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Human Papilloma Virus, Squamous Cell Carcinoma Antigen and Epidermal Growth Factor Receptor as Biological Markers in Sinonasal Inverted Papilloma

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Abstract: ***Sinonasal inverted papilloma (SIP) has a recurrent tendency and potential for transformation into squamous cell carcinoma and is a locally aggressive benign tumor. In the progression from benign SIP to malignancy, human papillomavirus (HPV) and epidermal growth factor receptor (EGFR) are suggested to play a major role. This work aims to assess their role as tumor marker in SIP. Immunohistochemical analysis for EGFR and PCR detection of HPV – DNA were estimated in nasal biopsies from 35 SIP, 35 NP and 30 control patients (turbinate surgery candidate). Serum SCCA1 was also assessed. HPV-DNA was detected as 63%, 8.6% and 0 % in SIP, NP, and control patients. EGFR intensity revealed statistically significant difference between SIP and other groups. Significant elevated serum SCCA level (6.14 ± 2.69 ng/ml) was found in SIP group, compared to 2.41 ± 1.12 ng/ml and 0.68 ± 0.25 ng/ml in NP and control group. There was a significant decrease of its level postoperatively. The sensitivity was 80.0 and the specificity was 93.8. These results may suggest SCCA and EGFR are possible reliable tumor markers in SIP monitoring and management. High prevalence of HPV-DNA points to its possible role as etiological factor for SIP.***

Keywords: Sinonasal inverted papilloma, squamous cell carcinoma antigen, human papilloma virus, epidermal growth factor receptor, biological markers.

1- Introduction

Sino-nasal inverted papilloma (SIP) has been a benign tumor defined by inward epithelial growth into the base layers of the nasal and paranasal sinus tissue [1]. It

accounts for the nose and paranasal sinuses' 2nd common benign tumor and 0.5– 4% of all nasal tumors. The high rate of recurrence, invasiveness, and correlation of SIP with squamous cell sinonasal carcinoma is well known[2]. The pathogenic origin of SIP has not been fully understood and controversial opinions discuss the relation between HPV and development of SIP. Many authors studied the implication of HPV in the etiology of SIP, but the data found in the literature are still contradictory [3]. An epitheliotropic DNA virus that can affect people's epidermis or mucosa is HPV. Viral HPV sequences are incorporated into cellular DNA and play a key role in promoting tumor growth and malignant transformation [4]. In the literature, HPV detection rates range greatly from 0-72 %, with an average of 25% [5]In patients with pulmonary, head and neck, gynecologic, and esophageal (SCC), SCCA was elevated and was used as a tumor marker. However, in patients suffering from some benign skin and pulmonary lesions and SIP patients, Serum levels of this antigen have also been documented to be high [6]. 88% of SIP was shown to play a key role in oncogenesis by activating somatic EGFR mutations; however, HPV infection provides another potential mechanism of activation of the EGFR pathway [7]. The aim of this work had been to evaluate the possible position of (HPV) and (EGFR) and (SCCA), as tumor marker in SIP patients which may points to their significance in diagnosis, management and follow up of these cases.

2- Material and methods

Following approval by the Ethics Committee of the hospital and all participants' written informed consent, this cohort prospective study (non-randomized trial) was conducted. Between April 2018 and May 2020, thirty-five patients in each group of (SIP), nasal polyposis (NP) and thirty control patients involved in the research were all treated at the Departments of Otorhinolaryngology, Al A_zhar University Hospitals. Patients with skin, renal and pulmonary disorders were exempt for possible uncertainty. In order to confirm the diagnosis of SIP and endorse definite surgical treatment, SIP patients must have documented pathological outcomes. Patients with NP must have a history of failure of medical treatment, endoscopic nasal examination, and pathological outcome to support polypoid nasal lesion, and surgery. Thirty patients (scheduled for turbinate surgery) were enrolled as a control. Data were registered and a preoperative CT scan was performed for the IP patients and divided into four stages depending on the stages of the staging system of Krouse [8]. Specimens from nasal biopsies were examined for HPV-DNA by Nested PCR. Tissue preparation and DNA isolation had been carried out in compliance with the manufacture of the instrument. The integrity of extracted DNA had been evaluated by amplification of a beta-globin fragment of 268 bp. All samples were sufficient for polymerase chain reaction (PCR) analysis following a DNA integrity evaluation. In order to detect HPV DNA in the samples, nested PCR to use the consensus MY09/MY11 primer pair (outer primers) and the GP5Ş/GP6Ş primer pair (inner primers), targeting around 150 bp HPV L1 gene fragment, had been applied. A 25 mL

