

Human topoisomerase II- α is highly expressed in sinonasal-inverted papilloma, but not in inflammatory polyp

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Abstract

Sinonasal-inverted papilloma is a benign tumour with a high rate of recurrence, but possible malignant transformation. Therefore, investigation of predisposition to malignant transformation of sinonasal-inverted papilloma gives clinicians the opportunity for adequate treatment. Topoisomerase II- α (topoII- α) and Ki67 are markers of cell proliferation in both normal and neoplastic tissues and its level of expression could be used as a predictive parameter. Our goal was to investigate by immunochemistry the expression level of topoII- α in inverted papilloma, inflammatory nasal polyp and normal sinonasal epithelium and to compare it with expression level of Ki67. TopoII- α nuclear immunostaining showed a differential positivity in the investigated cases. The topoII- α index was 30.6 ± 12.8 in inverted papilloma, 10.7 ± 6.6 in the adjacent epithelium of inverted papilloma, but only 2.3 ± 2.0 in the normal sinonasal epithelium. The differences in topoII- α expression between inverted papilloma and normal sinonasal epithelia were statistically significant. In inflammatory nasal polyp group, topoII- α index was 2.4 ± 2.1 , and the difference in the topoII- α index between inverted papilloma and inflammatory polyp group was also statistically significant. Nuclear immunostaining for Ki67 followed a similar variation. The Ki67 index was 50.0 ± 20.3 in inverted papilloma, 9.0 ± 6.6 in the adjacent epithelium of inverted papilloma and 2.4 ± 0.9 in normal sinonasal epithelium. The differences in Ki67 expression between inverted papilloma and either adjacent or normal sinonasal epithelia were statistically significant. Significant correlation coefficients were found between topoII- α and epithelial thickness ($r = 0.70, P < 0.0001$), and between Ki67 index and epithelial thickness ($r = 0.71, P < 0.0001$). In the inflammatory nasal polyp group Ki67 index was 5.9 ± 3.4 . The difference in the Ki67 index between inverted papilloma and inflammatory nasal polyp groups was statistically significant. Significant correlation coefficient was found between topoII- α index and Ki67 index in inverted papilloma ($r = 0.42, P < 0.05$). These results suggest that the inverted papilloma contains a significantly higher cell population with proliferative activity by comparison with normal sinonasal and inflammatory polyp epithelia, showing a significant correlation between topoII- α and Ki67 expression, and indicating that topoII- α could be an independent prognostic factor for a putative malignant transformation.

Keywords: inverted papilloma • nasal polyp • sinonasal epithelium • topoisomerase II- α • Ki67

Introduction

Inverted papilloma (IP) is a benign tumour of the mucous membrane in sinonasal tract, accounting 0.5–4% of all nasal tumours

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[1–4]. Macroscopically, the tumour resembles a polyp, though its colour usually varies from gray to pale pink. They are usually more vascularized than the inflammatory nasal polyps (INP), another type of sinonasal lesion with no reported malignant transformation. The origin of IP is in the Schneiderian membrane, which originates ectodermally from the nasal placode. Microscopically, IP is characterized by thickened epithelium, showing extensive invaginations of the hyperplastic epithelium into the underlying stroma. The cells show minimal nuclear

atypia with specific higher mitosis at the basal layer level [5, 6]. IP has a tendency to recurrence and malignant transformation. Reported recurrence rate are as high as 70%. Almost all recurrent tumours are found at the site of the previous surgery. The incidence of focal malignancy within IP or adjacent sites largely ranges from 1% to 53% [7, 8]. Finding additional markers for malignant transformation tendencies of IP could offer to the clinicians a better management of the disease and therefore appropriate treatments.

Any attempt to describe new markers requires parallel testing and comparison with markers being in clinical use. Ki67 is a nuclear antigen expressed in all phases of the cell cycle, except G0. Therefore, Ki67 levels reflect higher activity in cell division and have been revealed to be a useful marker of cell proliferation. It was detected as overexpressed in many human tumours, including head, and neck cancers [9–11]. Ki67 reactivity is now widely accepted as a marker of proliferative activity and correlates well with other cell kinetic measurements [12, 13].

