Detection of human papilloma virus-DNA in sinonasal inverted papilloma by PCR

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Background: The exact etiology of inverted papilloma (IP) is still unclear. Studies using in situ hybridization (ISH) and polymerase chain reaction testing (PCR) have detected HPV in up to 86% of IPs. But other various factors such as smoking have also been implicated. Mostly HPV-6, 11, 16 and 18 have been found to be correlated with IP. The presence of HPV-DNA in IP have been found to be associated with higher chance of recurrence and malignant transformation. Several methods are used for HPV detection includes ISH, PCR, immunohistochemical (IHC) staining for P16 protein and others. Till now PCR is the most accurate method as it is a highly-sensitive, widely-available and cost-effective.

Objective: This study aims to detect HPV-DNA and its subtypes in sinonasal IPs specimens by PCR.

Study design: A prospective case control study.

Methodology: The study included 26 patients, 21 cases presented unilateral nasal mass that was proved pathologically to be IP and 5 controls. IP was managed in all cases by endoscopic medial maxillectomy. Two sections at least were taken from the specimen. One section was stained by Hematoxylin and Eosin (H&E) for pathological confirmation and the other was used for PCR. Patients were followed up for 12 months to detect recurrence and malignant transformation. HPV-DNA was extracted from tissue samples and was detected by PCR amplification using consensus primers (My09, My11). Each HPV-DNA was examined separately for the genotype 6, 11, 16, 18 by specific primer.

Results: Inverted papilloma was detected in 76.2% of cases (n=16), exophytic papilloma in 9.5% (n=2) while oncocytic papilloma was detected in 14.3% (n=3) of cases. Squamous type represented 9.5% (n=2). Intermediate (transitional or cuboidal) type represent 28.6 (n=6) while the mixed types possess the highest percentage; 61.9% (n=13). HPV-DNA was detected in 28.6% (n=6) out 21 cases of IP, while none of the controls demonstrated HPV-DNA. Using PCR, 14.3% (n=3) of the positive cases was positive for HPV-6, 9.5% (n=2) was positive for HPV-11 and 4.8% (n=1) was positive for HPV-18. Recurrence was noted in 4.7% (n=1) of cases during follow up period as proved by biopsy. While, no malignant transformation was noticed.

Conclusion: HPV could be detected in 28.5% of IP with subtypes 6, 11 and, 18. The correlation of HPV and IP is not fully understood. So, the etiology of inverted papilloma is still unclear and, need more researches and more number of cases with another method of detection which may be more accurate such as E6, E7 mRNA.

Keywords: Inverted papilloma, polymerase chain reaction testing, PCR, Human papilloma virus, HPV.

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Introduction

The inverted papilloma (IP) is a benign epithelial tumor of the nasal mucosa and paranasal sinuses. It arises from the lateral nasal wall or within the maxillary sinus. [1] It comprises about 0.5 - 4% of primary nasal tumors. [2] It is known by its local aggressiveness, associated malignancy, high rate of recurrence. [3]

Pathologically sinonasal papilloma is classified according to their pattern of growth into papilloma with endophytic growth that is known as inverted papilloma (IP) and papilloma with exophytic growth that is called fungiform papilloma. The third type is known as cylindrical cell papilloma. [4]

Its exact etiology is still uncertain. Studies using in situ hybridization (ISH) and polymerase chain reaction testing (PCR) have detected HPV in up to 86% of IPs. But other various factors such as smoking, exposure to certain chemicals, allergy and chronic inflammation have also been implicated. [5]

Mostly HPV-6, 11, 16 and 18 have been found to be correlated with IP. The presence of HPV-DNA in IP have been found to be associated with higher chance of recurrence and malignant transformation. [6]

In meta-analysis, Syrjänen have found HPV-6 and 11 in 31.5% of sinonasal IP, whereas 27.8% of sinonasal IP contained HPV-16 and 18. Different patterns of HPV subtypes were also found. [7]

Several methods are used for HPV detection includes ISH, PCR, immunohistochemical (IHC) staining for P16 protein and others. [8] Till now PCR is the most accurate method as it is a highly-sensitive, widely-available and cost-effective. [8] This study aims to detect HPV-DNA and its subtypes in sinonasal IPs specimens by PCR.

