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Analysis of Human Papillomavirus in Schneiderian Papillomas as Compared to Chronic Sinusitis and Normal Nasal Mucosa

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Master Supervisors: Saul Frenkiel MD, Eduardo Franco PhD
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# Table of Contents

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Table of Contents</td>
<td>2</td>
</tr>
<tr>
<td>Abstract – English</td>
<td>3</td>
</tr>
<tr>
<td>Abstract – French</td>
<td>4</td>
</tr>
<tr>
<td>Introduction</td>
<td>5</td>
</tr>
<tr>
<td>Methods</td>
<td>12</td>
</tr>
<tr>
<td>1.) Identification of study groups</td>
<td>12</td>
</tr>
<tr>
<td>2.) Specimen extraction from paraffin blocks</td>
<td>13</td>
</tr>
<tr>
<td>3.) Line blot assay</td>
<td>14</td>
</tr>
<tr>
<td>4.) Statistical analysis</td>
<td>15</td>
</tr>
<tr>
<td>Results</td>
<td>16</td>
</tr>
<tr>
<td>Discussion</td>
<td>20</td>
</tr>
<tr>
<td>Study Limitations</td>
<td>25</td>
</tr>
<tr>
<td>Future Directions</td>
<td>26</td>
</tr>
<tr>
<td>Summary</td>
<td>27</td>
</tr>
<tr>
<td>Acknowledgements</td>
<td>27</td>
</tr>
<tr>
<td>References</td>
<td>28</td>
</tr>
</tbody>
</table>
Abstract:

Schneiderian papillomas (SP) are tumors arising from the surface epithelium (Schneiderian epithelium) of the nasal cavity and paranasal sinuses. Evidence points towards a viral etiology, specifically Human papillomavirus (HPV). Although substantial data indicates HPV as a likely etiology, little is known about the role of HPV in benign nasal pathologies or in normal nasal mucosa. Objective: to characterize the role of HPV in SP, chronic sinusitis (CS) and its prevalence in normal nasal mucosa. A case controlled study was undertaken, matching patients with SP to patients with chronic sinusitis (CS). Patients with normal nasal mucosa served as a control group. All patients had their tissues analyzed for the presence of various HPV types using polymerase chain reaction (PCR) coupled with a line blot assay. Results: A total of 168 patients were identified (74 SP, 74 CS, 20 control). Of these, 70 (41.7%) had detectable DNA, and 9/70 (12.9%) had detectable HPV of types 6, 11, and 16. None had detectable HPV type 18. Significant differences were detected in the presence of HPV in CS, SP and control groups, as well as in the presence of low risk versus high-risk types amongst investigation and control groups. Conclusions: Significant differences exist in the distribution of HPV between SP, benign nasal pathologies such as CS and normal nasal mucosa. HPV may play an important role, at least as cofactor, in the development of SP, with types 6, 11 and 16 more pivotal than other types. Line blot assay may provide a useful technique in identifying HPV in SP.
Abstract:

Les papillomes Schneideriens (PS) sont des tumeurs provenant de l’epithelium recouvrant la cavite nasal ainsi que les sinus. Les preuves pointent vers une etiologie virale, notamment le virus de papillome humain (VPH). Malgre le fait que des donnees substantielles inculpent le VPH comme etiologie probable, le role to VPH dans la muqueuse nasale normale, ou dans les pathologies nasales benignes, demeure. Objectif: Characteriser la relation entre le VPH et PS, la sinusite chronique (SC) et la muqueuse nasale. Une etude du type cas-controles a ete entreprise en accordant les patient avec les PS avec les patients souffrants de SC. Les patients avec une muqueuse nasale normale ont servis de contrôles. Les tissus provenant de chaque patient ont ete analyses par l’analyse de ligne taché a fin de detecter la presence des divers sous-types du VPH.

Resultats: Un total de 168 on été identifiés (74 PS, 74 SC, 20 contrôles). De l’ADN a été detecté dans les tissus de 70 (41.7%) de ces patients et les sous-types 6, 11 et 16 du VPH futs detectés chez 9/70 (12.9%). Le sous-type 18 du VPH n’a été detecté chez aucun de ces patients. Des differences considerables ont été detectees dans la presence du VPH entre les groupes de SC, de PS et de controle, ainsi que dans la presence de sous-types a faible risque et a haut risque entre le groupe sous etude et le group de controle.

