RESEARCH ARTICLE

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Factors Associated with Dysplastic Changes in Sinonasal Inverted Papilloma: Study of Tumor Infiltrating Lymphocytes (TILs) FOXP3, CD4, CD8, and expression of p53

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Abstract

Objective: This study examine FOXP3, CD4, CD8 and p53 expression in the transformation of the Sinonasal Inverted Papilloma (SIP) malignancy into sinonasal carcinoma. **Materials and Methods:** This study used a cross-sectional approach. The research sample from thirty-six paraffin block preparations with the diagnosis of SIP. Then, immunohistochemical staining was performed using FOXP3 mouse monoclonal antibody (236A/E7), CD8 rabbit monoclonal antibody (CD8/1179R), CD4 mouse monoclonal antibody (4B12) and p53 rabbit monoclonal antibody. **Results:** There was a significant difference between Foxp3 expression in SIP without dysplasia and SIP with dysplasia ($p= 0.013$). There was no significant difference between the expression of CD4 and CD8 in the two groups with p-values 0.1 and 0.062, respectively. The mean percentage of positive p53 expression in SIP without dysplasia was 0.45+0.63 and in the SIP with dysplasia 29.31+38.96. There was a significant difference between the two groups ($p<0.001$). **Conclusion:** FOXP3 and p53 were overexpressed in SIP with malignant transformation. FOXP3 together with p53 status is associated with dysplastic changed in the SIP. FOXP3 and p53 status could be potential biomarker of malignant transformation in sinonasal inverted papilloma.

Keywords: Sinonasal papilloma- inverted papilloma- dysplastic changes,- FOXP3- CD4- CD8- p53

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Introduction

Sinonasal papilloma is a sinonasal tumor which accounts for 5% of all sinonasal tumors (Barnes, 2002). Sinonasal inverted papilloma (SIP) is the most common histological variant of sinonasal papilloma characterized by hyperplastic epithelial growth towards the stroma (Hunt et al., 2017; Bishop, 2017). Inverted papilloma is generally benign, but in some cases can have an aggressive characterized by postoperative recurrence, and undergo malignant transformation. Transformation of inverted papilloma to carcinoma is found in 5-15% of cases. (Bishop, 2017) The most common type of malignancy that can arise from SIP is squamous cell carcinoma (Nudell et al., 2014).

The mechanism of malignant transformation of inverted papilloma has not yet been clearly formulated. Inflammation, imbalance of cell proliferation and apoptosis as well as the role of intercellular adhesion molecules are thought to play a role in malignant transformation (Yoon et al., 2013). Until now there are no definite histologic parameters that can predict malignant transformation in SIP (Lee et al.,2019). Chronic inflammation is often found together at the margins of papilloma tissue causing hypothesized role of chronic inflammation in the pathogenesis and malignant transformation of SIP. In addition, the tumor microenvironment (including immunomodulation) has been shown to play a role in carcinogenesis. Regulatory T lymphocytes are lymphocytes that play a role in peripheral immune tolerance which prevents excessive immune system activation. In relation to cancer progression, regulation of the immune system by regulatory T cells prevents the development of resistance to anti-tumor responses. Forkhead box transcription factor P3 (FoxP3) is a specific marker for regulatory T cells and is a key intercellular molecule in regulatory T cell physiology. Regulatory T cell infiltration in various types of solid tumors is associated with a worse prognosis (Sakata et al., 2020).

Various biomolecular markers that have been investigated in relation to malignant transformation in SIP are p53, p21, p27, TFPI-2, p63, bcl-2, and FoxM1. (Re et al., 2017). Due to the unavailability of a risk factor assessment system that is good enough to predict the recurrence and transformation of malignancy, patients

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with inverted papilloma are advised to be monitored in the long term even for life (Udager et al., 2017; Suh et al., 2014).

This study aimed to examine the clinicopathological factors that play a role in the transformation of the SIP malignancy into sinonasal carcinoma seen from the clinical, histopathological, biomolecular (especially p53), and tumor microenvironment (especially the immune response that plays a role, including FOXP3, CD4, and CD8) aspect.