Topoisomerases are essential nuclear enzymes acting in several major cellular events driving to cell proliferation, such as DNA replication, transformation, recombination and finally cell division. Topoisomerases control DNA topology by temporarily cutting single or double-strands of DNA in order to relax or induce supercoils. Elevated level of topoll- α is a very efficient marker for cell proliferation in both normal tissue, breast carcinoma and possibly other solid tumours, such as testicular teratoma and transitional cell carcinoma [14–17]. Topoll- α might show a predictive value in lesions prone to develop malignancy [18] or as a target in cancer therapy [19].

Little is known about the transformation mechanism in IP. In order to provide some basic insight into how to manage IP, and to evaluate both the pathological changes and tendency for malignant transformation, we investigated the expression of topoll- α , Ki67 and epithelial thickness and compared it to adjacent areas, INP and normal sinonasal epithelia (NSE).

Materials and methods

Patients

As shown in Table 1, samples consisted in IP of 23 patients (18 males and 5 females) with a male to female ratio of 3.6:1, aged from 43 to 82 years. There were 13 left-sided lesions and 10 right-sided lesions. The follow-up period ranged from 6 to 80 months (mean of 21.8 months). The most frequent symptom was unilateral nasal obstruction, followed by rhinorhea, facial pain, snoring, postnasal drip, sleep apnea and earache. One patient without clinical nasal symptoms was diagnosed by computer tomography (CT) examination for a neurological problem. CT scan of the paranasal sinuses was available in all cases. In one patient, the preoperative radiologic evaluation also included magnetic resonance imaging. The distribution of paranasal sinus involvement was 75% in the maxillary, 42% in the

Table 1 Clinical data of 23 patients underwent for sinonasal-inverted papilloma

Clinical data	Number	%
Age (years)		
Range	43–82	
Mean \pm SD	61.3 \pm 12.6	
Sex		
Male	18	68
Female	5	22
Symptoms		
Nasal obstruction	19	79
Rhinorhea	4	16
Facial pain	4	16
Snoring	3	12
Sleep apnea	1	4
Otagia	1	4
Postnasal drip	1	4
Neurological problems	1	4
CT Finding		
Maxillary Sinus	18	75
Nasal Cavity	15	62
Ethmoids Sinus	10	42
Sphenoid Sinus	1	4
Operation		
Medial Maxillectomy	10	43
Endoscopic surgery	13	57
Follow-up (months)		
Range	6–80	
Recurrence		
Cases	5	21.7
Mean (months)	51	

ethmoids and 4% in the sphenoids. Ten patients underwent medial maxillectomy and 13 resection, all with endoscopic approach. The recurrence rate was 20% (5 patients). Control group consisted of 10 patients with INP (5 men, and 5 women), aged 27–73 years, and 23 NSE from the IP specimens.

Epithelial thickness

Histological specimens were taken for assessment of epithelial thickness in IP, adjacent epithelium and NSE. Measurements were made from the basement membrane by using an eyepiece equipped with a linear micrometer, at a magnification 400 \times , under an Olympus LM50 microscope (Olympus Corp., Tokyo, Japan).

Topoisomerase II- α immunochemistry

Specimens from all patients with IP, INP and NSE were fixed in buffered 4% formaldehyde and embedded in paraffin blocks according to standard procedures. Sections (4–5 μm thick) were stained with haematoxylin-eosin. Representative paraffin blocks were then retrieved for immunostaining with a commercial kit, using rabbit polyclonal antibody against topoll- α (Novocastra Laboratories Ltd., Newcastle, UK). Sections were cut and applied to slides. Slides were heated at 60°C, for 4 hrs and deparaffinized in xylene. Sections were gradually hydrated in graded alcohols and washed in deionized water. After endogenous peroxidase activity blocking with 3% hydrogen peroxide, washing in deionized water, microwave oven heating at 92°C in sodium citrate, pH 6.0 and cooling at room temperature, the slides were washed in phosphate buffered saline (PBS) and incubated with anti-topoll- α antibody 1:80 dilution, for 1 hr, at room temperature, followed by HRP-conjugated secondary antibody (Zymed HRP polymer detection kit, Zymed Laboratories Inc., San Francisco, CA, USA), for 15 min. After washing in PBS, the colour was developed with 3,3'-diaminobenzidine. Slides were counterstained with Mayer's haematoxylin, dehydrated and mounted. Positive controls consisted in breast carcinoma specimens, previously homologated for positive topoll- α expression [16]. For negative controls the primary antibody was excluded during slide preparation.

