ORIGINAL CONTRIBUTION

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Human papillomavirus in rare unilateral benign intranasal tumours*

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SUMMARY

Background: *Inverted papilloma (IP), oncocytic papilloma (OP), respiratory epithelial adenomatoid hamartoma (REAH) and capillary hemangioma (CH) are benign and rare tumours. OP and IP are associated with squamous cell carcinoma (SCC). Human papillomavirus (HPV) may play a role in malignant transformation.*

Aim: *We aimed to investigate the presence of HPV, inflammation, epithelial dysplasia, and prognostic markers including proliferative cell nuclear antigen (PCNA), epidermal growth factor receptor (EGFR) and p53 in tissue specimens from rare unilateral intranasal benign tumours.*

Methods: *Presence of HPV DNA was detected by PCR. Proliferative cell nuclear antigen, EGFR, p53 expression and the presence of HPV type 16 in tissues were determined by immunohistochemical analysis.*

Results: *We determined the presence of HPV DNA in 2 of 6 IP cases, in one CH patient and one OP patient, but the REAH patient was negative. Histologically, only one specimen with IP was positive for HPV type 16 being the high risk type. The remaining cases were considered as low risk type HPV.*

Conclusion: *Although our patient numbers are limited, there is a significant association between IP and HPV. This is the first study showing the cooccurrence of CH and OP with HPV.*

Key words: nasal papilloma, human papillomavirus, intranasal, tumour

INTRODUCTION

Schneiderian papillomas are benign, locally aggresive lesions that arise in the sinonasal cavity and are associated with a high rate of recurrence. They are morphologically separated into 3 groups: oncocytic papilloma (OP), fungiform papilloma, and inverted papilloma (IP) [1,2]. Inverted papilloma may also be associated with squamous cell carcinoma (SCC) in approximately 5% of patients, although there may be a higher incidence in local recurrent cases [3].

IP is a rare tumour occurring in 0.5 to 7% of all intranasal tumours, thereby representing 4% of all nasal polyps. The incidence of IP is approximately 70% of all sinonasal schneiderian papillomas and for OP it is 3-5% [4,5]. IP is more prevalent in the fifth and sixth decades of life. Males are 4-5 times more frequently affected than females [2].

Respiratory epithelial adenomatoid hamartoma (REAH) was described by Wenig and Heffner in the sinonasal region in 1995 [6]. It is a rare type of hamartoma located in the nasal cavity and paranasal sinuses. REAH is not considered to be a neoplastic process, however there is no molecular evidence supporting or refuting this possibility [7].

Capillary hemangioma (CH) is a rare kind of tumour that may occasionally arise within the nasal cavity and paranasal sinuses. Katori et al. has reviewed nasal hemangiomas that appeared in the English literature since 1985. Among hemangiomas within the nasal cavity, six were capillary and three were cavernous hemangiomas [8].

Bacterial and viral infections, chronic inflammatory conditions, allergies, tobacco, and occupational exposures have all been cited as possible etiologic factors in developing unilateral intranasal masses. Increasing evidence is now pointing toward a viral origin. Although the etiology of IP is still unknown, recent studies using in situ hybridization and polymerase chain reaction (PCR) have detected human papillomavirus (HPV) in up to 86% of IPs [9]. However, no study has been performed in order to reveal the association between HPV and CH, OP and REAH.

More than 120 different types of the HPV have been isolated; more than 40 of these types infect the epithelial lining of the

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anogenital tract and other mucosal areas [10]. The presence of HPV DNA in sinonasal papilloma was reported for the first time in 1987 [11]. In particular, type 6, 11, 16, and 18 were the most frequently encountered viral types [12,13]. The frequency of HPV DNA in IP varies between 0–100%. In a recent review of the literature, the prevalence of HPV DNA in IP was found to be 33% [14]. This big variation may be due to the methods used or geographical differences. HPV may also play a role in malignant transformation. During tumour progression, several growth factors including epidermal growth factor receptor (EGFR) and transforming growth factor alfa (TGR-α), and p53 are thought to play a crucial role. It has been suggested that increased expression of these growth factors may give clues to malignant transformation [2].