Materials and Methods

This prospective study was carried out in the otolaryngology department, Faculty of Medicine, Fayoum University. It included 26 patients, 21 cases presented unilateral nasal mass that was proved pathologically to be IP and 5 controls. The local ethical committee approved this study. Written consents were obtained from all patients.

IP was managed in all cases by endoscopic medial maxillectomy. Two sections at least were taken from the tumor and routinely processed. One section was stained by Hematoxylin and Eosin (H&E) for pathological confirmation and the other was used for PCR. In the control cases, biopsies were taken from the inferior turbinate during septoplasty surgery.

Patients were followed up for 12 months to detect recurrence and malignant transformation. HPV-DNA and subtypes were searched for in cases of recurrence and/or malignant transformation.

For the histopathological features, IP slides were evaluated in each case commenting on the lesion pattern, epithelial type, pathological changes and grade of dysplasia.

PCR

HPV-DNA was extracted from tissue samples after deparaffinization using DNA extraction kit (QIA-amplification extraction kit (Qiagene, USA)). [6] The concentration of the extracted DNA was determined by using spectrophotometer at wave length 260 nm. Enzymatic amplification was performed by PCR using Taq polymerase enzyme and T-Gradient thermal cycler (Biometra, Germany). HPV was detected by PCR amplification using consensus primers (My09, My11). Each HPV-DNA was examined separately for the genotype 6, 11, 16, 18 by specific primers using PCR. Gel electrophoresis and ultraviolet light transillumination were used in detection of PCR amplified products. The amplified products of HPV by consensus primers gives 450 and genotype specific yielded 280, 360, 152, 217 for genotype 6, 11, 16 and 18 respectively **(Figs. 1-4)**.

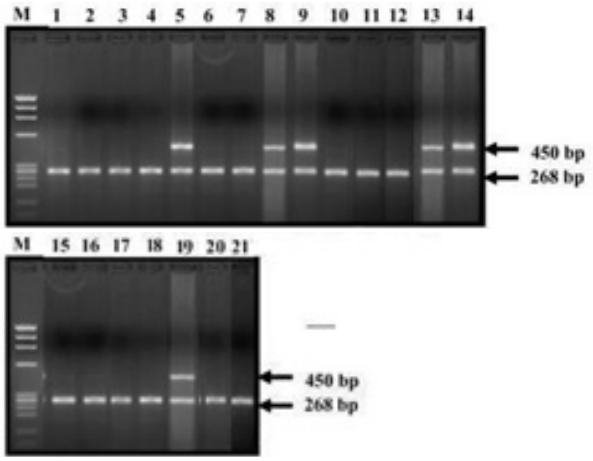

Fig 1. Agarose gel electrophoresis of HPV using consensus primers showing PCR amplification at 450 by and the house keeping gene at 268 bp.

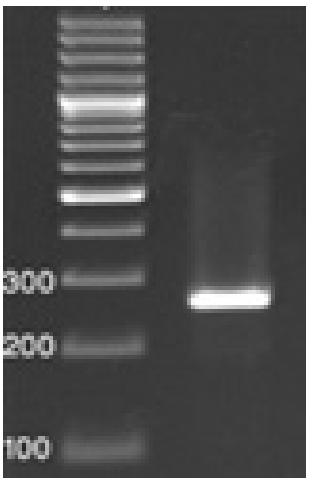

Fig 2. Sample with genotype 6 (280 bp).

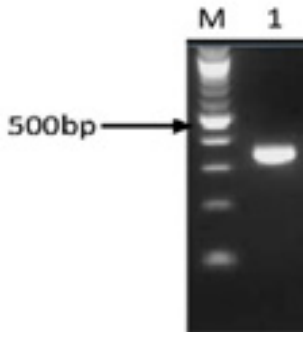

Fig 3. Sample with genotype 11(360 bp).

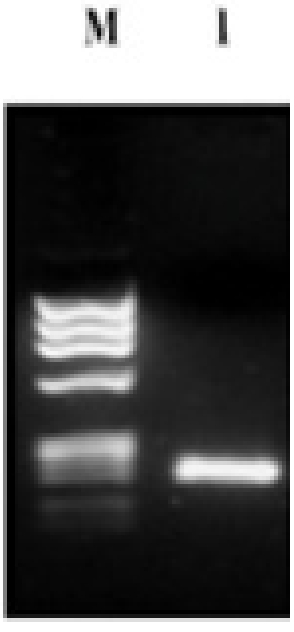

Fig 4. Sample with Genotype 18 (217 bp).