Ki67 immunochemistry

Representative paraffin blocks were retrieved for immunostaining with anti-human Ki67 monoclonal antibody (dilution 1:100, Zymed Laboratories Inc). The procedure was carried out in an automated immunohistochemical staining system (Ventana Medical Systems, Inc., Tucson, AZ, USA), in accordance with the manufacturer's instructions. For positive control, tonsil tissue was used, while as a negative control sections were prepared by excluding the primary antibody.

Quantitative and statistical analysis

The topoll- α , and Ki67 indexes were determined for each case in the areas of highest positive staining [13, 20]. ANOVA test was used for the comparison between topoll- α indexes in IP, adjacent areas and NSE and between Ki67 indexes in IP, adjacent areas and NSE. Student's t-test was used to evaluate the difference in topoll- α , and Ki67 indexes between IP and INP, and between IP and NSE with or without recurrence. Pearson correlation coefficients were calculated to evaluate the relationships between topoll- α index and epithelial thickness, between Ki67 index and epithelial thickness and between topoll- α and Ki67 indexes. A value of $P < 0.05$ was considered statistically significant. The WinSTAT for Excel was used for statistical analysis.

Results

Morphology of the epithelium differs in IP, INP and NSE

The morphology of the nasal sinus mucosa in normal areas and lesion areas was as classically described [6, 8]. Changes in the epithelial thickness, inflammatory infiltrations, oedematous connective tissue and inverted pattern for the epithelium, accompanied by large shaped masses of papillomatous epithelium embedded in mucosal stroma were shown (Fig. 1). The thickness of the epithelium was $348.0 \pm 131.1 \mu\text{m}$ in IP, $97.9 \pm 23.3 \mu\text{m}$ in adjacent epithelium and $51.0 \pm 14.1 \mu\text{m}$ in NSE. The differences in the epithelial thickness between IP areas and both adjacent epithelium and NSE areas were statistically significant ($P < 0.0001$).

Expression of topoisomerase II- α is highest in IP nasal areas

Topoll- α nuclear immunostaining was positive in all cases, but a higher number of cells were positive in IP specimens (Fig. 2). The mean values for topoll- α indexes were 30.6 ± 12.8 in IP, 10.7 ± 6.6 in the adjacent epithelium and 2.3 ± 2.0 in NSE, respectively. Those differences in topoll- α expression (Table 2) were statistically significant ($P < 0.0001$). The mean values for topoll- α index were 25.0 ± 15.8 in the 5 IP patients with recurrence, and 32.9 ± 11.9 in the 18 IP patients without recurrence, respectively. This difference was not statistically significant ($P = 0.23$). In INP group, mean value for topoll- α index was 2.4 ± 2.1 . The difference in the topoll- α index between IP and INP groups (Table 3) was statistically significant ($P < 0.0001$). Thus, the topoll- α was significantly highly expressed in all cases of IP.

Topoisomerase II- α expression correlates with Ki67 expression level

Nuclear immunostaining for Ki67 was positive in all investigated specimens, but highest in IP samples (Fig. 3). The mean values for Ki67 index were 50.0 ± 20.3 in IP, 9.0 ± 6.6 in the adjacent epithelium and 2.4 ± 0.9 in NSE. The differences in Ki67 expression (Table 2) were statistically significant ($P < 0.0001$). Mean values for Ki67 index were 45.0 ± 25.9 in the 5 IP patients with recurrence and 42.1 ± 26.3 in the 18 IP patients without recurrence. These differences were not statistically significant ($P = 0.8$). Significant correlation coefficients were found between topoll- α index and the epithelial thickness (Fig. 4A; $r = 0.70$, $P < 0.0001$) and between Ki67 index and epithelial thickness (Fig. 4B; $r = 0.71$, $P < 0.0001$). Higher topoll- α and Ki67 indexes were found for thicker epithelium in IP areas. Significant correlation coefficient was found between topoll- α and Ki67 indexes calculated for IP areas (Fig. 5, $r = 0.42$, $P < 0.05$). In the INP group, the