In the present study, we aimed to investigate the presence of HPV, inflammation, epithelial dysplasia, and prognostic markers including proliferative cell nuclear antigen (PCNA), EGFR and p53 in tissue specimens from patients with rare unilateral intranasal benign tumours such as IP, OP, CH and REAH.

MATERIAL AND METHODS

Patients

Patients visiting the Department of Otolaryngology, Adnan Menderes University Hospital, Aydin, Turkey, from 1999-2008, were included in the study. The total number of patients with unilateral nasal tumours was nine: 6 IP, REAH, OP and CH one case each. Five male and four female patients were included in the study. The average age of the patients was 54.22 ± 8.5 yrs with a range of 40-67 yrs.

In total, nine tissue samples were studied. Five were fresh tissue specimens and the others were in embedded paraffin. All samples were fixed in 10% neutrally buffered formalin, embedded in paraffin and processed for histological examination. Hematoxylin and Eosin stained sections were graded for dysplasia.

Table 1. Demographic and clinical characteristics of the patients.

Cases	Age/Sex	Post op. f/u period	Diagnosis	Recurrence	CT findings	MRI findings	Surgical approach
1	52/M	Four years	IP	+	Soft TM completely occupying left NC, aeration diminished in all	–	Lateral rhinotomy + ESS
2	51/M	Fifteen months	IP	–	Soft TM in left NC	Left nasal mass isointense in T1W images, isodense in T2W and enhances in solid fashion	ESS
3	67/M	Fifteen months	REAH	–	Obliteration of right OMC, polipoid soft TM in right NC	Non enhancing, non homogenius mass in the right posterior NC, located between IC and MC and also extended to NC	ESS
4	40/M	Ten months	IP	+	Lobulated soft TM in left NC. Located between the MC and IC.	–	Lateral rhinotomy
5	60/F	Ten months	IP	+	Expansile mass in the left NC and MS. Medial wall of MS is eroded.	Soft TM completely obstructing left MS and NC, also expanding to ES and sphenoid sinuses. Isointense in T1W images, hiperintense in T2W	ESS
6	58/M	Seven months	IP	+	Soft TM filling left NC, bilateral MS	–	ESS
7	51/F	Seven months	OP	–	Soft TM originating from left lateral nasal wall and ES	Hipertrophy of left IC	ESS
8	46/F	Six months	CH	–	Polipoid mass in the right anterior NC, which is not clearly distinguished from IC	–	Excision of the mass
9	63/F	Six years	IP	–	Solid TM occupying right NC and MS, destruction of right medial wall of MS, MC and IC	–	Refused surgery

NC: nasal cavity, MS:maxillary sinus, OMC:osteomeatal complex, f/u:follow-up

ESS: Endoscopic sinus surgery, MC:middle concha, IC:inferior concha, TM:tissue mass,

ES: ethmoid sinüs, FS:frontal sinüs, SS: sphenoidal sinüs

M: male, F: female

PCR analysis

DNA was extracted from the tissue samples using the NucleoSpin Tissue Kit (Macherey & Nagel, Düren, Germany) according to the manufacturer's instructions. We used a direct polymerase chain reaction (PCR) method for detecting HPV. Five L of DNA was used for PCR. To detect a broad range of HPV genotypes simultaneously, the consensus primers MY09 and MY11 were used. The primers are targeted to a conserved region of the L1 gene found in all HPV subtypes and amplify a fragment of 450 base pairs. Amplifications were carried out in a Mastercycler (Eppendorf, Hamburg, Germany) with denaturation at 94°C for 45 seconds, primer annealing at 55°C for 45 seconds, and DNA extension at 72°C for 1 minute, and last DNA extension at 72°C for 7 minutes. A total of 35 cycles were used for amplification. Amplicons were electrophoresed through a 2% agarose gel. Clinical samples were checked for DNA quality and the absence of inhibitors of amplification by analysis of the human ß-globin gene [15]. The HPV line assay kit (GenID GmbH, Straßberg, Germany) was used for HPV DNA amplification and genotyping with reverse dot blot hybridization by following the manufacturer's protocol. Briefly, 5 1 of each sample was amplified with biotinylated primers, which amplify the L1 open reading frame. Thereafter, the biotin-label amplified DNA was hybridized with sequence-specific oligonucleotide probes (SSOP) for HPV high-risk types 16, 18, 45, group detection of thirties and fifties high risk types (HPV types 31, 33, 35, 39 and 51, 52, 53), and the low-risk types 6 and 11.