Materials and Methods

This study was designed as a cross-sectional study at Department of Anatomical Pathology, Faculty of Medicine, Universitas Indonesia - Cipto Mangunkusumo Hospital Jakarta. This research approved by the Health Research Ethics Committee, Faculty of Medicine, Universitas Indonesia. In this study, we collected 36 paraffin block samples of patients with SIP during the period 2014 to 2019. Unstained slides were made from paraffin blocks and Foxp3, CD4, CD8 dan p53 immunohistochemistry staining was performed. In each case, slides were made from paraffin blocks then cut with a 3 µm thick microtome. The cut in the water bath was taken using a poly-L-lysinie slide, then deparaffinized. Immunohistochemical staining using Foxp3 mouse monoclonal antibody (236A/E7), CD8 rabbit monoclonal antibody (CD8/1179R), CD4 mouse monoclonal antibody (4B12) and p53 rabbit monoclonal antibody. FOXP3 and p53 were stained positive when stained on the cell nucleus, while CD4 and CD8 were stained positive when stained on the cell membrane. All cases were reviewed by 2 study pathologists to confirm the diagnosis of SIP and SIP with dysplastic changes using WHO Classification of Head and Neck Tumours criteria (Hunt et al., 2017). The TILs scoring method used is similar to the TILs scoring method based on the recommendations of the classification by Petersen (Petersen et al., 2006). Descriptive statistics were used to see the distribution of clinicopathological and biomolecular factors. Bivariate analysis was carried out on differences in clinicopathological and biomolecular factors using SPSS version 20 software. Significance was achieved when the p value <0.05 with 95% confidence interval.

Results

From the 36 cases of SIP, 15 cases (71%) were male in the SIP without dysplasia group and 6 cases (29%) were female. Similarly, a higher proportion of males than females was also found in the SIP group with dysplasia, with a proportion of 11 cases (73%) in males and 4 cases (27%) in females, respectively. The mean age found in the SIP without dysplasia group was 52.9 years with an age range of 31-75 years and the mean 47 years in the SIP with dysplastic group with an age range of 29-64 years.

Assessment of the percentage of Foxp3, CD4 and CD8 that were stained positive in the tumor stroma were grouped into 2 groups; low and high based on the Pettersen classification (Petersen et al., 2006; Kara et al., 2019). Low if the percentage less than 20% (<20%) and high if the percentage more than 20% (>20%). There was a significant difference between the expression of Foxp3 in the SIP without dysplasia and SIP with dysplasia (p= 0.013). There was no significant difference between the expression of CD4 and CD8 in the two groups with p-values of 0.100 and 0.062, respectively. The mean percentage of positive p53 expression in SIP without dysplasia was 0.45 (SD+0.63) and in the SIP with dysplasia 29.31 (SD+38.96). There was a significant difference between the two groups (p<0.001). The expression of FOXP3, CD4, CD8 and p53 in each group can be seen in Figures 1 and 2.

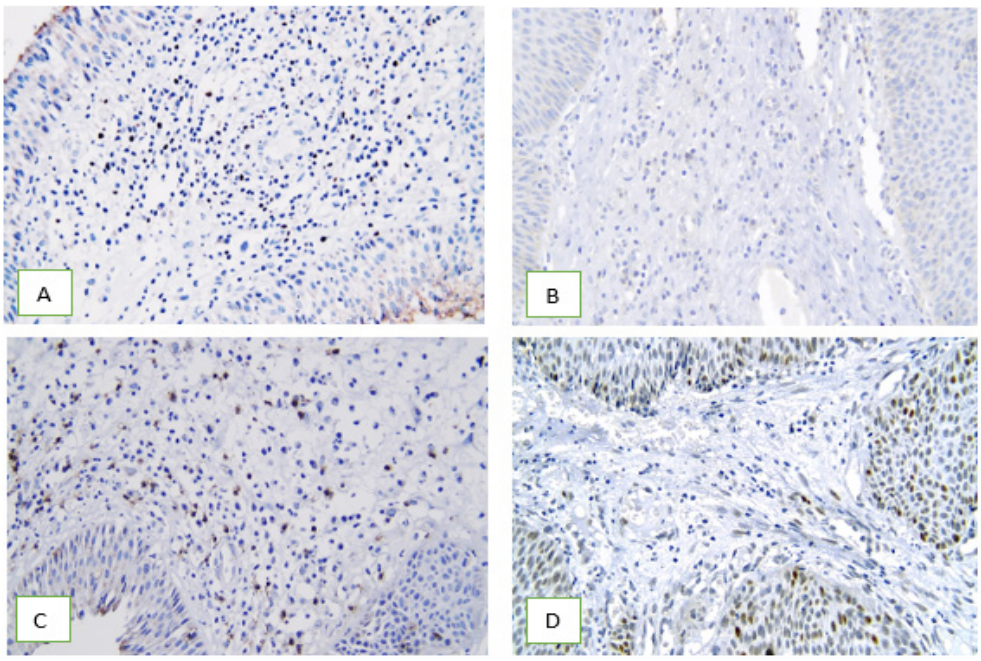

Figure 1. Foxp3, CD4, CD8 and p53 Expression in SIP with Dysplasia Group. A, FOXP3 expression at stroma; B, CD4 expression at stroma; C, CD8 expression at stroma; D, p53 expression in epithel with dysplasia (Objective 200x).