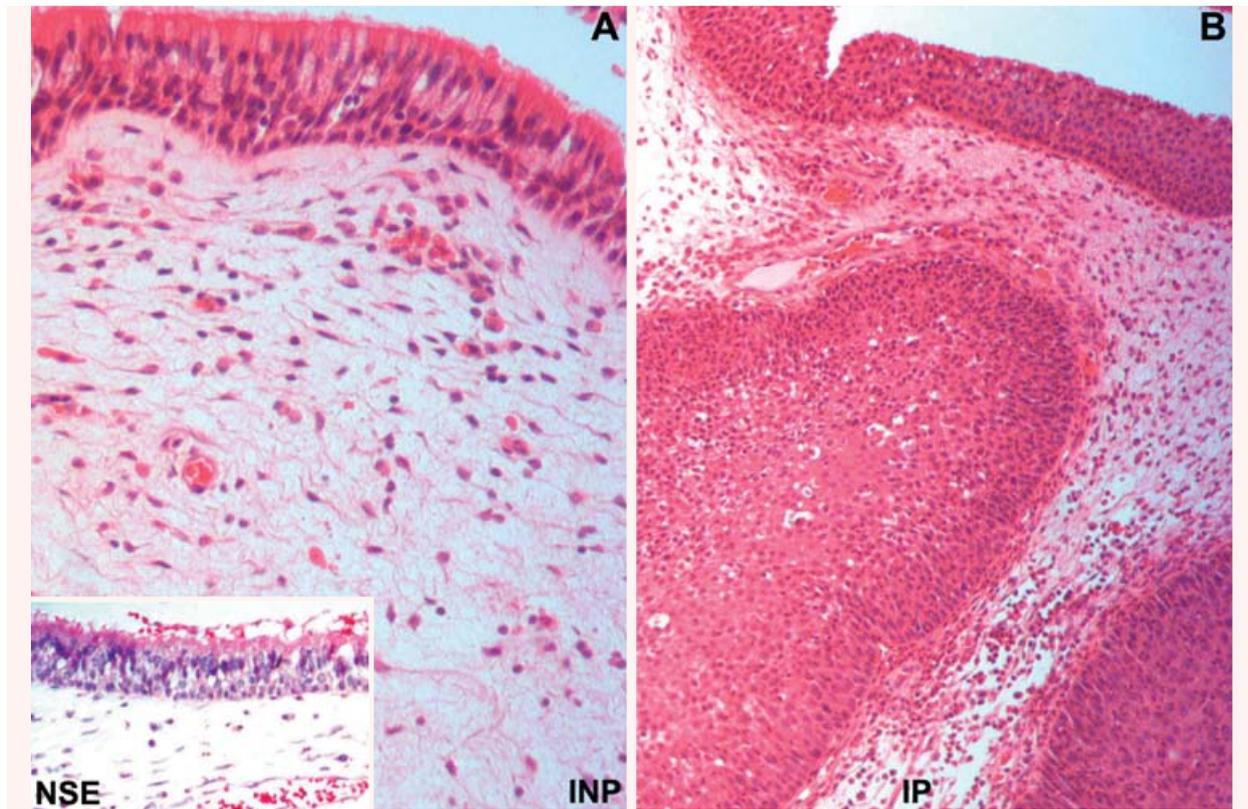


Fig. 1 Morphology of inflammatory nasal polyp and normal sinonasal epithelium as insert (A), and inverted papilloma (B), in specimens stained by haematoxylin-eosin. Original magnification: 200 \times (INP), 100 \times (NSE), 40 \times (IP).

mean value for Ki67 index was 5.9 ± 3.4 (Table 3). The difference in the Ki67 indexes between IP and INP group was statistically significant ($P < 0.0001$). Thus, Ki67, and topoll- α were significantly highly expressed in all cases of IP.

Discussion

The topoisomerase enzymes catalyze the breakage and rejoining of DNA and are therefore involved in many basic cellular processes, such as chromosome replication, condensation and segregation, as well as DNA repair and transcription [21]. Expression of topoll- α isoenzyme has been demonstrated to increase rapidly at the end of S to G2/M phase and decrease after the completion of mitosis. The enzyme is known to be a marker of cell proliferation in normal tissues [22]. High topoll- α expression is associated with high cellular proliferation and poor histological differentiation of the tumour [23]. High levels of the enzyme sensitize cells to the cytotoxic effects of topoisomerase inhibitors

[24]. Our results showed positive nuclear topoll- α staining for many cells in all 23 patients with IP, but for fewer cells in INP. These results suggested transformation of the IP, even though a benign one. IP epithelium was thicker than NSE. The epithelial thickness was highly correlated with the topoll- α index ($P < 0.0001$), meaning the thicker the epithelium, the higher the topoll- α index. The high topoll- α index includes enzyme expression in both covering epithelium and IP masses in the mucosa.