Immunohistochemistry

The sections (5 m thick) were mounted on poly-L-lysine coated glass slides, deparaffinized, and rehydrated. PCNA (code no: AM252-5M, RTU, Biogenex, Netherlands), EGFR (clone 111.6, ready to use; Lab Vision-Neo-markers, Fremont, CA, USA), p53 (code no: MS-1687-R7, Novocastra, Newcastle, United Kindom) and HPV type 16 (code no: AM 362-5M, Biogenex, Netherlands) immunohistochemical stains were applied to slides and detected with the "avidin-biotin-peroxidase complex technique".

The sections were evaluted by a pathologist in a blinded fashion. EGFR, p53 stains were analyzed semiquantatively and scored as a percentage of positive neoplastic cells. The percentage scoring of immunoreactive (IR) tumour cells was as follows: - (0-10 % IR cells) , + (11-50% IR cells), ++ (50-75% IR cells), +++ (>75% IR cells). HPV 16 stain was analyzed as negative or positive. The number of PCNA positive nuclei were calculated as percentage values.

RESULTS

A total of nine patients, six with IP, one REAH, one OP, and one CH in the nasal cavity were enrolled in the study. Some clinical characteristics of the cases are summarized in Table 1. Nasal obstruction was the most common complaint in the majority of cases.

The patients number 1, 4, 5 and 6 were recurrent cases that had previously undergone surgical procedures in other centers. The first six cases were operated by using different surgical approaches (lateral rhinotomy, medial maxillectomy, endoscopic resection). The ninth patient refused any kind of surgical treatment.

The diagnosis of the third patient changed from "nasal polyp" preoperatively to REAH postoperatively. The preoperative biopsy result was IP in the seventh patient and proved to be oncocytic papilloma postoperatively. In all other patients the diagnosis remained the same pre- and postoperatively.

Unilateral nasal mass was demonstrated by paranasal sinus computerized tomographic examination in all patients before the surgical intervention. In addition, magnetic resonance imaging was ordered for the second, third, fifth and seventh patient.

The presence of HPV as well as the inflammation, epithelial dysplasia, and prognostic markers including PCNA, EGFR and p53 of the study population are presented in Table 2. All samples were beta-globin positive except two parafin embedded specimens. In these two specimens, we failed to reveal HPV DNA isolation.

We detected HPV DNA in 2 of 6 IP cases, one OP and one CH patient by PCR. The REAH patient was negative for HPV. All tissues were tested by the HPV line assay kit. Histologically, only one specimen with IP was positive for

Table 2. Histopathological evaluation, HPV, prognostic markers of malignant transformation in IP and REAH patients.

Patient	Diagnosis	Inflamation	Edema	Dysplasia	EGFR %	P53	HPV	PCNA %
1	IP	Minimal-mixed	+	–	20	–	–	30
2	IP	Middle-mixed	+	–	–	–	HPV type 11	18
3	REAH	Minimal-monocytic	–	–	–	–	–	
4	IP	Middle-mixed	+	–	5	–	–	15
5	IP	Minimal-mixed	+	–	5	–	–	35
6	IP	High-mixed			–	–	–	20
7	OP	High-mixed	+	–	–	–	HPV-LR *	15
8	CH	Minimal-mixed	–	–	–	–	HPV-LR	35
9	IP	High-Mixed	+	–	8	–	HPV HR**	20

*LR=HPV low risk (6,11,40,42,43,44) EGFR= epidermal growth factor receptor PCNA=proliferating cell nuclear antigen

**HR=HPV high risk (16,18,31,33,35,39,45,51,52,56,58,59,66,68,73,82)

HPV type 16 which was a high risk type. The remaining cases were considered as the low risk type.
None of the specimens showed dysplasia. All tissue samples, except that from the CH patient, showed minimal degree of PCNA expression. None of the specimens was positive for p53. All cases except one showed mixed inflammation and edema findings.