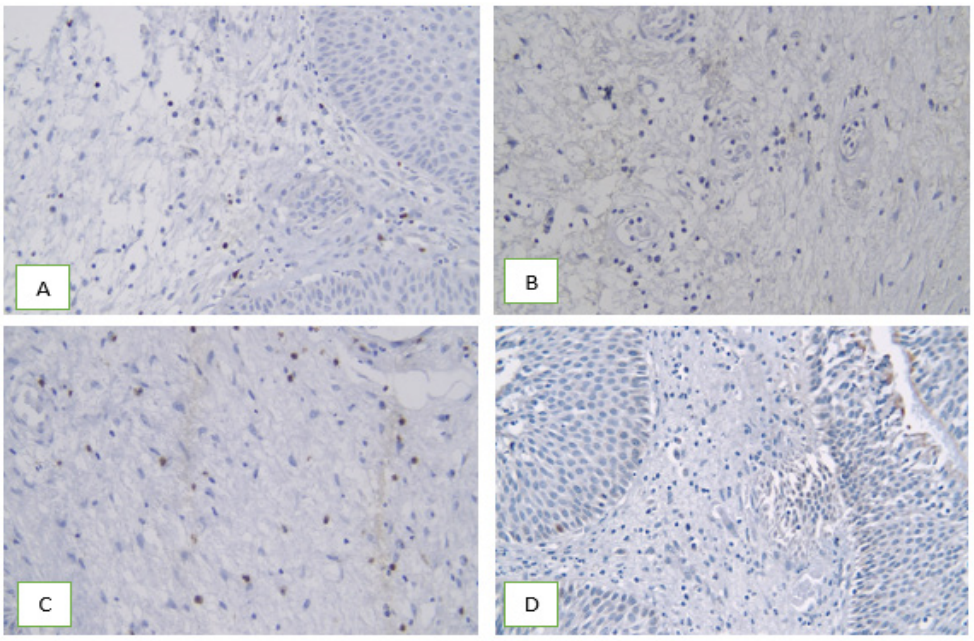

Figure 2. Foxp3, CD4, CD8 and p53 Expression in SIP without Dysplasia Group. A, FOXP3 expression at stroma; B, CD4 expression at stroma; C, CD8 expression at stroma; D, p53 expression in epithel without dysplasia (Objective 200x).

Discussion

Sinonasal Inverted Papilloma (SIP) is a benign neoplasm arising from the sinonasal surface epithelium and has a tendency to undergo malignant transformation. From the 36 samples diagnosed as SIP, most of the subjects were male (71%) with a male to female ratio of 2.6:1. The distribution of sex between groups (SIP without dysplasia and SIP with dysplasia) was almost the same and there was no significant difference between the two groups. Age distribution ranged from 31-75 years in the SIP without dysplasia and 29-64 years in the SIP with dysplasia. There was no difference in the age distribution between the groups. The distribution of sex and age is consistent with previous studies that SIP was found mainly in men (2.5-3.5 times more often than women) with a median age of the fifth decade of life (Barnes, 2002; Sakata et al., 2020).

In the literature, the rate of malignant transformation of SIP is reported between 5-27%. Malignant transformation occurs gradually starting from epithelial dysplasia - carcinoma in situ - invasive carcinoma. There is no widely accepted consensus in assessing epithelial dysplasia in SIP (Sun et al., 2014; Wang et al., 2016). Dysplasia is associated with worse prognosis, with a higher chance of recurrence and more aggressive behavior (Zhao et al., 2016; Orlandi, 2012). The presence of dysplasia will encourage surgeons to perform more operations. aggressively to prevent possible recurrence. (Lawson et al., 2008). In this study we found that the presence of dysplasia was associated with a higher probability of recurrence and malignant transformation.