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reaction mixture containing 100-200 ng of DNA template, 2 mM MgCl2, 10 pmol of each primer (MY09/MY11), 50 mM of each dNTP and 2 U of Taq DNA polymerase was performed in the first round of PCR reactions (5 Prime GmbH, Hamburg, Germany). The PCR amplification cycles would include initial denaturation at 94oC for 3 mins, accompanied by 40 cycles at 55oC for 1 min, 72oC for 1 min, 94oC for 1 min, and final annealing at 55oC for 1 min with elongation at 72oC for 5 mins. In a 50 mL reaction mixture consisting of 100-200 ng DNA template, 3 mM MgCl2, 25 pmol of each primer, 50 mM of each dNTP, and 2 U of Taq DNA polymerase, respectively, the second round of PCR reactions had been performed (5 Prime GmbH). Initial 3 mins denaturation at 94oC, accompanied by 40 cycles at 40oC for 2 mins, 72oC for 1.5 mins and 94oC for 1 min, and final annealing at 40oC for 2 mins with 5 mins elongation at 72oC, were included in the PCR amplification cycles. A 1.5 % agarose gel was run on the PCR products and stained with ethidium bromide. For EGFR evaluation, histopathological examination was done following preparation of Paraffin sections from patient`s biopsies and staining with eosinandhematoxylin. Immunohistochemical analysis has been carried out. for Monoclonal Mouse Anti-Human EGFR Clone H11. On a scale of 1 to 4, EGFR immune reactivity graded. Grade 1 for less than 5% staining, grade 2 for 5 to 20%, grade 3 for 21 to 50%, and grade 4 for more than 50%. The intensity had been graded as 1 for no staining, 2 for low intensity, 3 for moderate intensity and 4 for high intensity [7]. A pathologist who was away from the clinical results assessed the proportion and severity of staining. In order to determine their serum SCCA levels, venous blood specimens from enrolled patients in the SIP and NP groups were tested the day prior surgery and 4 weeks following surgery, with only one specimen from each control patient. Enzyme-linked Immunosorbent Assay (ELISA) conducted for serum SCCA1 estimation. Chi-square and Fisher-exact tests in each group statistically assessed the differences among patients with normal and increased serum SCCA levels in the SIP, NP and control groups. The Kruskale Wallis (Non-normal distribution) test and the Wilcoxon rank sum test subgroup analysis statistically assessed SCCA serum levels in the SIP, NP and control groups. Analysis (receiver operating characteristic curve; ROC) measured the sensitivity, specificity, positive and negative predictive values, and predictive accuracy of the outlier serum levels of SCCA (>3.5 ng/ml). The Wilcoxon signed rank test has been used to assess differences in the SIP and NP groups among pre and postoperative levels of SCCA. All analyses have been conducted using the Windows version 23.0 statistical package for social sciences (SPSS) software. At the $P < 0.05$ level, statistical significance was identified.

3- Results

Table 1 displays the clinical characteristics of 35 patients in each SIP, NP and control group of 30 patients. There were 20 men and 15 women participants in the SIP group, and their average age was substantially higher than that of the NP and control groups, with an average age of 55.83 ± 4.44, 46.85 ± 4.63 and 35.93±4.25 years, respectively.