DISCUSSION

This study was designed to investigate the presence of HPV, inflammation, epithelial dysplasia, and EGFR, PCNA, and p53 expression in tissue specimens from several patients with unilateral intranasal benign tumours.

Schneiderian papillomas are ectodermal lesions arising generally from the lateral nasal wall. These tumours show high recurrence rates, risk of malignant transformation, tendency toward multicentricity and local aggresiveness [16]. IP can also present with dysplasia or SCC. Recently, the interest of viral etiology in this disease type has been growing. Human papilloma viruses have been implicated in the etiology of IP. Multicentricity and high rates of recurrence for IP also point toward a field change and support a possible viral cause [17]. The role of HPV in the development of OP has not been demonstrated thoroughly yet [13]. In addition, the association of HPV in REAH and CH has not been demonstrated either.

We determined HPV DNA in two of six (33%) IP cases, in one CH, and one in OP patient by PCR. Our IP results were similar to recent studies that demonstrated an HPV association of 33% in IP [14]. Some investigators revealed the prevalence of HPV with higher rates. Guan et al. reported the prevalance of HPV type 57 was 78.3% in nasal IP cases by PCR [18]. However, Kraft et al. reported a positive rate of HPV in 11% of their cases [19].

In the over 100 types of HPV that are known to date, only a few are linked with malignant transformation. The viral subtypes 6, 11, 16, and 18 are the most frequently encountered [12,13]. Hwang et al. detected HPV 16 DNA in 2 out of 5 IPs with coexisting SCC [20], and Kashima et al. detected HPV 18 DNA in 1 out of 24 SCCs [21]. Katori et al. presented HPV 16 and/or HPV 18 DNA in 31% of their IP patients. However, in severe dysplasia/carcinoma, the rate increased to 42% - 50% [2]. Lu et al. detected HPV types 6 and 11 in higher rates in the recurrence group, while in the malignant group HPV type 16 and 18 were most frequently encountered [22].

In our study, two IP cases showed the presence of HPV. In the first specimen, which was detected by the HPV line assay kit as a high risk type HPV, proved histologically to be type 16. This case showed a mild degree of PCNA expression and positivity for EGFR.
Unfortunately, the patient refused surgical intervention and did not keep the follow-up controls. The second positive tissue specimen of HPV was detected by the HPV line assay kit as HPV type 11, and regarded as low risk. This specimen showed a mild degree of PCNA expression, dysplasia and p53 expression, but no evidence of EGFR was found. We have also identified a low risk group HPV in our OP and CH cases, however, unable to identify the type of HPV.

It has been suggested that increased expression of EGFR and TGF-α may give clues to malignant transformation and constitute useful early markers in IP carcinogenesis [23]. EGFR has a potent mitogenic activity and regulates the growth, proliferation and differentiation of a variety of cell types through binding to the external domain of the cell surface receptor. Regarding SCC of the nasal cavity and paranasal sinus, only a few reports concerning EGFR have been published. Chao et al. suggested a role for EGFR in the malignant transformation from IP to SCC of the nasal cavity [24]. In the present study, we determined an increase of EGFR in one IP patient, but this case did not show presence of HPV.

