

Possible Prognostic Significance of P53 and Ki 67 in Inverted Sinonasal Papilloma

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ABSTRACT

The aim of this study is to test the possible prognostic significance of p53 and Ki67 expression in inverted papilloma of the lateral nasal wall and adjacent sinuses regarding their malignant potential and recurrence. 49 biopsies of the lateral nasal wall and adjacent sinuses obtained from 41 patients from three hospitals were investigated. Immunohistochemically demonstrated p53 and Ki67 expression was measured and statistically evaluated. p53 immunoreactivity was demonstrated in most of papillomas with carcinomas but only in two benign papillomas, while Ki67 demonstrated stronger immunoreactivity in carcinomas and surrounding epithelium. Immunohistochemical staining of inverted sinonasal papillomas for p53 and Ki67 can give useful information concerning the existence of synchronous carcinoma and, in case of high Ki67, a hint toward possible recurrence.

Key words: head and neck tumors, nasal tumors, inverted papilloma, prognostic factors, Ki67, p53.

Introduction

Inverted papilloma of the nose and paranasal sinuses is a rare tumor of the head and neck region and constitutes 0.5–4% of all tumors of the nose and sinuses^{1–4}. Nevertheless, for decades it has been the subject of interest of many researchers due to several peculiar characteristics. It has been noted that this lesion has a marked tendency to recur as well as a significant association with squamous cell carcinoma of the paranasal sinuses^{1–4}. The reasons for such a behavior have been extensively examined but remained mostly unclear. Different prognostic parameters have been proposed so far. The rate of recurrence reported in the literature varies from 13 to 70% but neither histologic features nor clinical characteristics could be related to it^{1–2}. As the most important cause of recurring disease seems to be incomplete resection⁵. HPV infection proposed by some authors did not show any connection to the tendency to recur.

10–15% of inverted papillomas are reported to be associated with squamous cell carcinoma^{6,7} although rates as high as 50% have been found⁸. Squamous cell carcinoma can be associated with inverted papilloma in two ways: in the majority of cases it is present simultaneously either as a small focus or as the dominant part (synchronous carcinoma), in the remaining cases the carcinoma appears in the area where an inverted papilloma has previously been resected (metachronous carcinoma). Considering the close association of these two entities it has been speculated that inverted papillomas might act as precursor lesions for the development of malignant squamous cell carcinoma. New studies have therefore tried to elucidate the etiology of both benign inverted papilloma as well as carcinoma associated with inverted papilloma in order to better understand the nature of their association. The understanding of the crucial steps

in malignant alteration could help us identify the patients at higher risk for developing a malignant change.

Recognizing the role that the tumor suppressor p53 plays in a large number of human cancers^{8–10} the possible role of p53 in inverted papilloma has also been investigated. In various recently published papers the presence of mutated p53 has been demonstrated in inverted papillomas^{9,11–13}. The frequency of p53 mutations is significantly higher in inverted papillomas associated with malignant change (either synchronous or metachronous carcinoma) compared to the benign forms. The frequency of over expression of p53 in benign papillomas varies depending on the study from 0–26% but there is always a significant difference compared to those associated with carcinoma^{11,15,16}. The association of p53 mutations and the tendency to recur or advanced stage of disease at the time of diagnosis could not be demonstrated. This is in accordance with the observation that the tendency to recur in inverted papillomas is independent of malignant alteration^{1,2}.

The goal of our study was to investigate the potential prognostic significance of Ki67 and p53 expression in inverted sinonasal papilloma in respect to recurrence or malignant alteration.

Materials and Methods

Clinicopathological data

Patient data and paraffin blocks were retrieved from the files and archives of the ENT Departments of the University Hospital Šalata, Zagreb and the University Hospital Osijek as well as the Institutes of Pathology Medical faculty Zagreb, Clinical hospital Osijek and Semmelweis University Budapest. In the period from 1982 to 2000 there were 31 patients with inverted papilloma treated by the departments in Zagreb and Osijek and 10 patients were identified from the files of the Institute of Pathology Semmelweis University in Budapest.

The patients were 29 male and 12 female. Their age ranged from 18 till 82 with a mean of 55 years. Most frequent tumor location was the nose (39 out of 41 patients) in the majority cases extending to adjacent areas (maxillary sinus in 17 and ethmoid sinus in 15 cases). In 3 patients the tumor extended to the epipharynx and in 3 patients to the sphenoid sinus.