Conclusion: En ce qu’a trait à la presence du VPH, des differences considérables existes entre les papillomes Schneideriens et les pathologies nasales benignes telles que la sinusite chronique et la muqueuse nasale normale. Le VPH joue un role important dans le developpement de papillomes Schneideriens avec les sous-types 6, 11 et 16 etant plus souvent impliques. L’analyse de ligne taché peut se reviter une technique utile dans la detection du VPH dans les PS.
Introduction:

Schneiderian papillomas (SP) are uncommon tumors of the nose and paranasal sinuses, deriving their name from the involution of the surface epithelium lining the nasal cavity and sinuses (Schneiderian mucosa) into the underlying stroma. They represent approximately 0.5 - 4% of all primary nasal neoplasms.

Clinically, SP tends to present in the fifth to seventh decade of life, with a markedly male predominance. The most common symptom at time of presentation is nasal obstruction, with other symptoms including presence of a nasal mass, sinusitis, epistaxis, rhinorrhea, facial pain, epiphora, anosmia, parasthesia, diplopia and headaches.

SP typically arises from the lateral nasal wall, with frequent extension to involve the paranasal sinuses, mainly the maxillary and ethmoid sinuses. Other less common sites of involvement include the sphenoid sinus, frontal sinus, orbital structures and nasal septum. Multicentric lesions occur in 4-35% of patients.

SP has a typical gross appearance of a firm, bulky, polypoid mass, often times filling the nasal cavity and extending posteriorly. It may attach to normal structures via a stalk, or involve the epithelium more diffusely.

Histopathologically, SP can be divided into three types: inverting, fungiform and cylindrical, with inverting being the most common. The inverting type has an endophytic growth pattern, and is found almost exclusively on the lateral nasal wall, accounting for approximately 47% of SP. The fungiform subtype is an exophytic
growing subtype of SP, having a frequency of 50%, and are almost exclusively septal lesions\textsuperscript{19}. The cylindrical type is the rarest, accounting for only 3% of SP, and typically showing an endoexophytic growth pattern most commonly involving the lateral wall\textsuperscript{19}. Several systems have been developed in order to clinically stage inverted papillomas\textsuperscript{3,20,21}. The most recent of these systems\textsuperscript{22} is represented in table 1. Management of SP is controversial.

The treatment centers around surgical excision, with all but small septal lesions managed by lateral rhinotomy with medial maxillectomy\textsuperscript{9}. The choice of degree of surgical excision is one of debate. Most authors believe that the treatment of choice is the achievement of wide surgical margins obtained via lateral rhinotomy and medial maxillectomy\textsuperscript{11,14,15,23-25}. Recurrence rates have been reported between 28 and 74%\textsuperscript{14,26} attributed mostly to inadequate margin resection. Kristensen\textsuperscript{11} in reviewing the recurrence rates of several authors reported an average recurrence rate of 11% in patients treated with radical surgical excision, compared with an average of 64% recurrence in patients treated with limited excision. A previous study\textsuperscript{9} conducted by our group revealed a recurrence rate of 0% in patients treated with aggressive surgical resection as compared to 45.9% in those patients treated with limited resection. However, several authors, including many of those favoring wide excision, also recommend the use of limited resection in cases where there appears to be limited disease\textsuperscript{11,13,15,23-25}, with computed tomography helping to define those candidates seemingly suitable for more conservative management. Areas of the nose that would be more suitable to endoscopic treatment include small tumors of the nasal passage, middle turbinate, lateral nasal wall and septum\textsuperscript{27}. Kamel\textsuperscript{28} recommends the use of conservative endoscopic surgery in cases
of SP not involving the maxillary sinus. Previous work conducted by our group recommended aggressive surgical management, with the exception of septal lesions.

