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ORIGINAL ARTICLE

Immunohistochemical study for some markers of Human Papilloma Virus in nasal polyps

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ABSTRACT
This study was completed in laboratories of Biology Department in Faculty of Science. It explains Immunohistochemical study for some markersof human papilloma virus in nasal polyps. We used HPV and P53 markers for Immunohistochemical assay for the patients included in this study, 8.3 and 25 % of cases were positive for HPV and P53 respectively , Although immunohistochemistry marker is considered as highly sensitive for detection of HPV in nasal mass , also, The result illustrated Out of 60 of benign nasal polyp , 31(51.66 %) were inverted papilloma and 29 (48.33%) cases were inflammatory nasal polyp , 15 cases were positive for HPV (9 inverted papilloma and 6 inflammatory nasal polyp) and 5 cases positive for P53(4 inverted papilloma and 1 inflammatory nasal polyp) , The majority of age group included in this study was between (30-39) years.
Keywords: Immunohistochemical study,Nasal polyps,Human papilloma virus

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INTRODUCTION

The nasal polyp histology is typically characterized by pseudocyst formations consisting of edema formation, albumin accumulation, lack of collagen in the extracellular matrix and excessive infiltration of inflammatory cells mainly consisting of eosinophils, although the link between remodeling and inflammation is not fully elucidated , eosinophils are considered the most common and important inflammatory cells in nasal polyp pathogenesis, but there are currently no uniform cut off values for eosinophils to be used as the diagnostic criteria, due to geographic and ethnic differences over time (1).The most common high-risk HPV not only include types 16 and 18 but also include types 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68, an epidemiologic study using high-risk HPV DNA types 16, 18, 31, and 33 demonstrated that 21% of sinonasal carcinoma cases were positive for high-risk HPV, and this HPV infection may act as an oncologic agent (2).

MATERIAL AND METHODS

Immunohistochemical detection kits

Table 1: HPV and P53 kits

No.	Kit	Company/ Country
1	Dako monoclonal mouse anti-human papillomavirus (HPV)	Dako / Denmark
	Clone: K1H8 Code: M3528 Isotype: IgG, kappa Dilution: 1:50 Dako antibody Diluent	
2	Dako monoclonal mouse antihuman P53 protein	Dako / Denmark

	Clone: DO-7 Code number: M7001 Immunogen recombinant human wild -type P53 protein Isotype: IgG2b, kappa Dilution: Ready to use	

Histological study

After performing the surgery to remove the nasal polyp, the specimensends to the histopathology laboratory, where the specimens are preserved in formalin at a concentration of 10% for the purpose of tissue fixation, the specimen is placed in paraffin mold and treated with alcohol and xylene, specimens were exposed to thin section instrument to (four micron) slices in density, and assorted on charged slides, then hematoxylin and eosin staining procedures occur by the following steps:

1. Wax removal by oven for two hours at 60 C.
2. The slides were carried to xylene jar and two times alteration of xylene was performed for 5 min.
3. Rehydrated in two times modification of one hundred percent ethanol for 2min.
4. Put slides in 90% ethanol for 2min.
5. put slides in 80% ethanol for 2min.
6. Put slides in 70% ethanol for 2 min.
7. Put slides in distilled water for 2 min.
8. Put slides in hematoxylin for 3 min
6. Washed in in running tap water for 10 min.
7. Put in Eosin stain for 1 min.
8. Put slides in 70% ethanol for 2 min.
9. Put slides in 80% ethanol for 2min.
10. Put slides in 90% ethanol for 2min.
11. Put slides in100% ethanol for 2min.
12. Allowed the slide cool progressively at room temperature for at least 20 min.
13. Xylene and mounting.