Together 49 biopsies could be retrieved, 8 of which showed carcinoma.

Immunohistochemistry

The tissue specimen underwent standard procedure. In brief: after fixation in 10% neutral formalin the tissue was embedded in paraffin and cut on a sliding microtome in 5 um slices. The slices were mounted on silanised glass and stained with antibodies against p53 and Ki67 (DAKO, Glostrup, Denmark). The immunostaining was assessed histologically. For Ki67 first the area of strongest staining was identified and in this area the positive cells were counted establishing their percentage on 1000 cells

using an image analyzing system (ISSA, Vamstech, Zagreb). For p53 the expression was only established as being positive or negative.

Statistical Analysis

Statistical analysis was performed using Statistica 5.0 for Windows statistical program. For nominal p53 results we used Yates corrected one-tailed Chi-square test. The value of Ki67 was relative (percentage) and therefore we used nonparametric statistic (Mann-Whitney U test). Value $p < 0.05$ was considered as statistically significant.

Results

Surgical procedure

The procedures include both those performed for initial treatment as well as treatment of recurrent disease. Most frequently the patients were treated by polypectomy or FESS (Functional Endoscopic Sinus Surgery) (51% of cases). In 9 cases a combination of FESS and Caldwell-Luc operation was performed, while 7 patients were treated by Caldwell-Luc operation alone. Lateral rhinotomy with medial maxillectomy was used in only one case and radical maxillectomy in two. Both of the patients treated with radical maxillectomy had malignant disease; in one of these two cases, radical maxillectomy was accompanied by exenteration of the orbit.

Incidence of recurrence and malignancy

34 primary biopsies – 26 benign lesion. Eight patients suffered from malignant disease with 3 of them having in the first biopsy an inverted papilloma diagnosed (metachronous disease) while the rest presented with synchronous disease.

Among 49 biopsies from our patients 15 materials were taken for recurring disease. In 7 cases material of the primary lesion could not be retrieved. In 7 from 8 patients with benign disease recurrence presented only once and one patient had two recurrences. Patients with malignant disease suffered from multiple recurrences (data not show) (n=4) (Table 1).

TABLE 1
TYPE OF PATHOLOGY

	Papilloma	Carcinoma
Patients	41	8
Recurring disease	11	4
First diagnosis	44	5

Immunohistochemistry

Immunohistochemical reaction for Ki67 was assessed in the epithelial lining. The standard distribution included slight to moderate activity in basal cell layer, extending occasionally to two-thirds of epithelium thickness. In malignant epithelium the activity was much

higher and distributed throughout the tumor. A non-standard staining pattern was also appreciated in benign-appearing epithelium adjacent to tumor. In contrast to this the p53 staining was weak in non-malignant samples, whereas the tumours uniformly showed diffusely expressed immunoreactivity.

Statistical analysis

Primary lesions

Immunoreactivity for p53 was positive in the vast majority of carcinomas and negative in all papillomas except two (Table 2). The sensitivity of p53 test is 87,5% and specificity is 92%.

TABLE 2
P53 IN PRIMARY BIOPSY. DIFFERENCE IN IMMUNOREACTIVITY FOR P53 BETWEEN CARCINOMAS AND PAPILOMAS IS STATISTICALLY SIGNIFICANT, USING χ^2 TEST WITH YATES CORRECTION FOR NOMINAL RESULTS, P<0.001

	P53 poz	P53 neg
Benign	2	24
Malignant	7	1

Immunoreactivity for Ki67 was much higher in malignant than in benign lesions (Figure 1 shows the descriptive statistic of Ki-67). Because the value of Ki67 is expressed numerically, we wanted to check the validity of the test with ROC curve. It departs in its values from