Table I: Krouse Staging System of Inverted Papilloma

<table>
<thead>
<tr>
<th>Stage</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage I</td>
<td>Disease limited to nasal cavity alone</td>
</tr>
<tr>
<td>Stage II</td>
<td>Disease limited to the ethmoid sinuses and medial and / or superior aspect of the maxillary sinus</td>
</tr>
<tr>
<td>Stage III</td>
<td>Disease involving the lateral and / or inferior aspect of the maxillary sinus, or extending into the frontal and / or sphenoid sinus.</td>
</tr>
<tr>
<td>Stage IV</td>
<td>Disease spread outside of the confines of the nasal cavity and paranasal sinuses. Any specimen with coexistent malignancy.</td>
</tr>
</tbody>
</table>

The etiology of SP remains undetermined, but evidence points towards a viral causality, specifically human papillomavirus (HPV). Human papillomavirus (HPV) is a member of the papovaviridae family. It is a double-stranded circular DNA virus that infects vertebrates, leading to epithelial tumors, either benign or malignant. The circular DNA is covalently closed, and is approximately 55nm in diameter and 8 kilo-base pairs in length. To date, nearly 100 types of HPV's have been identified. As a group, HPV is heterogeneous, with each type having more than 10% dissimilarity in various nucleotide sequences.

The DNA of HPV is organized into three regions of unequal length, with all the protein coding sequences located on one strand (table II). The DNA contains open reading frames for both early (E) and late (L) genes. The early and late sections account
for approximately 50 and 40% respectively of the genome\textsuperscript{33-35}. Each of the various open reading frames has a specific function\textsuperscript{31,36-39}. The E genes encode nonstructural proteins regulating viral transcription and replication\textsuperscript{40}. They are expressed both in nonproductively infected as well as transformed epithelial cells. E1 is involved in the initiation of viral DNA synthesis. E2 is involved both in viral replication and regulation of transcription. The role of E4 is that of cytoskeleton alteration, and is involved in the maturation and release of papillomavirus particles\textsuperscript{41}. E5 causes papilloma induction through its interaction with growth factor receptors\textsuperscript{41}, while E6 and E7 cause papilloma formation and malignant transformation\textsuperscript{42}. The late region of the DNA contains two genes, namely L1 and L2, which are intimately involved in viral assembly. The L1 and L2 genes encode for the major and minor capsid proteins, which are only expressed in fully differentiated epithelial cells\textsuperscript{31,41}. Separating the early and late regions is the non-coding upstream regulatory region, or long control region, which accounts for about 10% of the genome. This section contains the viral origin of replication, as well as sequences that interact with both viral and host proteins in viral expression and replication.

The functions of viral oncoproteins E6 and E7 are critical in HPV’s role in carcinogenesis. These two oncoproteins promote tumor progression by altering the function of two key elements of tumor suppression, namely the p53 and retinoblastoma tumor suppressor (pRb) genes\textsuperscript{43}. P53 protein is normally induced following host DNA damage. It encodes for a transcription factor that activates a subset of genes that cause the arrest of cells in the G1 phase of the cell cycle, thereby allowing repair of the damaged DNA before entering the S phase of the cell cycle\textsuperscript{44}. If the repairs are not made, aberrant DNA may occur, and the possibility of tumor formation exists. E6 specifically targets
p53 for ubiquitin-dependent proteolysis, resulting in its destruction, and subsequent non-repair of damaged DNA\textsuperscript{43,45}. An inverse relationship between HPV infection and p53 mutation has been established in oropharyngeal carcinoma\textsuperscript{43}.

Cyclin-D is a protein regulating the phosphorylation state of pRb, which is necessary for the transition between G1 and S phases of the cell cycle. E7 has a similar sequence to cyclin-D, and causes inappropriate and heightened movement into the S-phase of the cell cycle. This has been clearly demonstrated in tonsillar squamous cell carcinoma\textsuperscript{44}.

**Table II: Organization of Human Papillomavirus Genome**

<table>
<thead>
<tr>
<th>Coding Region</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>E1</td>
<td>Initiation of viral DNA synthesis</td>
</tr>
<tr>
<td>E2</td>
<td>Viral replication and transcription regulation</td>
</tr>
<tr>
<td>E3</td>
<td>Unknown</td>
</tr>
<tr>
<td>E4</td>
<td>Cytoskeleton alteration</td>
</tr>
<tr>
<td>E5</td>
<td>Papilloma induction</td>
</tr>
<tr>
<td>E6</td>
<td>Papilloma formation and malignant degeneration</td>
</tr>
<tr>
<td>E7</td>
<td>Papilloma formation and malignant degeneration</td>
</tr>
<tr>
<td>Long control region</td>
<td>Viral origin of replication</td>
</tr>
<tr>
<td>L1</td>
<td>Viral assembly</td>
</tr>
<tr>
<td>L2</td>
<td>Viral assembly</td>
</tr>
</tbody>
</table>

