

**UNIVERSITY OF MEDICINE AND PHARMACY OF TÂRGU MUREŞ
ROMANIA**

**A CONTRIBUTION TO THE ETIOPATHOGENESIS,
DIAGNOSIS AND MANAGEMENT OF SINONASAL
INVERTED PAPILLOMAS**

PhD THESIS ABSTRACT

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Keywords: inverted papilloma, chronic rhinosinusitis, polyps, squamous cell carcinoma, human papilloma virus (HPV), squamous metaplasia, laser assisted microdissection, polymerase chain reaction, enzyme – linked immunosorbent assay (ELISA)

INTRODUCTION

Since 1991, based on histopathological features, three different types of sinonasal papilloma have been classified by the World Health Organization as exophytic or fungiform papilloma, oncocytic or columnar cell papilloma and inverted papilloma. Inverted papilloma is the most common type and accounts for approximately 70 % of all sinonasal papillomas. It is an uncommon epithelial neoplasm of great interest for the clinicians due to its aggressive clinical behavior, high rate of recurrence and an association with squamous cell carcinoma. Its etiology is controversially discussed in the literature. Theories like viral infections, environmental carcinogens, proliferation of nasal polyps, allergy, chronic inflammation, have been raised.

The mechanism responsible for association of chronic rhinosinusitis with inverted papilloma is yet poorly understood. Some studies suggest that inverted papilloma may be an inflammatory polyp and not a true papilloma, an unusual viral – influenced expression of inflammation or a morphologically altered polyp.

Biomolecular studies show that human papilloma virus may be involved as an important etiological factor in the pathogenesis of inverted papilloma.

The most frequent site of origin is the lateral nasal wall and the ostio-meatal complex. The nasal septum, frontal and sphenoid sinuses are rarely affected. The tumor may disturb adjacent structures extending beyond the paranasal sinuses into the nasopharynx, pterygomaxillary fossa, orbit or brain

Aggressive surgery such as medial maxillectomy via lateral or sublabial rhinotomy has been used as gold standard to treat inverted papilloma.

Because of their tendency to recur, their potential for malignant transformation or association with malignant tumors, a long term follow-up is required after surgery.

Accurate preoperative evaluation with CT and MR imaging and the development of endoscopic sinus surgery has influenced the rhinosurgeons toward the use less invasive intranasal endoscopic approaches to resect sinonasal inverted papilloma

AIM OF THE STUDY

The aim of this study was to bring by clinical and biomolecular research work contribution to the etiopathogenesis of inverted sinonasal papilloma and to develop finally an algorithm for management of patients with sinonasal inverted papilloma.

MATERIAL AND METHOD

Over a 13-year period (January 1999 – December 2011), 79 patients with histologically proven inverted papilloma were analysed retrospectively.

All patient clinical data were retrospectively analysed. The lesions were classified into stages according to the staging system for inverted papilloma proposed by *Krouse*. Every single case was evaluated, and after corroboration of the preoperative nasal video-endoscopy, preoperative CT scans with the intraoperative findings and finally with the histopathological result, the tumor stage has been established.

Patient's complaints, the presence of bone erosions and the association to squamous cell carcinoma were also analysed.

The surgical management that was chosen with the follow-up results, are described and discussed.

The second part of the study was a histological reexamination of all available slides, which especially focuses on the overlying epithelium of the inverted papilloma, the ipsilateral neighboring polyps and the contralateral polyps in patients with inverted papilloma and bilateral chronic rhinosinusitis with polyps. In all cases representative sections of the papillomas were available for examination.

Because human papilloma viruses (HPV) currently seem to be an important candidate as a cofactor in the pathogenesis of the inverted papilloma, we tried to identify the presence of viral DNA in the squamous metaplastic epithelium of the inverted papilloma and the contralateral inflammatory polyp by patients with inverted papilloma and bilateral chronic rhinosinusitis with polyps using polymerase chain reaction – enzyme linked immunosorbent assay (PCR – ELISA).

Serial 10 µ – thick sections cut from representative paraffin – embedded tissues and mounted on pretreated specimen slides were deparaffinated with xylene, rehydrated through a series of alcohol and stained with methylene blue.

DNA was extracted by proteinase K digestion from microdissected tissue fragments using the commercial QIAamp DNA mini Kit (Qiagen, Hilden, Germany).