Immunohistochemical staining method

1. Slide baking: the slides were placed in vertical position in the hot air oven at 60˚C for1 hour.
2. Twice in Xylene for 5 minutes each.
3. Put slide in ethanol 100% for 2min
4. Put in ethanol 90% for 4 min.
5. Put the slide in distilled water for 2 min
6. Retrieval solution: Pretreatment with heat mediated antigen retrieving solution was done according to manufacturer's data sheet. Water filled metal plate with Kaplan jars containing the retrieval solution were heated until 90-95˚C, the slides were put in Kaplan jars and continued heating at the same temperature for 20 minutes, followed by 20 minutes cooling at room temperature. Wash the slides in distal water.
7. Cooling for 20 min then Wash buffer for 10 min.
8. Peroxidase block: 2-3 drops of peroxidase block were used to cover the whole tissue sections in each slide, put in a humid chamber and then incubated at 37˚C for 15 minutes to block endogenous peroxidase activity.
9. Washing buffer for 10 min
10. 100µl of diluted primary antibody was applied to each slide. For all
primary antibodies the slides were incubated within a humid chamber at room temperature and then they kept overnight.
11. In next day morning washing for 10 min and Put the slides in HRP solution for 30 min then Washing for 10 min.
12. Add chromogen (1 mil substrate with one drop from chromogen) for 20 min and Washing with distilled water for 2 min then Put slides in ethanol for 2 min let the slide dry.
13. Xylene and one to two drops of DPX mounting medium were applied to the xylene wet sections then Cover slip.

RESULT AND DISCUSSION

Immunohistochemical expression of P53 and HPV in clinico-pathological variables of nasal polyp patients

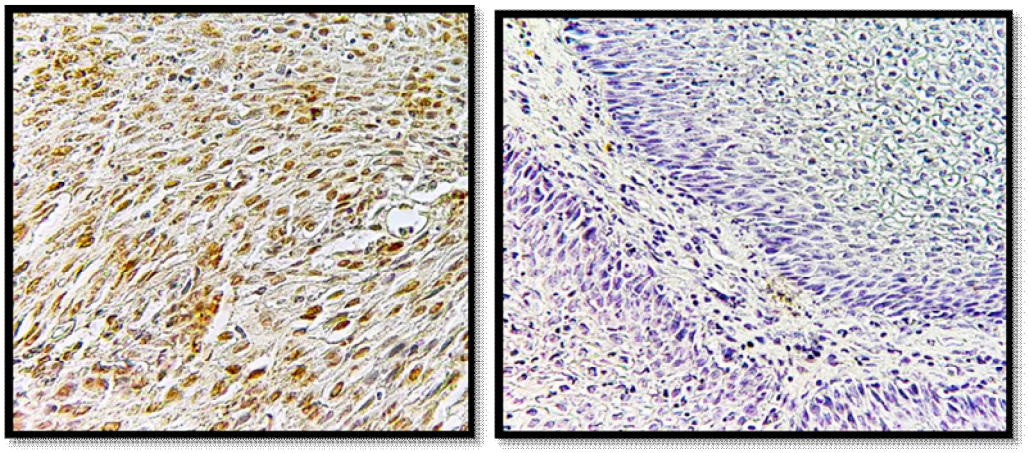
Out of 60 of benign nasal polyp, 31(51.66 %) were inverted papilloma and 29 (48.33%) cases were inflammatory nasal polyp, 15 cases were positive for HPV (9 inverted papilloma and 6 inflammatory nasal polyp) and 5 cases positive for P53(4 inverted papilloma and 1 inflammatory nasal polyp), The majority of age group included in this study was between (30-39) years Table (2).The current results reported the inverted papilloma causes higher then of the inflammatory nasal polyp which similarity with other studies , (3) he showed the inverted papilloma was the commonest benign neoplastic condition (46.2%) in study at Karbala , also , (4) she found the inverted papilloma was the commonest benign nasal polyp (48.7%) in study at Hilla general teaching hospital, while (5) they reported that that the inflammatory nasal causes were 73 (81.1%) compared with the inverted papilloma causes which was 17 (18.8%) . We used HPV and P53 markers forImmunohistochemical assay for the patients included in this study, 8.3 and 25 % of cases were positive for HPV and P53 respectively , Although immunohistochemistry marker is considered as highly sensitive for detection of HPV in nasal mass , other recent studies which pointed out the increasing prevalence of the human papilloma virus in the area of head and neck tumor , (6) they found the study in Czech republic confirmed HPV positivity in 8% of patients with oral squamous cell carcinoma , also , (7) she founded that the percent of 17.2 % of 64 specimens were positive for HPV marker and negative for P53, this can be explained by the viral infection not always overexpress P53, due to the cellular host's dependence on viruses for viability, viruses have developed various strategies to recruit and utilize the host's machinery for their own purposes , as a transcription factor , p53 plays an important role in how cells undergo cellular differentiation , therefore, it is not surprising thatviral targets involving p53 are prominent (8).