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SIP's clinical appearance relies on the sites of involvement, involving unilateral nasal obstruction (82.8%), frontal headache (68.6%), epistaxis (51.4%), rhinorrhea (31%) and hyposmia (12%). HPV-DNA was detected in 63% (22 out of 35 cases) of SIP, 8.6% (3 out of 35 cases) of NP while none of the control patients have HPV-DNA ($p<0.001$). In terms of immunohistochemical staining EGFR intensity, all NP and control samples did not stain; 7 patients (20.0%) did not stain in the SIP group; 13 patients (37%) had low staining; 11 patients (31%) had moderate staining; four patients (12%) had high staining; and there was a substantial statistical difference among the 2 groups (table 1). Elevated basal SCCA level (6.14 ± 2.69 ng/ml) was found in SIP group, compared to 2.41 ± 1.12 ng/ml of the NP group and 0.68 ± 0.25 ng/ml of control group which was a substantial ($P < 0.001$) difference. There was also a substantial difference in serum SCCA levels among preoperative and postoperative measures in the SIP and NP groups ($P < 0.001$). The average preoperative and postoperative serum SCCA levels in the SIP group were 6.14 ± 2.69 and 1.47 ± 0.45 ng/ml, respectively, and 2.41 ± 1.12 and 1.42 ± 0.64 ng/ml, respectively, in the NP group (Table 2). SCCA levels yielded an area under the curve (AUC) of 0.945, a sensitivity of 80.0, a specificity of 93.8 at cut-off 3.5 ng/ml, using the receiver operating characteristic (ROC) curve analysis for SIP (figure 1).

Table (1): Demographics, clinical and laboratory findings of studied cases

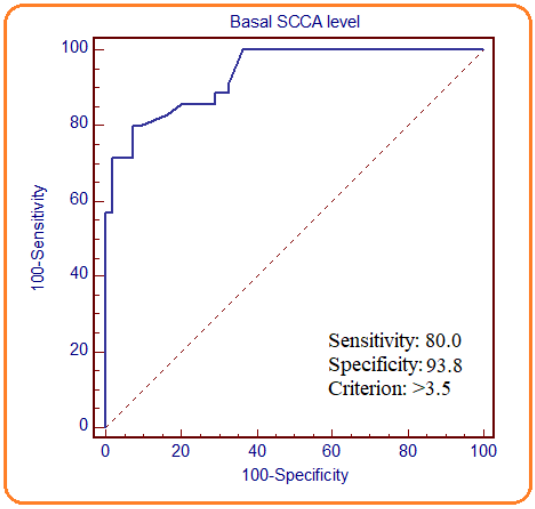
	Control group 30	NP group 35	SIP group 35	
Age (years)	35.93±4.25	46.85±4.63	55.83±4.44	<0.001
Gender (M/F: %)	19/11	25/10	20/15	0.458
Symptoms				
Nasal obstruction	0	24 (86.6%)	29 (82.8%)	
Epistaxis	3 (10%)	4 (12%)	18 (51.4%)	
Rhinorrhoea	25 (83%)	5 (14.3%)	11 (31%)	<0.001
Hyposmia	7 (23%)	6 (17.1%)	4 (12%)	
Frontal headache	18 (60%)	18 (51%)	24 (68.6%)	
Krouse Stages				
1			4 (11.5%)	
2			8 (22.9%) 22 (62.9%)	
3			1 (2.8%)	

4				
HPV-DNA				
PCR+ve	0 (0%)	3 (8.6%)	22 (62.9%)	<0.001
PCR -ve	30(100%)	32 (91.4%)	13 (37.1%)	
EGFR intensity				
No staining	30 (100%)	35 (100%)	7 (20%)	
Low staining	0	0	13 (37%)	<0.001
Moderate staining	0	0	11 (31%)	
High staining	0	0	4 (12%)	
SCCA level (ng/ml)	0.68±0.25 (0.40-1.20)	2.41±1.12 (0.80-5.10)	6.14±2.69 (2.10-10.2)	<0.001

Table (2): The level of SCCA pre and post-operative serum

	SCCA levels		
	Pre-operative	Post-operative	
SIP	6.14±2.69	1.47±0.45	<0.001
NP	2.41±1.12	1.42±0.64	<0.001

Figure (1): ROC curve analysis for levels of SCCA in studied groups. Analysis of the ROC curve yielded a 0.945 area under the curve.