Extracted preparations were subjected to PCR. The primers GP5 and GP6 which are specific for HPV were used for amplification. Successful amplification of a beta-globin fragment indicated that the samples were adequate for PCR analysis. Amplified products were separated electrophoretically on a 2% agarose gel stained with ethidium bromide.

Pure epithelial cell samples were captured by a manual needle – based microdissection method (figure 1, 2, 3) and by laser assisted microdissection technique.

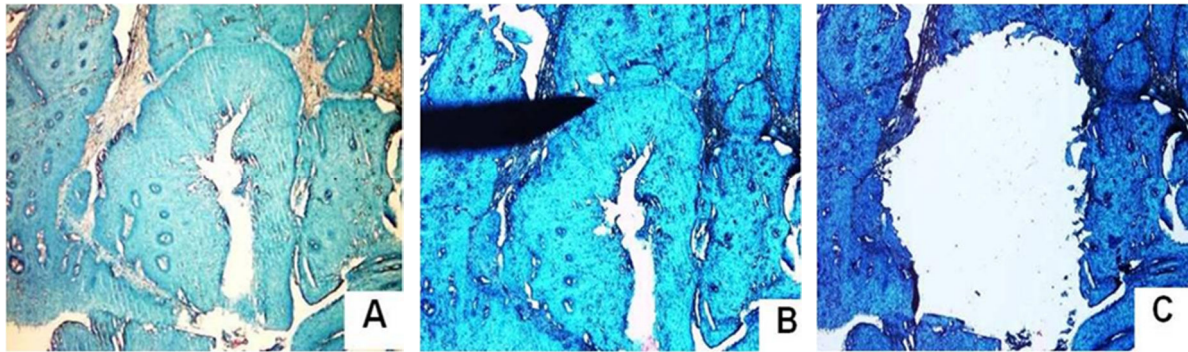


Figure 1, 2, 3 – methylene blue stain of inverted papilloma before (A, B) and after (C) manual needle – based microdissection

After standard PCR, we detected and identified the presence of the subtypes of HPV – DNA (HPV – 6, HPV – 11, HPV – 16, HPV – 18, HPV – 31, HPV – 33) in the squamous metaplastic epithelium of the inverted papilloma and contralateral inflammatory polyps by polymerase chain reaction – enzyme linked immunosorbent assay (PCR – ELISA).

The PCR product was denaturated, labeled with Digoxigenin – DIG and immobilized via biotin to a streptavidin – coated microtiter plate. The immobilized PCR product was then detected with an antibody against digoxigenin that was conjugated to peroxidase. Finally the probe is visualized by virtue of peroxidase metabolizing to form a colored reaction product (figure 4).



Figure 4 – digoxigenin labeled PCR product immobilized via biotin to a streptavidin – coated microtiter plate

RESULTS AND DISCUSSIONS

Out of the 79 patients, 51 were men (64.5 %) and 28 women (35.5 %), confirming the male predominance ($Z = -5.411$; $p < 0.0001$). The peak incidence was registered in the 7th decade of life (60-69 years).

The most common symptom was nasal obstruction (59.50 %) followed in decreasing order by rhinorrhea (41.70 %) and headache (21.5 %). Smell disorders mostly due to the severe nasal obstruction was associated in 13.9 % cases and intermittent nasal bleeding in only 5.1 % cases.

Forty-three patients (54.3 %) were admitted with a stage II inverted papilloma with tumor involving the ostiomeatal complex, ethmoid sinuses and/or the medial portion of the maxillary sinus. Eleven patients (14 %) were admitted with extranasal inverted papilloma extension beyond the sinuses or even malignant transformation, representing stage IV inverted papillomas.

Regarding the adjacent bone erosions that can occur, clinical and/or radiological evidence of bone destruction was found in 18 cases (22.7 %), 9 of whom were found with stage II inverted papillomas, 4 with stage III and 5 with stage IV inverted papilloma.

In concordance with the location of the tumor, bone destructions predominantly involved the lateral nasal wall and the lamina papyracea.

Two patients had evidence of skull base erosions, one of them presenting a dura exposure with dura defect. None of these two patients revealed clinical signs of meningism or any other neurological deficit. In one case inverted papilloma extended into the pterygomaxillary fossa, in two cases there was tumor extension into the soft tissue of the cheek, one case was with tumor extension into the orbit.