Results

This study included 21 cases having IP; 18 males and 3 females. It also included 5 control males. **(Table 1)** summarizes the demographic data of both groups.

Table 1. The demographic data of both groups.

		Cases		Control	
		N	%	N	%
Age	Range	27-80 years		20-42 years	
	Avg	47 years		28.5 years	
Gender	Male	18	85.7%	5	100%
	Female	3	14.3%	0	0%
Total		21	100%	5	100%

Histopathology:

On examining the histopathology, the following lesion patterns were noticed **(Table 2)**. Inverted papilloma was detected in 76.2% of cases (n=16), exophytic papilloma in 9.5% (n=2) while oncocytic papilloma was detected in 14.3% (n=3) of cases.

Table 2. The lesion patterns noticed by histopathology.

Lesion pattern	Number of cases	Percentage
Inverted papilloma	16	76.2%
Exophytic papilloma	2	9.5%
Oncocytic papilloma	3	14.3%
Total	21	100%

The epithelial types are shown in **Table 3**. Squamous type represents 9.5% (n=2). Intermediate (transitional or cuboidal) type represent 28.6 (n=6) while the mixed types possess the highest percentage; 61.9% (n=13).

Table 3. The epithelial types by histopathology.

Epithelial type	Number of cases	%
Squamous	2	9.5%
Intermediate (transitional or cuboidal)	6	28.6%
Mixed (Squamous, columnar or squamous and intermediate)	13	61.9%
Total	21	100%

HPV-DNA was detected in 28.6% (n=6) out 21 cases of IP, while none of the controls demonstrated HPV-DNA **(Table 4)**.

Table 4. HPV-DNA.

	Control	IP cases
Negative	5	15
Positive	0	6
Total	5	21

Using PCR, 14.3% (n=3) of the positive cases were positive for HPV-6, 9.5% (n=2) were positive for HPV-11 and 4.8% (n=1) was positive for HPV-18 **(Table 5)**.

Table 5. Results of PCR and genotyping.

	N	PCR and genotyping		
Negative	15	71.4%		
			HPV-6	3	14.3%
Positive	6	28.6%	HPV-11	2	9.5%
			HPV-18	1	4.8%

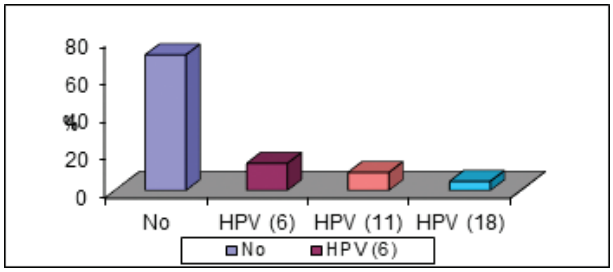

Fig 5. Results of PCR.

Recurrence was noted in 4.7% (n=1) of cases during follow up period **(Table 6)** as proved by biopsy. HPV-DNA was not detected in that specimen. While, no malignant transformation was noticed.

Table 6. Recurrence rate.

	Number of cases	%
Negative	20	95.3%
Positive	1	4.7%
Total	21	100%

Discussion

The etiology of IP is still uncertain. There are major controversies whether, HPV is involved in the pathogenesis of IP or not. Studies have detected HPV in 0-86 % of IPs using ISH and PCR. But also HPV can be detected in normal mucosa. [9,10] Other various factors have been also suggested such as smoking, exposure to certain chemicals, allergy and chronic inflammation, these factors have not been proved yet. [5] Published reports have found that HPV type 6, 11, 16 and 18 are correlated with IP. It is also known that detection of HPV-DNA in IP have been found to be associated with higher chance of recurrence and malignant transformation. [6]