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4- Discussion

Different etiological factors have been suggested for SIP. The role of HPV in SIP pathogenesis and etiology has become a matter of discussion in many clinical and pathologic studies. A comparative investigation of HPV-DNA prevalence in patients with SIP, NP and control groups was included in this study. None of twenty controls were positive for HPV. This finding is in consistent with that obtained from a study on 216 normal tissue sinonasal and 91 sinonasal polyps' samples [9]. In contrast to these results, 6 positive cases for HPV were reported out of 46 healthy persons (13%) biopsied from there nasal mucosa[10]. In another study, HPV also found up to 60 % (9/15) of nasopharyngeal mucosa samples [11]. The high prevalence of HPV-DNA in oral mucosa was found in other studies that reinforce the idea that the virus colonization alone is not enough to produce obvious mucosal changes [12]. HPV-DNA in this study was found in SIP and NP cases as 62.9% and 8.6% respectively, which possibly shows the impact of HPV infection in patients with SIP. This is close to recent study when the prevalence and distribution forms of HPV were examined in the Chinese population's SIP and found higher than that of the control group (64.7%)[13] .HPV infection is detected in up to 33.3% of SIP and 21.7% of sinonasal carcinomas[14]. Our findings are not similar that obtained in other work where 10.3 % of SIP and 22.7 % of SIP-associated SCC were detected with HPV-DNA [15].This low incidence was also reported recently where only 8.3% and 33.3% of SIP and SIP-associated SCC were HPV positive respectively[16]. The role of HPV in SIP was not proved as the virus was isolated only in 13% of SIP and there was no detection in any of the SCC cases associated with IP [17]. Also, HPV- DNA was found only in 12.2% of SIP patients suggesting that it may be only one of its possible etiological factors[18]. A higher result found in a retrospective study on Iranian patients where the HPV- DNA was reported in 18.9% and 100% of SIP and SIP-associated SCC respectively[19]. In addition, HPV was isolated in (30.3%) SIP patients and (60%) SIP-associated SCC patients, but these results were not a statistically significant predictor for recurrence or as a risk factor for SIP associated SCCs[10]. The reasons for this discrepancy are unknown. In various studies, the reported HPV positivity rate seems to be significantly different. This may represent variations in the detection method of HPV or integrity of various HPV subtypes. Our results in in terms of immunohistochemical staining of EGFR intensity report significant statistical differences among the control and the study group. In comparison with these results, 86 out of 156 cases of SIP and SIP-associated SCC (55%) displayed some degree of EGFR staining, with an average 21.7 % stain area. In a higher percentage of specimens, SIP-associated SCC specimens stained positive for EGFR than patients with IP, (71%) and (50%) respectively, however this variation was not statistically significant. For SIP and SIP-associated SCC, the average percentage of staining was 20% vs. 34% [5]. In HPV-positive SIP vs. HPV-negative samples, the incidence of EGFR staining was greater (56.2% vs. 23.6%)[18]. The low HPV prevalence in this series suggests that it is not a primary cause for SIP; low-risk HPV can, however,

contribute to SIP development by raising EGFR expression and activation that may predispose to malignant transformation. On the other hand, all cases of the analysis of 58 SIP and 22 SIP-associated SCC reported either an EGFR mutation or an HPV infection. In all cases of SIP-associated SCC and in all cases of SIP, except for one patient with weak positive HPV infection, HPV and EGFR mutations were mutually exclusive [15]. We examined if serum SCCA levels were associated with the status of the disease and compared these levels with similar common sinonasal diseases,NP and control groups. Our results showed a substantial rise in basal SCCA levels in patients with SIP relative to other groups, that were in line with previous studies [20][21] , [22][23] Furthermore, levels of SCCA in SIP were not linked to the Krouse staging system [8]. This controversy was documented in a later study; where the level of SCCA correlated more with the volume of tumors measured by MRI [24]. SCCA level measurement has been found in current research to be helpful for SIP detection with high sensitivity (80%) and specificity (92.7%) which was close to a recent study where the sensitivity and specificity were (83.3%, 94.7%) respectively. [24]. Due to its direct release into circulation from the squamous epithelium, high serum SCCA levels in SIP patients may be triggered. Endoscopic examination, imaging, and biopsy are useful preoperative diagnostic procedures for SIP and are usually recommended. However, often it is hard to suspect SIP on clinical and radiological examination, especially in recurrent cases with extensive fibrosis. In addition, since the treatment choices and disease prognosis vary from other nasal lesions, it is essential that patients with SIP are diagnosed promptly before treated with proper wide surgical excision. Serum SCCA may be utilized in SIP patients as a useful biologic marker.