The HPV life cycle is such that infection occurs in the basal layer of epithelial tissue, particularly in stem cells. The exact mechanism is unclear, but evidence suggests that there is no specificity of cellular binding by HPV\textsuperscript{30,46}. After the initial
infection, the viral DNA becomes latent, lasting for weeks until the first evidence of infection becomes apparent in the form of papilloma formation\textsuperscript{30,47}. After a period of latent infection, HPV becomes activated through an unknown mechanism, resulting in increased transcription and papilloma formation.

HPV is known to play a role in cervical and anogenital cancers\textsuperscript{48-50}. Over 95\% of patients with carcinoma of the uterine cervix display DNA of high-risk HPV types\textsuperscript{51}. As well, studies have revealed that up to 73\% of anogenital carcinoma patients have detectable HPV\textsuperscript{52}. Typically, HPV types 6 and 11 are associated with more benign cervical and anogenital pathologies such as condylomata, low-grade cervical intraepithelial neoplasia and benign anal intraepithelial neoplasia\textsuperscript{52-53}. HPV types 16 and 18 are more associated with more malignant pathologies such as high-grade cervical intraepithelial neoplasia, cervical carcinoma and anal carcinoma\textsuperscript{52-53}.

Anogenital and cervical epithelia and upper aerodigestive tract mucosa are similar in that both are primarily squamous cell epithelia. Studies have shown further similarity with respect to histopathologic characteristics and molecular biologic properties\textsuperscript{44,55}. From this it is plausible that some of the mechanisms of HPV activity in anogenital and cervical epithelia may be extrapolated to the epithelia of the upper aerodigestive tract.

HPV has been linked with several head and neck neoplasms\textsuperscript{32,36-64} including laryngeal papillomatosis, laryngeal carcinoma, tonsillar carcinoma, verrucous carcinoma of the larynx and oral cavity carcinoma. The exact relationship between HPV and these clinical entities remains to be fully understood.

Little is known about the role of HPV in other benign nasal pathologies such as chronic sinusitis (CS) or its prevalence in normal nasal mucosa. Buchwald et al.\textsuperscript{45},
utilizing both in-situ hybridization and PCR did not detect any HPV DNA in 21 specimens of normal nasal mucosa. Weiner et al.\textsuperscript{29} found no evidence of HPV DNA in 84 normal turbinate specimens. In contrast, Bryan et al.\textsuperscript{62} detected HPV in 9 of 14 specimens of normal mucosa. These, however, were obtained from nasopharyngeal biopsies, which may represent a different viral reservoir. To our knowledge, and extensive research of the literature, examination of HPV in patients with chronic sinusitis has not been conducted.

The purpose of this current study was to evaluate the presence of HPV in SP, as compared to benign nasal pathology and normal nasal mucosa to determine if any difference exists between infectivity of HPV types across the spectrum of intranasal mucosal neoplasia.

A second objective was to evaluate line blot assay, a technique not previously used in the analysis of HPV in Schneiderian papilloma, in order to assess its utility in this area of investigation. Line blot assay has been used to investigate anogenital disease associated with HPV infection, as well as esophageal disease\textsuperscript{66,67}. Line blot assay utilizes a reverse hybridization reaction of biotin-labeled amplified DNA with oligomer probes\textsuperscript{67}. The amplified DNA is hybridized with all the relevant probes in a single reaction, thus reducing contamination and processing time. This current study is the first to utilize this technology in detecting HPV in SP.
Methods:

1.) Identification of study groups:

A retrospective chart review of patients diagnosed with SP at the Sir Mortimer B. Davis – Jewish General Hospital at McGill University between 1978 - 1999 was undertaken. Pathologic examination of the excised tissues was done post-operatively, concentrating on cytology, tissue architecture and histologic subtype. All patients were then matched in a case-controlled manner with patients diagnosed with chronic sinusitis, and their diagnoses pathologically confirmed. Chronic sinusitis was defined as persistent inflammation of the nose and paranasal sinus mucosa for greater than three months duration. Chronic sinusitis was chosen to represent benign nasal pathology due to its relative frequency in the general population. Patients were matched according to gender, age of the patient and age of the specimen (ie year of surgery). A third group of twenty patients, in whom no known nasal mucosal disease occurred were used as the control group. These consisted of patients undergoing routine nasal septoplasty. Samples from the control group consisted of biopsies from both the ethmoid sinuses, namely the bulla ethmoidalis, and inferior turbinates The study groups are summarized in table III. Using a polymerase chain reaction technique coupled with a line blot assay\textsuperscript{66,67}, archival formalin-fixed, paraffin embedded pathologic tissue samples were examined for the presence of human papillomavirus.
Table III: Study groups

<table>
<thead>
<tr>
<th>Study Group</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Primary investigation group</td>
<td>Patients with pathologic confirmation of Schneiderian Papilloma (SP) either malignant or benign variant</td>
</tr>
<tr>
<td>Co-investigation group</td>
<td>Patients with pathologic confirmation of Chronic Sinusitis (CS); inflammation of the nasal cavity and paranasal sinuses for 3 months or longer</td>
</tr>
<tr>
<td>Control</td>
<td>Patients with otherwise normal nasal mucosa undergoing routine nasal septoplasty for septal deviation</td>
</tr>
</tbody>
</table>

2.) Specimen extraction from paraffin blocks:

The paraffin blocks were prepared in the Department of Pathology at the Jewish General Hospital (Montreal, Canada). The blocks were sectioned using a disposable microtome blade. A new blade was used for each specimen. Sections were cut 20μm thick, and then transferred directly into a microcentrifuge tube. A negative control block was interspersed every ten blocks to monitor for contamination.

The samples were then transferred in a sealed container to the Department of Microbiology at Notre Dame Hospital (Montreal, Canada) for the remainder of the testing. Each sample was extracted with 1 ml of octane, and then mixed on a rotating shaker at room temperature for 15 minutes, or until the paraffin was solubilized. The tissue was then centrifuged at 12000 x g for two minutes, and the solvent removed with a pipette. If any paraffin remained, these steps were repeated. The tissue was then suspended in 0.5 ml 100% ethanol and centrifuged for 2 minutes at 12000 x g, and the ethanol removed. Ten μl of acetone was added and then dried (tubes open) in a 55°C oven for 60 minutes. The dried pellet was suspended in a digestion solution with a final concentration of 200 μg/ml proteinase K and 1% Laureth-12 in digestion buffer. This was
incubated for 3 hours at 55°C, and then spun at 12000g for 5 minutes. The protease was then heat inactivated by incubating the tubes at 95°C for 10 minutes. Any debris was removed. Each tube was assigned a reference number, such that samples were essentially randomized, and the technician performing the analysis was blinded as to the tissue source. The quantity of DNA was measured in a spectrophotometer.

3.) Line Blot Assay:

A sample of extracted DNA from each specimen was amplified using the PGMY-09/11 L1 consensus PCR protocol. The protocol uses 50 pM of each of primer pools PGMY-09 and PGMY-11 in a 1X PCR buffer containing 4 mM of MgCl₂, 7.5 U of AmpliTaq Gold DNA polymerase, 600 μM dUTP, and 200 μM each dATP, dCTP and dGTP, 50 μM KCl, 10mM Tris (pH 8.3) and biotinlabeled primer PGMY. The line blot assay was completed as previously published³⁷,³⁸. Amplification was performed in a Percon Elmer TC 9600 thermal cycler using the following profile: activation of AmpliTaq Gold at 95°C for 9 minutes, denaturation at 95°C for 1 minute, annealing at 55°C for 1 minute and extension at 72°C for 1 minute. Twenty-five microliters of AMPLICOR solution was added to 50 microliters of PCR amplified products. Seventy microliters of denatured PCR product was subsequently added to each individual well of an AMPLICOR typing tray containing 3 ml of a hybridization solution (4 x SSPE, 0.1% sodium dodecyl sulfate; each SSPE is equivalent to 0.18 M NaCl, 10 mM NaH₂PO₄ and 1 mM EDTA, pH 7.7) that was pre-warmed to 53°C and a strip of both HPV oligonucleotide and β-globin probes. The probe mixtures for the following HPV genotypes were fixed on distinct lines on each individual strip: types 6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 45, 51, 52, 53, 54, 55, 56, 57, 58, 59, 66, 68, MM4, MM7, MM8 and
MM9. The tray was then incubated in a water bath for 30 minutes at 53°C. The hybridization fluid was then gently aspirated from each of the wells, and 3 ml of washing solution containing 1 x SSPE and 0.1% sodium dodecyl sulfate was added at room temperature, and again aspirated. The washing buffer was aspirated and 3 ml of AMPLICOR streptavidin-horseradish peroxidase conjugate was added, and the tray shaken for 10 minutes on a shaker platform. This step was repeated once. After aspiration of the washing buffer, 3 ml of citrate buffer was added to each well and again aspirated. The substrate was prepared by mixing 0.01% hydrogen peroxide and 0.1% ProClin in a 0.1M citrate solution with 0.1% tetramethylbenzidine in dimethylformamide. Three ml of substrate was added per well. The trays were shaken at 70 rpm for five minutes at room temperature. The substrate was then removed, the strips rinsed with distilled water and stored in citrate buffer and the results read within 30 minutes. Measures to avoid false positivity secondary to contamination were strictly adhered to.