The mechanism of malignant transformation occurs due to an imbalance between cell proliferation and apoptosis, and inflammation-mediated oncogenesis and the role of adhesion molecules (Pereira et al., 2017; Li et al., 2020). HPV infection has been known to trigger tumorigenesis in various organs by interfering with p53 so that tumor suppression does not occur and cell proliferation continues. In this study, it was found that

Table 1. Relationship between Clinicopathological Characteristics with SIP

Parameter		SIP without dysplasia (n=21)	SIP with dysplasia (n=15)	p-value
Age (mean+SD)		52.9+11.75	47.0+10.18	0.126
Gender (n (%))	Male	15 (71%)	11 (73%)	0.900
	Female	6 (29%)	4 (27%)	
Foxp3 expression (n (%))	Low	20 (95.2%)	9 (60%)	0.013
	High	1 (4.8%)	6 (40%)	
CD4 expression (n (%))	Low	20 (95.2%)	15 (100%)	0.100
	High	1 (4.8%)	0 (0%)	
CD8 expression (n (%))	Low	16 (76%)	15 (100%)	0.062
	High	5 (24%)	0 (0%)	
P53 expression (mean+SD)		0.45±0.63	29.31±38.96	<0.001

p53 expression in the SIP with dysplasia was higher (mean 29.31%) than the SIP without dysplasia (mean 0.45%) and there was a significant relationship between p53 expression in both groups ($p<0.001$). These results complement previous studies that p53 expression does play a role. P53 as a transcription factor that controls cell proliferation and apoptosis was more commonly found in SIP with dysplasia and was associated with malignant transformation (Li et al., 2020). In studies linking the involvement of HPV in SIP, similar results were obtained where HPV infection was more common in the SIP with dysplasia than in the SIP without dysplasia (Almangush et al., 2018). This is in accordance with the literature which states that HPV infection triggers tumorigenesis through the E6/E7 protein produced and interferes with p53 activity (Xu et al., 2010).

In this study, TILs were also assessed through FOXP3, CD4 and CD8. TILs are known as a prognostic factor in several head and neck malignancies. High CD4 and CD8 cell infiltration is associated with better clinical outcomes in head and neck malignancies and other tumors, whereas high FOXP3 infiltration is associated with worse clinical outcomes. It is known that immune cell infiltration in ICH may reflect a T-cell-mediated immune response and suggest the involvement of HPV infection in its pathogenesis (Katori et al., 2006). Several hypotheses suggest that HPV stimulates an effective and prognostically beneficial anti-tumor response through activated CD4 and CD8 responses. and a high CD8/FOXP3 ratio (Lin et al., 2013).

In this study, Foxp3 expression showed a significant difference in the SIP with dysplasia and without dysplasia ($p=0.013$), where Foxp3 expression with high expression in the SIP with dysplasia was found to be higher than in the SIP without dysplasia. In the SIP without dysplasia, in general, the results of low FOXP3 expression were obtained. In addition, in this study, the expression of CD4 and CD8 in the two groups did not show a significant difference, but the results showed that in the SIP with dysplasia, the expression of CD4 and CD8 generally showed low expression. This is in line with several studies which state that Foxp3 as a T regulator (Treg) can suppress the immune response through inactivation of CD4 and CD8 T cells so that CD4 and CD8 expression will decrease (Cools et al., 2008).

As the role of lymphocytes as cells that mediate inflammation to eliminate tumor cells, this study found an increase in Foxp3 expression in the SIP environment with dysplasia that suppresses the activation of CD4 and CD8 cells to eliminate tumor cells, causing tumor progression to malignancy.This indicates that Foxp3 in this study is associated with worse clinical outcomes characterized by the high expression of Foxp3 in the dysplasia group. The findings of this study are comparable to those of studies conducted on other tumors which suggest that Foxp3 is associated with a worse prognosis in some tumors and can be considered as a prognostic factor (Zhang et al., 2010). In this study, it was also found that the high expression of p53 was comparable to the high expression of Foxp3 in the SIP with dysplasia. In a study conducted by Elliot et al, stated that high FOXP3 expression was found in the SIP with HPV involvement and this could indicate HPV-mediated immune system activation which in turn could interfere with the activity of p53 as a tumor suppressor and is associated with a worse prognosis. (Elliot et al., 2019).

In conclusion, Foxp3 and p53 with high expression were found in SIP with dysplasia and malignant transformation. Foxp3 together with p53 are associated with dysplastic changes in SIP. FOXP3 and p53 can be potential biomarkers of malignant transformation in SIP.

Author Contribution Statement

LR, YDBS, AFM and DS were involved in the method's conceptualization and design; LR, YDBS, AFM and DS were involved in data curation, analysis, and interpretation. LR and YDBS gave it a thorough conceptual and editing evaluation; The final version of the essay was revised and approved by all authors.

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Study Approval

This work was permitted by the research committee of the Faculty of Medicine Universitas Indonesia.

Ethical approval

The Faculty of Medicine's Ethics Committee waived informed consent for this study (Protocol 20-02-0118 – Registry No. KET-137/UN2.F1/ETIK/PPM.00.02/2020).

Availability of Data

On reasonable request, the associated author will release the datasets used in this work.

Conflict of Interest

All authors state that they have no conflicting interests.

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