All INP specimens stained positively for topoll- α , but the index was significantly lower in this group than in IP one. When we consider for the IP areas, the enzyme expression in the covering thickened epithelium only, the topoll- α index was still lower in INP specimens. Therefore, IP significantly differed from INP in terms of both morphology and proliferation markers. In our group of 23 patients there was no malignant transformation during the relatively short follow-up period. The patients will be further followed-up for at least 5 more years to check our hypothesis: topoll- α is a marker for putative malignant transformation of IP. This hypothesis was suggested by the distribution of the results. There were eight patients with topoll- α index higher than 30, and Ki67 index below 60; four with Ki67 index

Fig. 2 Expression of topoll- α in normal sinonasal epithelium (A), epithelium of inflammatory nasal polyp (B), adjacent epithelium of inverted papilloma (C) and inverted papilloma area (D, E). Original magnification: 400 \times (A, B, D and E), 200 \times (C).

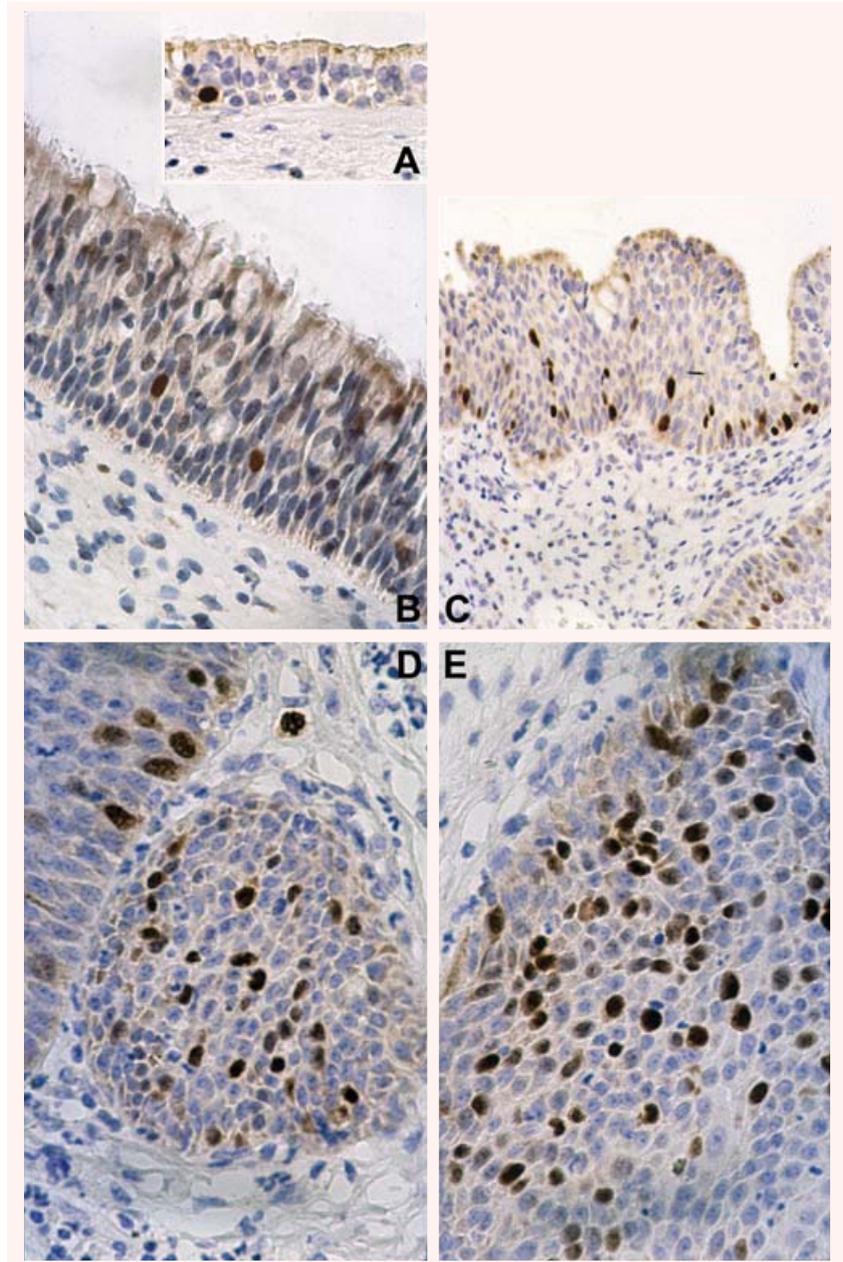


Table 2 Topoisomerase II- α and Ki67 indexes in inverted papilloma, adjacent and normal sinonasal epithelia

Type of sinonasal epithelium	Topoll- α index	<i>P</i>	Ki 67 index	<i>P</i>
Normal	2.3 \pm 2.0		2.4 \pm 0.9	
Adjacent	10.7 \pm 6.6		9 \pm 6.6	
Inverted	30.6 \pm 12.8	<i>P</i> < 0.0001	50 \pm 20.3	<i>P</i> < 0.0001

Table 3 Topoisomerase II- α and Ki 67 indexes in inverted papilloma and inflammatory nasal polyps

Type of sinonasal epithelium	No. of cases	TopoII- α index	<i>P</i>	Ki 67 index	<i>P</i>
Inflammatory polyps	10	2.4 \pm 2.1		5.9 \pm 3.4	
Inverted	23	30.6 \pm 12.8	<i>P</i> < 0.0001	50 \pm 20.3	<i>P</i> < 0.0001