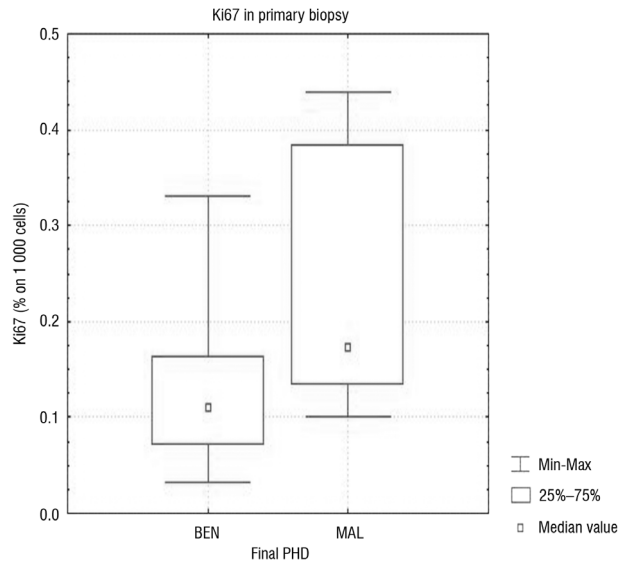


Fig. 1. Ki67 distribution in primary biopsy, descriptive statistic. There is statistically significant difference in Ki67 expression between benign and malignant lesions. The value of Ki67 was relative (percentage) and therefore we used nonparametric statistic Mann-Whitney U test, p=0.019. Median value in benign lesions is 10.2 %, and in malign lesions is 17%.

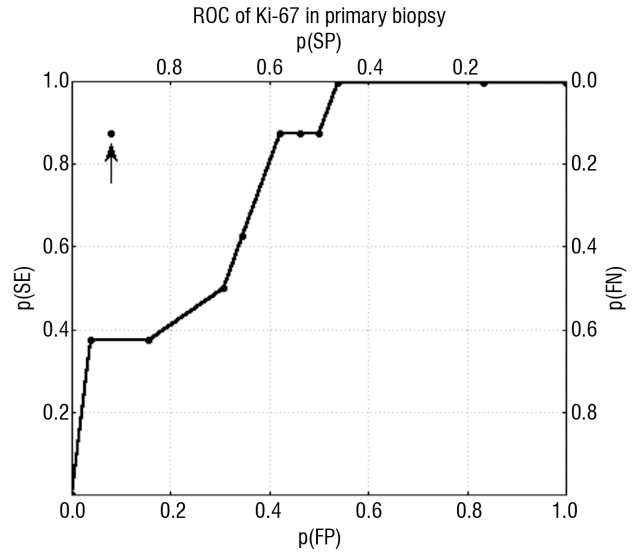


Fig. 2. ROC curve of Ki-67 in primary disease. With a hypothetical cutoff at 10% Ki-67 positive cells in biopsy, all patient with malignant disease should be recognized, meaning that the sensitivity of this test is 100%, but the specificity only 46%. The arrow shows the characteristic of p53. Taking together Ki67 and p53 values did not bring any new information about the biological behavior of lesions.

high sensitivity and specificity of the test. The Receiver Operating Characteristic Curve of Ki-67, and position of p53 characteristic showed that with a hypothetical cutoff at 10% Ki-67 positive cells in biopsy, all patient with malignant disease should be recognized, meaning that the sensitivity of this test is 100%, but the specificity only 46% (Figure 2). Taking together Ki67 and p53 values did not bring any new information about the biological behavior of lesions.

Recurring lesions

In recurrence specimens (15 biopsies, from 12 patients) despite an obvious tendency in line with the above results, there was no statistical significant difference in p53 or Ki67 expression between benign and malignant lesions (Table 3 and Figure 3), possibly because of the small specimen number.

TABLE 3
P53 IN RECURRENCE BIOPSY. THERE WAS NO STATISTICAL SIGNIFICANT DIFFERENCE IN P53 EXPRESSION BETWEEN BENIGN AND MALIGNANT LESIONS, χ^2 TEST WITH YATES CORRECTION, P=0.072.

	p53 poz	p53 neg
Benign	2	7
Malign	5	1

In 4 patients with malignant disease a progression of Ki67 immunoreactivity, from 5.5% to 21% was observed in subsequent materials.

