spicy food, and radiation and virus infections. The differences in anatomical sites of cancer are related to the local factors (such as climate and lifestyle), genetic and mode of viral infection transmission (15). Viral infections are important risk factors for nasopharyngeal and sinonasal carcinoma, such as Epstein-Barr, herpes virus, and HPV infections, particularly the high-risk HPV types (HR-HPV) (16,17).

Detecting of HPV16/18 DNA in Sinonasal and Nasopharyngeal Lesions:

The results of present study coincide with previous studies used PCR and ISH and found that HR- HPV types were prevalent in malignant tumors of sinonasal and nasopharyngeal more than sinonasal benign tumors. A study havebeen found HR-HPV type 16 with a detection rate of (20 %) as the most common type in sinonasal squamous cell carcinomas (SSCC)(18), another study(19) found thathigh-risk HPV DNA positivity insinonasal carcinomas was (21%). Also, oncogenic HPV types 16 and 18positivity were (22.2%) in sinonasal squamous cell carcinomas (SSCC) cases,whileHPV types 16 and 18werenot detected in a sinonasalpapilloma(20)**.**Moreover, high-risk HPV-16 was the most common genotype seen in (16.4%) of NPC patients. in UK (21).A study inmoroccohas revealed that the detection of HR-HPV DNA in NPC cases was (34%)(22).However, one studyhas failed to find an association between HR-HPV and NPC since it has found HR-HPVs detection rates were equally in NPCs (31%) and nasopharyngeal controls (35%)(23).

In contrast to the results of present study, it has been found that HR- HPV types 16 and18, were more common in inverted papilloma (29.4 %), than SSCC (25.0 %)(24).Also,the results of the present study are in

contrast toanother study revealed that HPV16 in nasal polypswas the most common type with a detection rate of 76%(25).

According to the odds ratio values, thepresent study suggests astrong association between HR-HPV 16/18 and (SNC-NPC, SNP) progression. And thelow association between HPV HR-HPV 16/18 and NP progression.

It has been found that HPV high loads and integration may play significant roles in HPV-mediated cell malignancy (26) by activities of two viral oncoproteins, E6 and E7. HPV E6 can induce the degradation of p53 by binding to the ubiquitin ligase (E6AP), resulting ininhibition of p53-dependent signaling upon stress stimuli, and leads to tumorigenesis (27) and the viral E7 protein binds to and inactivates pRb, activating E2F but independent of cyclin-dependent kinase. The inactivation of host pRb by HPV E7 resulting intransfer cell from G1to S phase. (24). According to previous researches, a higher frequency of HR-HPV types was detected in dysplastic and carcinoma of IP (28).One explanation for increased HR-HPV detection rates in benign and malignant tumors of sinonasal tract was hypothesized by that the increasing detection rates of HR HPV types in dysplastic IP and IP with SCC in sinonasal tract as compared with non-dysplastic IP due to the viral tendency for integration and " hit and run" phenomenon in dysplastic IP and IP with SCC that result in losing most of LR-HPV infection as infected cells are shed (29; 26).

Alteration of p53 – Expression in Relation with Sinonasal and Nasopharyngeal Lesions:

According to the statistical analysis of the odds ratio values the present study suggestingimplication of p53 in the pathogenesis and carcinogenesis of SNC-NPC while not implicated inthe pathogenesis and tumorgenesis of SNP and INP.

The present results are consistent with the results of theprevious study that havefound the positivity to p53 in SSCC was (62.5 %)(24) and the current results consist with another study found p53 expression in SSCC was (83 %)(30). Also, the present resultsare in line with the study that found the expression of p53 in NPC was (85.5%) (31)although their results are higher than the present finding.

Another study found overexpressed p53 in (73%) of NPCcases (23).In contrast to the results of present study,one studyfound p53 expression inSSCC was (33.3%)(33).