Although a benign epithelial neoplasm, inverted sinonasal papilloma has a significant malignant potential. Out of these 79 cases, seven patients (8.8 %) had concomitant inverted papilloma and squamous cell carcinoma.

Out of the seven cases with inverted papilloma and squamous cell carcinoma, two presented with multiple clinical and radiological evidence of focal bone erosions, while five showed no bone destruction.

All but two patients underwent surgical treatment: 29 patients were treated exclusively by an microscopic/endoscopic endonasal approach and 48 patients were treated by a combined microscopic/endoscopic and external approach or exclusively by an external approach.

In the twenty-nine patients treated via an microscopic/endoscopic endonasal approach recurrence occurred in 5 cases (17.2 %), the mean follow-up period was 19.9 months (range 6 – 58 month, median 19.0 month). By the patients treated with an external approach (48 cases) the recurrence occurred in 8 cases (16.7 %), the mean follow-up period was 24.2 months (range 6 – 51 month, median 21.5 month). The postoperative follow-up periods for the

patients operated exclusively by an endonasal approach and those operated by an external approach were not statistically significant (Mann-Whitney statistic = 536.5, $p = 0.093$). Tumor recurrence rates for the two different surgical approaches were not statistically significant ($p > 0.05$).

Our present study shows that in 47 cases (59.5 %) there was inverted papilloma associated with polypoid degenerations of the mucosa, while 22 (27.9 %) of these patients presented clinically with bilateral chronic rhinosinusitis including polyps. Out of the 22 cases with inverted papilloma and bilateral chronic rhinosinusitis with polyps, predominant squamous epithelial metaplasia was found in the inverted papilloma cases ($n = 16$). The ipsilateral neighboring polyps contained transitional epithelium (9 cases) and respiratory epithelium (10 cases), while only in 3 cases squamous metaplasia was predominant. The contralateral polyps showed normal respiratory epithelium in most specimens (17 cases), transitional epithelium in 4 cases and in only one case squamous metaplastic epithelium.

Of the **22** samples with inverted papilloma, **12** were HPV DNA positive (54.5 %): **4** cases with the association HPV - 6 + HPV - 11 + HPV - 16 + HPV - 18, **5** cases with the association of HPV - 16 + HPV - 18, **2** cases with the association HPV - 6 + HPV - 11 + HPV - 16 and **1** case with HPV - 16.

Of the **22** samples with inflammatory polyps, **4** were HPV DNA positive (19 %): **1** case with the association HPV - 6 + HPV - 11 + HPV - 16 + HPV - 18 and **2** cases with the association HPV - 16 + HPV - 18 and **1** case with HPV - 16.

Of the **7** cases with inverted papilloma and squamous cell carcinoma, **3** were HPV DNA positive (42.8 %): **2** case with HPV - 16 + HPV - 18 and **1** case with HPV - 16.

CONCLUSIONS

Inverted papillomas of the nose and paranasal sinuses are locally aggressive, uncommon benign epithelial tumors that can mimic sinonasal malignant growth.

Bilateral inverted papilloma and inverted papilloma of the nasal septum are uncommon and rare.

Wide extent of the tumor and/or bone destruction cannot be taken as indicators for association or transition to malignancy.

The mechanism involved in the pathogenesis of focal bone erosions is debated, but may be related by long-standing pressure generated by the expanding mass or to inflammatory mediators.

Preoperative computer tomography assessment of sinonasal inverted papilloma with evaluation of bone destructions and tumor extension is important in the planning of surgical treatment.

Our results supports transnasal micro-endoscopic surgery to manage inverted papilloma, this approach is an important surgical alternative for selected patients.

Close follow-up after surgery is advocated to detect early recurrence or possible transformation to malignancy. Recurrence can be minimized by an appropriate surgical planning.

Although its etiology is yet unclear, IP is most likely of heterogeneous origin, with HPV related and HPV non related tumors.

HPV infection is not limited to squamous epithelium and inversion but inversion seems to be closely associated with high risk HPV subtypes (HPV – 16).

Association of inverted papilloma with chronic rhinosinusitis including polyps is more likely to be indirect (independent lesions that share common etiologies) rather than direct (stages along polyp with respiratory epithelium – transitional epithelium – squamous metaplasia – inverted papilloma, continuum).

Further work is needed in order to understand and to define the relationship of IP to rhinosinusitis with polyps.