5- Conclusion

The level of HPV, EGFR and serum SCCA can be a reliable parameter to be followed by IP patients or can be used as a diagnostic marker to distinguish SIP patients from NP patients if a definite pathological confirmation diagnosis cannot be obtained by a clinical and initial tissue biopsy. Further biomedical researches on a large scale for detection of biomarkers for SIP may help for detection of major prognostic and possible targeted therapeutic consequences for the clinical treatment of the SIP and SIP related SCC.

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Institutional Review Board Statement: The study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Ethics Committee – Al_azhar university hospital (ENT. _44_0000044). Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: The data presented in this study are available on request from the corresponding author.

Conflicts of interest:

The authors declare that they have no conflicts or competing interests

6- REFERENCES

[1] J. W. Wood and R. R. Casiano, "Inverted papillomas and benign nonneoplastic lesions of the nasal cavity," *American Journal of Rhinology and Allergy*, vol. 26, no. 2. pp. 157–163, Mar. 2012, doi: 10.2500/ajra.2012.26.3732.

[2] T. Nukpook*et al.*, "Prevalence and association of Epstein-Barr virus infection with sinonasal inverted papilloma and sinonasal squamous cell carcinoma in the northeastern Thai population," *Infectious Agents and Cancer*, vol. 15, no. 1, Jun. 2020, doi: 10.1186/s13027-020-00308-5.

[3] A. Marza, C. Sarafoleanu, and G. Musat, "Sinonasal inverted papilloma – what's new," *Romanian Journal of Rhinology*, vol. 9, no. 36, pp. 170–176, Nov. 2019, doi: 10.2478/rjr-2019-0022.

[4] C. Long *et al.*, "Clinical evidence-based review and systematic scientific review in the identification of malignant transformation of inverted papilloma," *Journal of Otolaryngology - Head and Neck Surgery*, vol. 49, no. 1, Apr. 2020, doi: 10.1186/s40463-020-00420-x.

[5] G. C. Lin *et al.*, "Epidermal growth factor receptor, p16, cyclin D1, and p53 staining patterns for inverted papilloma," *International Forum of Allergy and Rhinology*, vol. 3, no. 11, pp. 885–889, Nov. 2013, doi: 10.1002/alr.21215.

[6] C. Promsopa, S. Suwansri, and P. Khuntikij, "The serum squamous cell carcinoma antigen level in inverted sinonasal papilloma and nasal polyps patients," *World Journal of Otorhinolaryngology - Head and Neck Surgery*, Mar. 2020, doi: 10.1016/j.wjorl.2020.02.002.

[7] A. M. Udager, J. B. Mchugh, K. S. J. Elenitoba-Johnson, and N. A. Brown, "Oncoscience 908 www.impactjournals.com/oncoscience EGFR mutations in sinonasal squamous tumors: oncogenic and therapeutic implications," 2015. [Online]. Available: www.impactjournals.com/oncoscience/.

[8] J. H. Krouse, "Endoscopic treatment of inverted papilloma: Safety and efficacy," *American Journal of Otolaryngology - Head and Neck Medicine and Surgery*, vol. 22, no. 2, pp. 87–99, 2001, doi: 10.1053/ajot.2001.22563.

[9] W. Lawson, N. F. Schlecht, and M. Brandwein-Gensler, "The role of the human papillomavirus in the pathogenesis of schneiderian inverted papillomas: An analytic overview of the evidence," *Head and Neck Pathology*, vol. 2, no. 2, pp. 49–59, 2008, doi: 10.1007/s12105-008-0048-3.