**Statistical analysis:**

Statistical analysis of the data was evaluated using $\chi^2$ for an expected frequency of 5 or more per cell. In cases of smaller numbers per cell, the Fisher exact test was employed. A $p<0.05$ was accepted as statistically significant.
Results:

The cases of seventy-four patients, 50 males and 24 females, diagnosed with SP between 1978 and 1999 were reviewed. Demographics are presented in table IV. These were matched to 50 males and 24 females diagnosed with CS over the same period. The average age was 54.6 years. Of the patients diagnosed with SP, 8 (10.8%) patients were diagnosed as having malignant variants (MSP), and 66 (89.2%) patients were diagnosed with benign variants (BSP). The control group consisted of 20 patients undergoing routine septoplasty for septal deviation, 13 males and 7 females, with an average age of 37 years. Samples from the control group consisted of biopsies from both the ethmoid sinuses (bulla ethmoidalis) and inferior turbinates.

Table IV: Demographic data amongst study groups

<table>
<thead>
<tr>
<th>Study group (N=168)</th>
<th>Female</th>
<th>Male</th>
<th>Average Age (years)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Schneiderian Papilloma (n=74)</td>
<td>24</td>
<td>50</td>
<td>54.6</td>
</tr>
<tr>
<td>Chronic Sinusitis (n=74)</td>
<td>24</td>
<td>50</td>
<td>54.6</td>
</tr>
<tr>
<td>Normal Nasal Mucosa (n=20)</td>
<td>7</td>
<td>13</td>
<td>37.0</td>
</tr>
</tbody>
</table>

Of the 168 total patients, 70 (41.7%) had detectable levels of DNA (table V). The age distribution of specimens was 3 months to 21 years. No DNA was detectable in specimens that were older than twelve years. The results of the breakdown of HPV positivity is illustrated in table VI. Of the 74 patients diagnosed with SP, 28 (37.8%) had detectable DNA. Of the 8 patients with MSP, 3 (37.5%) had measurable DNA. Of these patients, 1 (33.3%) tested positive for HPV 16. Of the 66 patients with BSP, 25 (37.8%)
had detectable DNA. Of these patients, 2 (8.0%) tested positive for HPV 6, 3 (12%) tested positive for HPV 11, and 1 (4.0%) tested positive for HPV 16. All patients with SP with positive testing for HPV were of the inverting histologic type. Of the 74 patients with CS, 22 (29.7%) had detectable DNA. Of these patients, 1 patient (4.5%) tested positive for HPV 16. In the 20 control patients, all 20 (100%) had detectable DNA. Of these patients, 1 (5.0%) tested positive for HPV 16. Of all 70 patients, 2 (2.9%) tested positive for HPV 6, 3 (4.3%) tested positive for HPV 11, and 4 (5.7%) tested positive for HPV 16. Overall HPV positivity was 12.9% (9 of 70 patients). Thus, in comparing HPV positivity amongst all SP (ie BSP plus MSP) to CS and the control group, the rate of positivity were 7/28 (25%), 1/22 (4.5%) and 1/20 (5%) respectively. As such the prevalence of HPV positivity in SP was 5 times that of either CS or the control group. None of the 70 patients with detectable DNA tested positive for HPV 18.