The measurement of p53 protein and PCNA index may be helpful in estimating prognosis and directing clinical treatment in carcinoma or displasia [25,26]. Affolter et al. investigated a largely normal to an high p53 overexpression status in IP samples which could be associated with HPV infection [27]. The mechanisms of action of HPV in oncogenesis have been attributed to its ability to render the p53 tumour suppressor gene of the host cell ineffective [11]. Katori et al. has suggested that investigation of p21, p53 and HPV would be helpful in distinguishing the lesions that have the potential of carcinoma development and dysplasia [2]. Dysplasia and the presence of p53 were not demonstrated in any of our cases. In the literature, there is no study investigating the relation between HPV and REAH. In our histological examination of one specimen diagnosed as REAH, it showed edema, dysplasia. Besides the absence of EGFR and PCNA expression, as well as having a low degree of inflammation, a monocytic cell infiltration support that REAH cases do not have a tendency for malignant transformation.

Oncocytic papilloma shows similar clinical characteristics as IP. The etiology of OP remains unknown [14]. This is the first study that showed the presence of HPV in an OP case. This may suggest that HPV plays a role in developing OP as demonstrated previously in IP etiology.
The recurrence rate changes from 25% to 35%, and simultaneous malignant transformation has been observed in some of the cases [16]. In our OP patient, a high level and a mixed type of inflammation and edema were observed. Additionally, no evidence of cancer markers (EGFR and p53), but only low levels of PCNA were detected.

Hemangiomas are benign tumours that originate in vascular tissues of skin, mucosa, muscles and glands [8]. Traumatic or

hormonal etiology have been implicated in the development of these tumours, however the role of microbiological agents has not been determined [28].

Our study is the first demonstration of the positivity of HPV in CH. In previous studies, it has been revealed that some viruses and bacteria cause damage to the endothelium of bloodvessels [29,30]. We suggest that the finding of positivity of HPV in CH should be investigated in many other cases, since we have only one patient with CH in our study group.

In conlusion, this is the first study showing the cooccurrence of HPV in CH and OP. It is suggested that, although our patient numbers are limited, there is a significant association between IP and HPV. It is recommended that in benign unilateral nasal tumours HPV should also be studied in etiology. These findings should be confirmed by further studies with larger number of patients.