Papilloma may be exophytic or inverted depending on the site of HPV infection. In the nasal septal mucosa, it is exophytic while in the lateral nasal wall and/or paranasal sinus mucosa, it is IP. For unknown reasons, IP epithelium tends to be nonkeratinized. Hence, viral replication and reinfection rarely or never occur. As the superficial epithelial cells are shed, HPV can be lost from the lesion. This partly explains why HPV 6/11 infection rate in IP is lower than in exophytic papilloma. The progression of IP to dysplasia and malignancy may be due to secondary infection or integration of HPV-16/18. It is also suggested that carcinomas may develop in IP because of decreasing cellular apoptosis, which is triggered by HPV infection. [11]

In our study, the lesion pattern was inverted pattern in 76.2% (n=16) of cases, exophytic in 9.5% (n=2) and oncocytic papilloma in 14.3% (n=3) of cases. Yoskovitch et al has publish a similar report. [12] They have found 76.4% IP, 18.1% fungiform type and 5.5% mixed type. In another report including 43 cases, IP was found to be 79%, exophytic type was 12% while the mixed type was 9%. [13]

On reviewing the literature, HPV subtypes found in IP were 6, 11, 16 and 18. One study [9] reported the presence of subtypes 6,11,18 in 29.4% of IP cases out of 68 patients. Another report [14] has found types 6,11,16 in 32.8% of cases out of 67. Also, Kim and coworkers, [15] reported the presence of subtypes 6,11,16 and 18 in 25% of cases out of 28. In meta-analysis by Syrjänen, [7] a total of 1041 IPs were analyzed. HPV-6 and 11 was detected in 31.5% of IP whereas HPV-16 and 18 was detected in 27.8% of IP. On the other hand, Judd et al, [16] have not found HPV-DNA in 9 cases of IP using PCR, ISH, and IHC. That results may be due to the low number of cases. In our study, 28.5% of cases were positive for subtypes 6,11,18 out of 21 cases which is nearly the same results as the published reports.

HPV can be detected by PCR, ISH, IHC staining for p16 protein and E6/E7 mRNA method. [8] Testing for HPV E6/E7 transcripts by RNA-ISH is an ideal platform for HPV detection. It confirms the presence of integrated and transcriptionally active virus by permitting the visualization of viral transcripts directly in tissue sections. It is also technically feasible and easily transferrable into the diagnostic pathology laboratory. There was also a high rate of concordance (99%) between the E6/E7 mRNA method and HPV-DNA. [17] Though, we could not use it in our study because of the unavailability.

PCR and ISH are the most commonly used methods in literature because of their high sensitivity and specificity and estimation of viral load. But, ISH has a reduced specificity at low viral load, so we preferred the PCR as it is more accurate than ISH.

The present study included a comparative investigation of HPV-DNA prevalence in patients with IPs and a control group. None of five controls were positive for HPV using PCR. Lawson and co-workers [11] reported that none of 216 sinonasal tissue samples and 91 sinonasal polyps was related to HPV infection. In contrast to these findings, Jenko and associates, [9] reported 6 (13%) positive cases for HPV out of 46 healthy persons biopsied from there nasal mucosa using PCR. Also, Bryan et al, [10] found HPV as high as 60% (9/15) of specimens of nasopharyngeal mucosa. Studies using PCR revealed a high prevalence of HPV-DNA in a histologically normal oral mucosa. The previous results support the idea colonization of the virus by itself in normal mucosa is not sufficient to produce obvious histologic changes. [18]

In view of the clinical and morphological evidence of IPs, some doubts arise as to whether HPV infection is the most decisive etiological factor. We raised the following questions: Why is the virus in IP is site specific to the lateral wall of the nasal cavity? Why is IP invariably unilateral? Why IP does not appear in children who are more susceptible to viral infections than adults? Bearing these doubts in mind and comparing the morphological characteristics of IP with laryngeal papillomas and anogenital warts (condylomas), which are certainly related to HPV infection, we cannot satisfactorily answer these questions. It is therefore not surprising that there is no generally accepted view about the pathogenesis of these lesions. We think the etiology of IP and its relation to HPV need more researches, larger number of cases and more methods of detection.

Conclusion

HPV could be detected in 28.5% of IP with subtypes 6, 11 and, 18. The correlation of HPV and IP is not fully understood. So, the etiology of inverted papilloma is still unclear and, need more researches and more number of cases with another method of detection which may be more accurate such as E6, E7 mRNA.

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