Regarding benign tumors, the present results are compatible to study that have found the positivity to p53 inSIP was 35.5 %(34)and also are consisted with astudythat found p53 expression in SIP was 26.3%(35). Another study found p53 positivity in IP was19%(36) is lower than the result of the present study whereas the same study has found p53 positivity in NP was 40%(36).Also, the present study is consistent with astudyfound p53 expression in NP patients was 42.9% (37). and the present resultsdoes not consistwith the same study that has found p53 expression in IP was75% (37).

The p53, as a tumor suppressor protein, plays an important role as a guardian of the genome and significant to cellular anti -carcinogenesis. (38). Some studies indicated the deletion of p53 function is the most common molecular alterations in HNSCC, which is mediated by p53gene mutations, loss of heterozygosity of p53, or binding to HR-HPV oncoprotein E6 (39,40). The HPV-mediated tumorigenesis was demonstrated by the activities of viral oncoproteins, namely E6 and E7. HPV oncoprotein E6 can induce the degradation of p53 by binding toubiquitin ligase (E6AP)then inhibit p53-dependent signaling upon stress stimuli and contribute to tumorigenesis (24,41).

Several studies have found that HNSCC patients with HPV infection are less likely to harbor p53 mutations as compared with those patients without HPV infection (3, 42). The present study suggesting no relationship between HPV infection and p53 dIsregulation in benign and malignant tumors.

Alteration of p16- Expression in Sinonasal and Nasopharyngeal Lesions.

The CDK-inhibitor p16 is a tumor suppressor protein, the main function of p16 is negative regulation of the activity of specific cyclin and cyclin-dependent kinases (CDK) complexes such as cyclin D1, CDK4, and CDK6thus preventing uncontrolled proliferation (43; 44).

According to the statistical analysis of the odds ratio values the present study suggesting theimplication of p16 with the pathogenesis and carcinogenesis of SNP while notimplication with the pathogenesis andtumorgenesis of SNC-NPC and INP.

The results of thecurrent study are consistent with theprevious study found that p16-IHC expression in nasopharyngeal tumors was44% (45) and alsoconsistwitha study found that p16 was detected in 37% of SNC cases (19).The present resultsalso areconsistent with another study found p16 expression was 58.3% in HNSCC(46). In contrast to the results of the present study,one study found weak or negative p16 staining in non-keratinized and undifferentiated SNC types while more likely p16 positivity inkeratinized SNC (47) and also contrast withanother study found the p16 positivity in IP with carcinomatous degeneration was seen in 14% of cases(27).

Regarding benign tumors of sinonasal tract, the results of p16-IHC expression of the current study are consistent withprevious study resultsthat found p16 in IPs was positive in 62.5% (48) and consistent with another studythatfound p16-IHC expression was seen in 88.8% of SNPs cases(59). And also the present results are consistent with a study found an abundant p16 expression in normal respiratory mucosa as well as in SNPsand their adjacent mucosa. (43).Also, the presentresultsarein line with the results of astudy found p16-positivity was 64% in IP patients (27) and also in line with a studyfound that p16 over-expression was traceable non-significant in nasal polyps and nasal turbinates by western blot (25) while in a site analogous to sinonasaltract,one studyfound p16 expression wasobserved in (28%) of tumour-free tonsil samples(50). The loss of p16 (INK4a) due to homologous deletion, point mutation or methylation has also been widely observed in some malignant tumors such as theesophagus, lung, and HNSCC (9,10,11). In addition, the absence of p16 is often a critical event in tumor progression,where at least deletion of one copy is sufficient in the premalignant lesions for giving a selective advantage to cell tumorgenesis (8). The previousstudy has found that knockout mice of p16 have increased cancer susceptibility and more spontaneous tumors when exposed to carcinogenic agents compared to wild-type mice (44).

Several studies have indicated that the replication of HPVs interferes with the normal cell cycle control mechanisms and have suggested that malignant transformation is closely related to these processes and the oncogenic potential of HPV probably due to their ability to modify cell cycle checkpoints, resulting in accumulation and transmission of genetic abnormalities (51,52). Several other studies have evaluated the relationship between the tumor suppressor protein p16 and HPV, and have suggested the expression of p16 as a surrogate marker for HPV infection and proposed the over-expression of p16 reflect the biologically active HPV infection giving the functional inactivation of pRb (by HPV oncoprotein E7) induced p16 upregulation (12,13).