Table 2: p53 and HPV markers in clinico-pathological variables of nasal polyp patients

Age groups	P53		HPV	
	Positive (n=5) 60 (100%)	Negative (n=55) 60 (100%)	Positive (n=15) 60 (100%)	Negative (n=45) 60 (100%)
10 - 19	0 (0%)	5 (8.3%)	1 (1.6%)	4 (6.6%)
20 - 29	0 (0%)	8 (13.3%)	1 (1.6%)	7 (11.6%)
30 - 39	3 (5%)	16 (26.6%)	6 (10%)	13 (21.6%)
40 - 49	1 (1.6 %)	13 (21.6%)	3 (5 %)	11 (18.3%)
50 - 59	0 (0%)	8 (13.3%)	1 (1.6%)	7 (11.6%)
60 - 69	1 (1.6 %)	3 (5%)	2 (3.3%)	2 (3.3%)
70 - 79	0 (0%)	2 (3.3%)	1 (1.6%)	1 (1.6%)
Diagnosis				
IP	4	27	9	22
NP	1	28	6	23

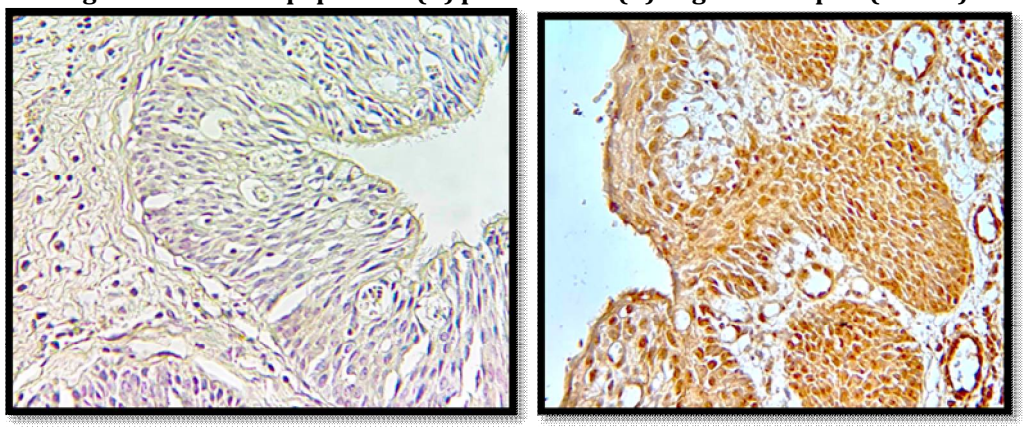
Detection of HPV and P53 microscopically for nasal polyp tissue

The microscopic examination of the tissue sections showed that the positive results for HPV and p53 were appearedin all presented cases discontinuous cell clusters stained , limited to the epithelium , The signals were located in nuclei and cytoplasm , within the epithelium the signals frequently occurred in middle and apical cell layers, seldom in basal cell layers , the stained regions were mostly apparent in epithelial sections also showing epithelial changes, as stratification and ciliumde generation , the stroma consisted of firm and fibrotic tissue and showed minor inflammatory cell infiltration , in nasal polyps cytoplasmatic and intranuclear signals were detected in all cell layers, without clear accentuation. In the stained regions no signs of epithelial stratification or degeneration could be found, beyond the epithelial signals also cells near to the basement membrane in the oedematous stroma stained HPV positive, there was an obvious subepithelial infiltration of inflammatory cells in nasal polyp figure (1-4). Many studies (9) also they used the microscopic examination of the tissue sections for HPV and p53 in sinonasal mass.