REFERENCES

1. Christensen WN, Smith RR. Schneiderian papillomas: a clinicopathologic study of 67 cases. Hum Pathol. 1986; 17: 393-400.
2. Katori H, Nozawa A, Tsukuda M. Markers of malignant transformation of sinonasal inverted papilloma. Eur J Surg Oncol. 2005; 31: 905-911.
3. Ringertz N. Pathology of malignant tumors arising in the nasal and paranasal sinus cavities and maxilla. Acta Oto-laryngol. 1938; 27: 31-42.
4. Lawson W, Ho BT, Shaari CM, Biller HF. Inverted papilloma: a report of 112 cases. Laryngoscope. 1995; 105: 282-288.
5. Liu CY, Tsai TL, Hsu CY, Lin CZ. Oncocytic Schneiderian papilloma. J Chin Med Assoc. 2004; 67: 255-257.
6. Wenig BM, Heffner DK. Respiratory epithelial adenomatoid hamartomas of the sinonasal tract and nasopharynx: a clinicopathologic study of 31 cases. Ann Otol Rhinol Laryngol. 1995; 104: 639-645.
7. Ozolek JA, Hunt JL. Tumor Suppressor Gene Alterations in Respiratory Epithelial Adenomatoid Hamartoma (REAH) Comparison to Sinonasal Adenocarcinoma and Inflamed Sinonasal Mucosa. Am J Surg Pathol. 2006; 30: 1576-1580.
8. Katori H, Tsukada M. Lobular capillary hemangioma of the nasal cavity in child. Auris Nasus Larynx. 2005; 32: 185-188.
9. Tang AC, Grignon DJ, MacRae DL. The association of human papillomavirus with Schneiderian papillomas: a DNA in situ hybridization study. J Otolaryngol 1994; 23: 292-297.
10. Steben M, Duarte-Franco E. Human papillomavirus infection: epidemiology and pathophysiology. Gynecol Oncol. 2007; 107: 2-5.
11. Sauter A, Matharu R, Hörmann K, Naim R. Current advances in the basic research and clinical management of sinonasal inverted papilloma. Oncol Rep. 2007; 17: 495-504.
12. Furuta Y, Shinohara T, Sano K et al. Molecular pathologic study of human papillomavirus infection in inverted papilloma and squamous cell carcinoma of nasal cavities and paranasal sinuses. Laryngoscope. 1991; 101: 79-85.
13. Gaffey MJ, Frierson HF, Weiss LM, Barber CM, Baber GB, Stoler MH. Human papillomavirus and Epstein-Barr virus in sinonasal Schneiderian papillomas. An in situ hybridization and polymerase. Am J Clin Pathol. 1996; 106: 475-482.
14. Syrjanen KJ. HPV infections in benign and malignant sinonasal lesions. J Clin Pathol. 2003; 56: 174-181.
15. Giovannelli L, Campisi G, Lama A et al. Human papillomavirus DNA in oral mucosal lesions. J Infect Dis. 2002; 185: 833-836.
16. Kaufman MR, Brandwein MS, Lawson W. Sinonasal Papillomas. Clinicopathologic Review of 40 Patients With Inverted and Oncocytic Schneiderian Papillomas. Laryngoscope. 2002; 112: 1372-1377.
17. Mirza N, Kathleen M, Yuichi S, Kroger H, Kennedy D. Identification of p53 and human papilloma virus in schneiderian papillomas. Laryngoscope. 1998; 108: 497-501.
18. Guan Y, Zang C, Zhang C. Study on correlation between HPV57 infection and the etiology of IP. Lin Chuang Er Bi Yan Hou Ke Za Zhi. 2006; 20: 922-924.
19. Kraft M, Simmen D, Casas R, Pfaltz M. Significance of human papillomavirus in sinonasal papillomas J Laryngol Otol. 2001; 115: 709-714.
20. Hwang CS, Yang HS, Hong MK. Detection of HPV in sinonasal inverted papillomas using polymerase chain reaction (PCR). Am J Rhinol. 1998; 12: 363-366.
21. Kashima HK, Kessis T, Hruban RH, Wu TC, Zinreich SJ, Shah KV. Human papillomavirus in sinonasal papillomas and squamous cell carcinoma. Laryngoscope. 1992; 102: 973-976.
22. Lu SS, Zou R, Xu JW. Relationship between prognosis of nasal inverted papilloma and human papillomavirus types. Zhonghua Er Bi Yan Hou Tou Jing Wai Ke Za Zhi. 2005; 40: 195-198.
23. Mendenhall WM, Hinerman RW, Malyapa RS et al. Inverted papilloma of the nasal cavity and paranasal sinuses. Am J Clin Oncol. 2007; 30: 560-563.
24. Chao JC, Fang SY. Expression of epidermal growth factor receptor in the inverted papilloma and squamous cell carcinoma of nasal cavity. Eur Arch Otorhinolaryngol. 2008; 265: 917–922.
25. Mumbuc S, Karakok M, Baglam T, Karatas E, Durucu C, Kibar Y. Immunohistochemical analysis of PCNA, Ki67 and p53 in nasal polyposis and sinonasal inverted papillomas. J Int Med Res. 2007; 3: 237-241.
26. Guichard C, Gilain L, Abd-Al Samad I et al. Epithelial cell proliferation, apoptosis, and apoptosis inhibition in inverted papillomas. Laryngoscope. 1998; 108: 716-720.
27. Affolter A, Helmbrecht S, Finger S, Hörmann K, Götte K. Altered expression of cell cycle regulators p21, p27 and p53 in tumors of salivary glands and paranasal sinuses. Oncol Rep. 2005; 13: 1089-1094.
28. Truss L, Dobin S M, Donner LR. Deletion (21)(q21.2q22.12) as a sole clonal cytogenetic abnormality in a lobular capillary hemangioma of the nasal cavity. Cancer Genet Cytogenet. 2006; 170: 69-70.
29. Muhlestein JB. Chronic infection and coronary artery disease. Med Clin North Am. 2000; 84: 123-148.
30. Leowattana W. Chronic infections and atherosclerosis. J Med Assoc Thai. 2001; 84: 650-657.

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