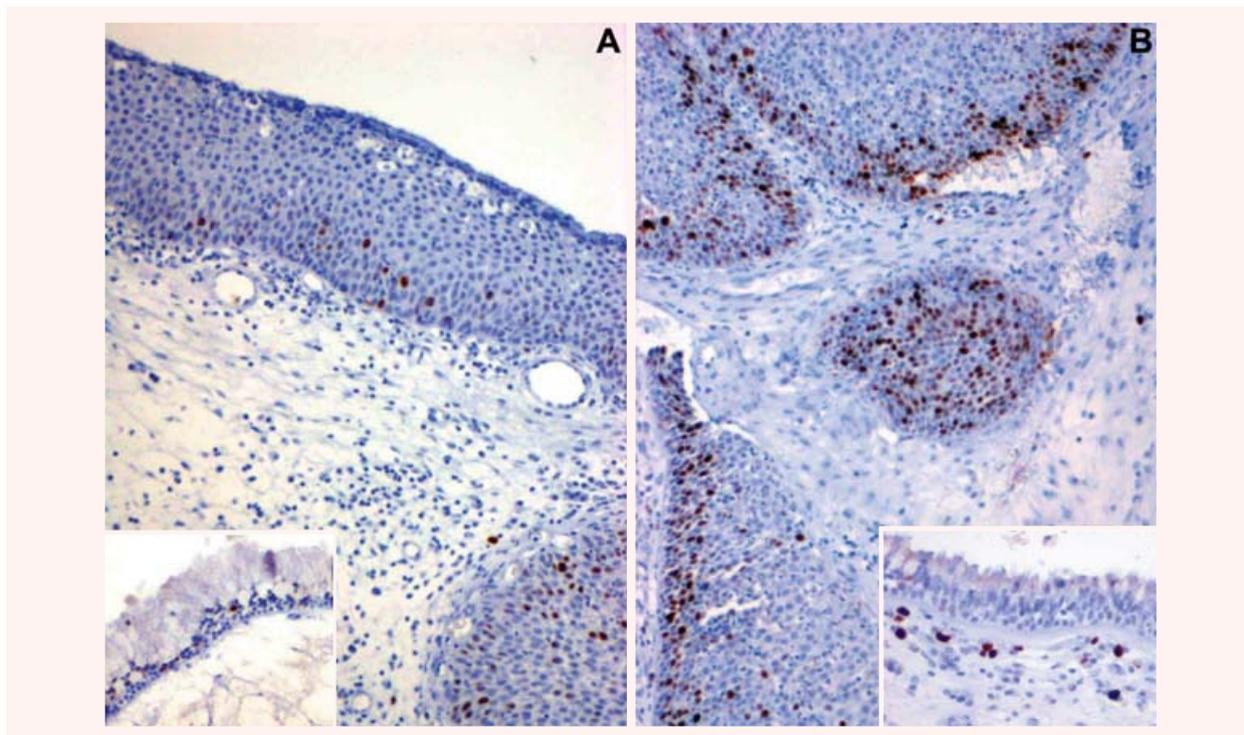


Fig. 3 Expression of Ki67 in normal sinonasal epithelium (insert in **A**), adjacent epithelium of inverted papilloma (**A**) and inflammatory nasal polyp (insert in **B**). See the Ki67 positive nuclei for the infiltrated cells in sub-epithelial stroma of inflammatory nasal polyp, and the faint staining for few nuclei in the epithelium. Original magnification: 100 \times (**A**, insert), 100 \times (**A**), 100 \times (**B**), 400 \times (**B**, insert).

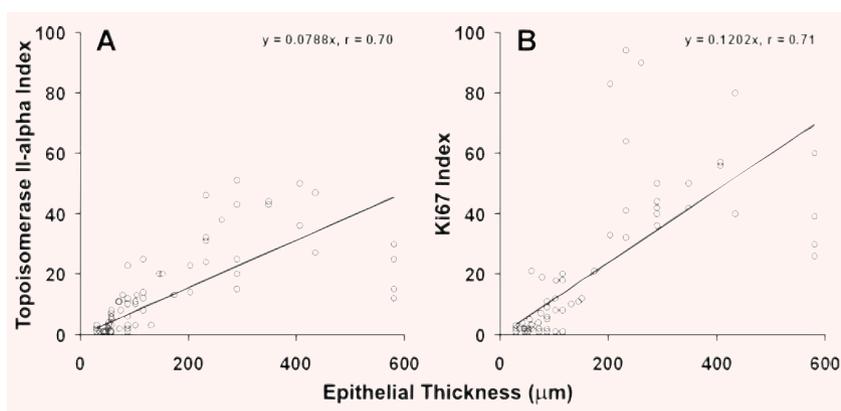


Fig. 4 Correlation plots for topoisomerase II- α index versus epithelial thickness (**A**), and Ki67 index versus epithelial thickness (**B**).

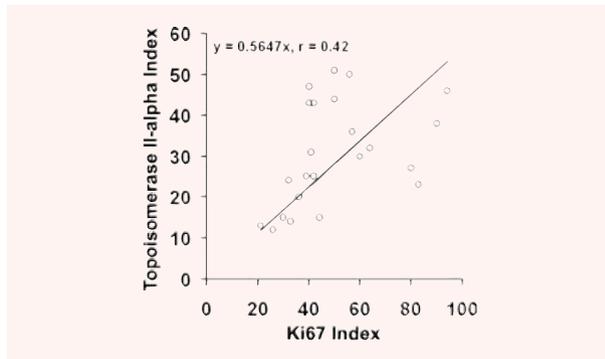


Fig. 5 Correlation plot for topoisomerase II- α index versus Ki67 index.

higher than 60, and topoll- α index below 35; while both indexes were at highest level in only two patients. IP is considered to be a benign, but locally aggressive tumour with a 2–53% rate of transformation into squamous cell carcinoma [7]. Malignant

transformation of an IP was pathologically confirmed [8, 25], refuting the theory that it is simply the intermingling of two separate primary tumours arising within a bed of metaplastic epithelium.

Different results were noted for all 10 INP cases considered in our study. INP is an oedematous tissue lined by respiratory non-proliferating epithelium. The polyps are often attached to the mucosa by a stalk and appear as intranasal grape-like masses, usually bilateral. Histologically, they consist of respiratory epithelium surrounding an inflammatory stroma, infiltrated by inflammatory cells with a predominance of eosinophils. Unlike IP, INP is not associated with malignancy [26, 27]. Our results are aligned with those facts.

To our knowledge, this is the first study to examine the expression of topoll- α in IP compared to NSE areas and INP. The high topoll- α index of IP showed in the present study, may explain the invaginated appearance of sinonasal epithelium occupying the lamina propria and giving the overlying mucosa a polypoid or nodular form. The high expression of topoll- α in IP compared to INP can explain the rate of malignant transformation of IP.

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