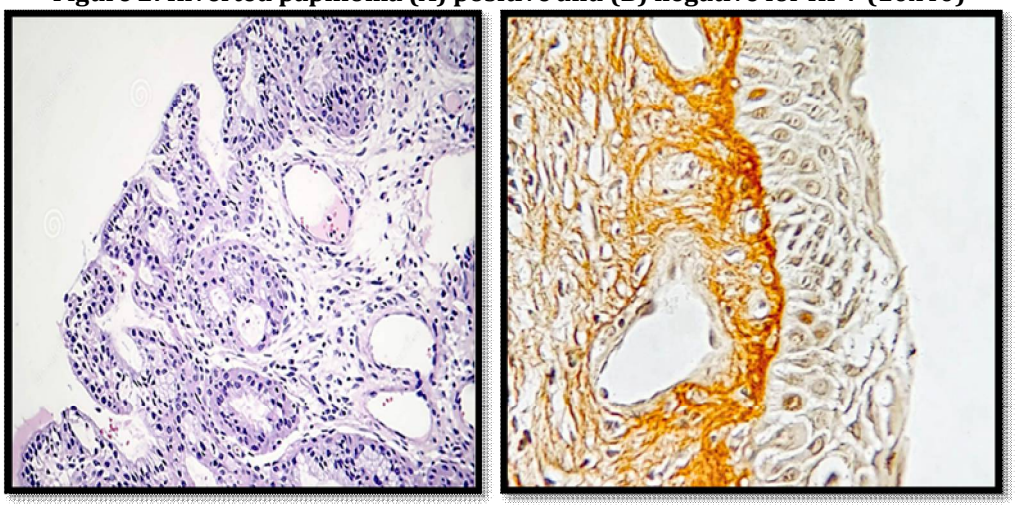
A B

Figure 1: Inverted papilloma (A) positive and (B) negative for p53 (10x40)



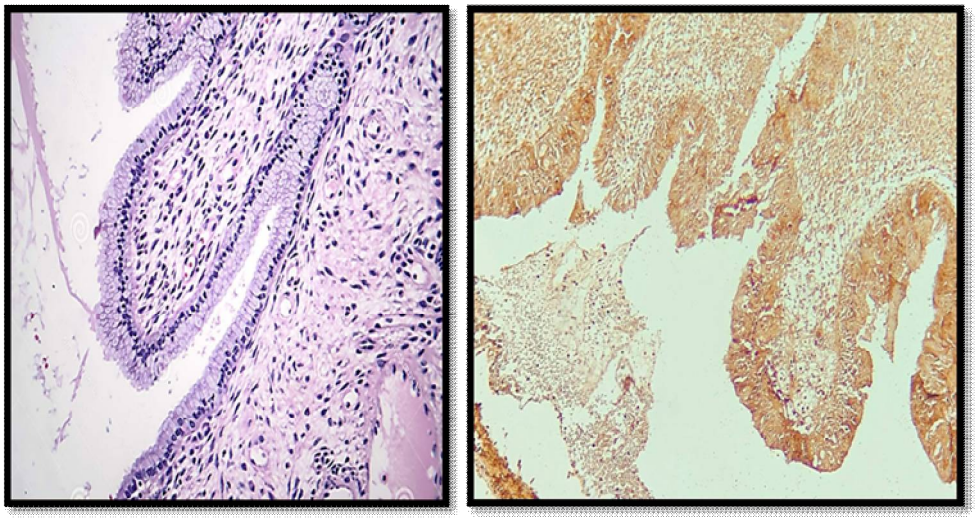
A B

Figure 2: Inverted papilloma (A) positive and (B) negative for HPV (10x40)



A B

Figure 3: Inflammatory nasal polyp (A) positive and (B) negative for p53 (10x40)



A B

Figure 4: Inflammatory nasal polyp (A) positive and (B) negative for HPV (10x40)

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