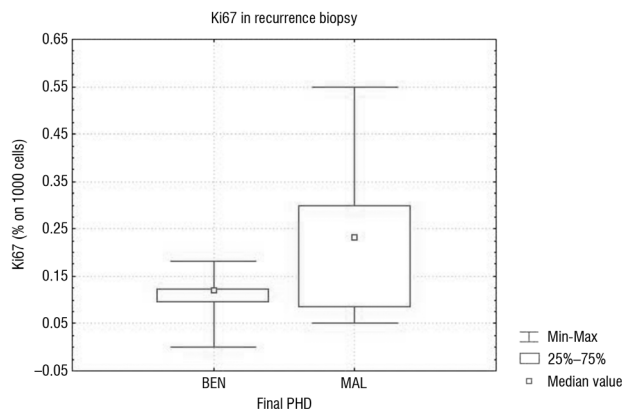


Fig. 3. Ki67 distribution in recurrence biopsy. There was no statistical significant difference in Ki67 expression between benign and malignant lesions. According to Mann-Whitney U test $p=0.146$. Median value in benign lesions is 12 %, and in malignant lesions is 23 %.

Discussion

Inverted papilloma of the nose and paranasal sinuses is a rare tumor of the head and neck region showing a high recurrence rate and the association with carcinoma. Considering the close association of inverted papillomas and carcinomas it has been speculated that inverted papillomas might act as precursor lesions for the development of squamous cell carcinoma. Different parameters have been evaluated trying to understand the crucial steps in their malignant alteration and/or their propensity to recur. So far no prognostic parameter could be identified despite the fact that e.g. p53 alteration has been found in carcinomas associated with inverted papillomas^{11–15}. Interestingly, the frequency of p53 overexpression in squamous cell carcinomas of the maxillary sinus not associated with inverted papilloma is also reported in a wide range. Some authors did not find any p53 overexpression in these carcinomas and hypothesized a different pathogenesis of carcinomas associated with in-

verted papillomas compared to those that arise independently⁶. On the contrary, some authors report even higher frequencies of p53 overexpression in maxillary sinus carcinomas not associated with inverted papilloma. The association of p53 mutations and the tendency to recur or advanced stage of disease at the time of diagnosis could not be demonstrated indicating that the tendency to recur in inverted papillomas is independent of malignant alteration^{1,2}.

Our results are in accordance with these data showing p53 immunoreactivity in almost all cancers and in only 2 out of 41 benign papillomas. The staining for Ki67 brings additional information identifying, with high sensitivity but low specificity, the population of cancer bearing patients. In this a very important element represents the fact that altered immunoreactivity can be demonstrated outside the area of frankly malignant epithelium, in contrast to p53 overexpression limited to tumor tissue only. This means that in patients with Ki67 expression above 10% and its irregular distribution in the epithelium a synchronous carcinoma should be searched for. The drawback of low specificity is not so important in rare events like carcinomas connected with inverted papillomas. In addition, the obvious tendency (showing no statistical significance) of papillomas with high Ki67 to recur should not be neglected.

The diagnosis of inverted papilloma is rarely made preoperatively. This pathology is rarely suspected and it is most often confused with inflammatory polyposis explaining at least partially why polypectomy was so often used as the initial procedure in our study. The number of patients with recurrent disease in our study was low so that it was not possible to evaluate the role of different surgical techniques in the prevention of recurrence.

From our data we can conclude that immunohistochemical staining of inverted sinonasal papillomas for p53 and Ki67 can give useful information concerning the existence of synchronous carcinoma, and, in case of high Ki67 a hint toward possible recurrence.

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MOGUĆA PROGNOŠTIČKA VRIJEDNOST P53 I KI67 U INVERTNOM PAPILOMU NOSA I SINUSA

S A Ž E T A K

Cilj ovog rada je testirati moguću prognostičku vrijednost ekspresije p53 i Ki67 u invertnom papilomu lateralne nosne stjenke i sinusa obzirom na maligni potencijal i rekurenciju. Analizirano je 49 biopsija lateralne nosne stjenke i sinusa dobivenih od 41 bolesnika iz tri bolnice. Mjerena je i statistički analizirana ekspresija p53 i Ki67. p53 imunoreaktivnost je nađena u većini papiloma s karcinomima, a u samo dva benigna papiloma, dok je Ki67 pokazao snažniju imunoreaktivnost u karcinomima i okolnom epitelu. Imunohistokemijsko bojenje invertnog papiloma nosa i sinusa na p53 i Ki 67 može dati korisnu informaciju vezanu za postojanje sinkronog karcinoma, a u slučaju visokog Ki 67 ukazati na moguću rekurenciju tumora.