[10] K. Jenko*et al.*, "In inverted papillomas HPV more likely represents incidental colonization than an etiological factor," *VirchowsArchiv*, vol. 459, no. 5, pp. 529–538, Nov. 2011, doi: 10.1007/s00428-011-1139-1.

[11] B. R. L. S. Bevan I, J. & Crocker, and S. Young L, "Detection of HPV 6 and 11 in tumours of the upper respiratory tract using the polymerase chain reaction," 1990.

[12] S. M. Amin, K. H. Abdel Maged, A. Y. Naser, and B. H. Aly, "Laryngopharyngeal Reflux With Sore Throat: An Ultrastructural Study of Oropharyngeal Epithelium," 2009.

[13] Y. Zhang *et al.*, "The prevalence and distribution of HPV types in nasal inverted papilloma in Chinese Han population," 2017.

[14] K. Syrjänen and S. Syrjänen, "Detection of human papillomavirus in sinonasal papillomas: Systematic review and meta-analysis," *Laryngoscope*, vol. 123, no. 1. pp. 181–192, Jan. 2013, doi: 10.1002/lary.23688.

[15] A. M. Udager*et al.*, "Human papillomavirus (HPV) and somatic EGFR mutations are essential, mutually exclusive oncogenic mechanisms for inverted sinonasal papillomas and associated sinonasal squamous cell carcinomas," *Annals of Oncology*, vol. 29, no. 2, pp. 466–471, Feb. 2018, doi: 10.1093/annonc/mdx736.

[16] M. Alireza, A. Mohammad, R. Maryam, and Z.-M. Ali, "Investigating Human Papilloma Virus Types in Sinonasal Papilloma Using Polymerase Chain Reaction: Is It Really a Prerequisite for Nasal Papilloma Formation?," *Maedica*, vol. 15, no. 1, pp. 76–80, 2020, doi: 10.26574/maedica.2020.15.1.76.

[17] S. Mohajeri*et al.*, "Human papillomavirus: An unlikely etiologic factor in sinonasal inverted papilloma," *Laryngoscope*, vol. 128, no. 11, pp. 2443–2447, Nov. 2018, doi: 10.1002/lary.27207.

[18] A. Scheel *et al.*, "Human papillomavirus infection and biomarkers in sinonasal inverted papillomas: Clinical significance and molecular mechanisms," *International Forum of Allergy and Rhinology*, vol. 5, no. 8, pp. 701–707, Aug. 2015, doi: 10.1002/alr.21524.

[19] S. Jalilvand, M. Saidi, Z. Shoja, N. Ghavami, and R. Hamkar, "The prevalence of human papillomavirus infection in Iranian patients with sinonasal inverted papilloma," *Journal of the Chinese Medical Association*, vol. 79, no. 3, pp. 137–140, Mar. 2016, doi: 10.1016/j.jcma.2015.11.003.

ISSN 2515-8260 Volume 08, Issue 03, 2021

[20] R. Yasumatsu*et al.*, "Clinical value of serum squamous cell carcinoma antigen in the management of sinonasal inverted papilloma," *Head and Neck*, vol. 27, no. 1, pp. 44–48, Jan. 2005, doi: 10.1002/hed.20115.

[21] M. Suzuki, Z. Deng, M. Hasegawa, T. Uehara, A. Kiyuna, and H. Maeda, "Squamous cell carcinoma antigen production in nasal inverted papilloma," *American Journal of Rhinology and Allergy*, vol. 26, no. 5, pp. 365–370, Sep. 2012, doi: 10.2500/ajra.2012.26.3797.

[22] P. Matoušek, K. Zeleník, K. Šafarčík, L. Čábalová, and P. Komínek, "Squamous cell carcinoma antigen as a marker of sinonasal inverted papilloma," *European Archives of Oto-Rhino-Laryngology*, vol. 271, no. 3, pp. 535–538, Mar. 2014, doi: 10.1007/s00405-013-2604-z.

[23] Y. Yamashita *et al.*, "Squamous cell carcinoma antigen as a diagnostic marker of nasal inverted papilloma," *American Journal of Rhinology and Allergy*, vol. 30, no. 2, pp. 122–127, Mar. 2016, doi: 10.2500/ajra.2016.30.4287.