However, the validity of p16 expression as a surrogate marker for HR-HPV infection has not seen in aninverted papilloma. One study has suggested that p16 gene is not lost during the development of SNP, while in the carcinogenesis process a non-HPV related p16 expression was more likely to be inactivated not lost (27). The loss of p16 expression is the most common oncogenic alterations secondary to the loss of chromosomal locus 9p21 (53) and because of other factors such as RB abnormalities or deletion and methylation of the p16 gene can also induce p16 over-expression. Thus, it is not possible to indicate whether the p16 over-expression observed in SNP is HPV-related or not. However, p16 is one of the most frequently lost genes in head and neck cancers, thus it could provide false-negative results for the detection of HPV.

The p16 overexpression in nasal polyps may be a result of simultaneous inflammation and not induced by HPV oncogene dysregulation (50,25) thus, herein p16 expression can be anonspecific marker for detection HPV infections.

Association between HPV16/18 Infection and p16 Expression in Sinonasal and Nasopharyngeal Lesions.

The present study reveals significant relationships between HPV16/18 infection and the p16 expression in sinonasal and nasopharyngeal cancers.

The results of the present study agree with most previous studies that have found significant relationships between the presence of HPV16/18 and the p16 expression in sinonasal and nasopharyngeal cancers (19,46,54,55). The expression of p16 in HPV-positive carcinomatous tissues is reasonable because p16 over-expression has been accepted as a marker of HR-HPV-associated carcinomas, such as vulva, (56), cervix

(57), penis (58), oropharynx and sinonasal tract (59)and tonsil (60). However, a recent study has reported that p16 over-expression has frequently detected in normal tissues of the tonsil which were not associated with HPV infection (50). Also,another study found a significant relationship between HPV16/18 and over-expression of p16 in HNSCC (46). However, they also found a significant positive correlation between HPV 6/11 and p16 immuno-expression. However, thep16 expression should be still considered as asurrogatemarker for HR-HPV in sinonasal and nasopharyngeal cancers (50,61).The present studysuggests that p16 over-expression is a good marker for detecting HR-HPV insinonasal and nasopharyngeal carcinoma.

Association between HPV HPV16/18Infection and the p16 Expression in the Nasal Inflammatory Polyp Lesions.

The present study reveals significant relationships between theHPV HPV16/18infections and p16 expression in the nasal inflammatory polyps.

These results are supporting the hypothesis that p16 overexpression in nasal polyps may be a result of simultaneous inflammation and not induced by HPV oncogene dysregulation according toprevious study (50) that have reported p16 expression can be detected in tumor-free tonsil suggesting that p16 accumulation, if observed in tumor-free tissue, may relate to accelerated cell ageing, which induced by chronic infection of epithelium by bacteria, viruses and fungi as well as prolonged activation of lymphocytes and APC dendritic cells in the areas (25; 50). Thus, the present results proposed over-expression of p16 can be nonspecific marker for detection HR- HPV infections in the inflammatory polyps and p16 over-expression might be induced as a result of inflammation.

Conclusions:

The present results suggest implication of HPV16/18 in sinonasal and nasopharyngeal pathogenesis and tumorgenesis **,** significant expression of p53 in sinonasal and nasopharyngeal carcinoma suggesting low association between HPV16/18 and p53 dysregulation and mutation and also indicate implication of p53 over-expression withearly event in sinonasal and nasopharyngeal carcinogenesis, the present results indicated low implication of p16 over- expression with sinonasal papilloma pathogenesis and tumorigenesis whereas not implicated with sinonasal and nasopharyngeal pathogenesis and tumorgenesis, and there is a possible role of HPV16/18 in disregulation of p16 in sinonasal and nasopharyngeal carcinoma.

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