Table V: Summary of DNA positivity

<table>
<thead>
<tr>
<th>Study Group</th>
<th>DNA Positivity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Schneiderian Papilloma</td>
<td>28 / 74 (37.8%)</td>
</tr>
<tr>
<td>Chronic Sinusitis</td>
<td>22 / 74 (29.7%)</td>
</tr>
<tr>
<td>Normal Nasal Mucosa</td>
<td>20 / 20 (100%)</td>
</tr>
<tr>
<td><strong>Overall</strong></td>
<td>70 / 168 (41.7%)</td>
</tr>
</tbody>
</table>
Table VI: Summary of HPV testing

<table>
<thead>
<tr>
<th>Group (N=70)</th>
<th>HPV 6</th>
<th>HPV 11</th>
<th>HPV 16</th>
<th>HPV 18</th>
<th>All Types</th>
</tr>
</thead>
<tbody>
<tr>
<td>MSP (n=3)</td>
<td>--</td>
<td>--</td>
<td>1 (33.3%)</td>
<td>--</td>
<td>1/3 (33.3%)</td>
</tr>
<tr>
<td>BSP (n=25)</td>
<td>2 (8.0%)</td>
<td>3 (12.0%)</td>
<td>1 (4.0%)</td>
<td>--</td>
<td>6/25 (24%)</td>
</tr>
<tr>
<td>CS (n=22)</td>
<td>--</td>
<td>--</td>
<td>1 (4.5%)</td>
<td>--</td>
<td>1/22 (4.5%)</td>
</tr>
<tr>
<td>Control (n=20)</td>
<td>--</td>
<td>--</td>
<td>1 (5.0%)</td>
<td>--</td>
<td>1/20 (5.0%)</td>
</tr>
<tr>
<td>Overall (n=70)</td>
<td>2/70 (2.9%)</td>
<td>3/70 (4.3%)</td>
<td>4/70 (5.7%)</td>
<td>0/70 (0%)</td>
<td>9/70 (12.9%)</td>
</tr>
</tbody>
</table>

In examining the relationship between the CS, MSP, BSP and control groups, several observations were noted, as summarized in table VII. The presence of HPV was not determined to be clinically significant (p=0.096) between these four groups. However, if BSP and MSP were combined, there was a significant difference (p=0.046) with respect to the presence of HPV between groups. The presence of various types of HPV, specifically types 6, 11 and 16 was not determined to be clinically significantly different (p=0.122). In examining the difference between low risk types of HPV (type 6 and 11) and high-risk types (type 16), a significant difference (p=0.029) was found between all four groups. However, if both MSP and BSP were combined, no significance difference was found (p=0.076).
Table VII: Summary of statistical analysis

<table>
<thead>
<tr>
<th>Groups Being Compared</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Presence of HPV in CS, SP and control</td>
<td>0.046*</td>
</tr>
<tr>
<td>Presence of low risk versus high risk HPV types in CS, MSP, BSP and control</td>
<td>0.029*</td>
</tr>
<tr>
<td>Presence of HPV in CS, MSP, BSP and control</td>
<td>0.096</td>
</tr>
<tr>
<td>Presence of HPV types 6, 11 or 16 in CS, MSP, BSP and control</td>
<td>0.122</td>
</tr>
<tr>
<td>Presence of low risk versus high risk HPV types in CS, SP, and control</td>
<td>0.076</td>
</tr>
</tbody>
</table>

* Statistically significant p=0.05
Discussion:

Schneiderian papilloma is a benign, locally aggressive tumor of the nose and paranasal sinuses. The etiology of SP remains unconfirmed. Several possible etiologies exist, including viral infection, chronic inflammation, allergic proliferation and environmental carcinogen exposure. Evidence linking industrial carcinogens to an increased risk of SP was not evaluated in the current study. Increasingly, a viral etiology in the development of SP has gained popularity.

Kusiak first described the presence of intracytoplasmic or intranuclear inclusion bodies in SP consistent with HPV infection. Respler et al. first demonstrated the presence of HPV DNA in SP, with subsequent DNA hybridization studies and polymerase chain reaction studies alluding to human papilloma virus as a likely agent in the development of SP.

HPV analyses of cervical and ano-genital lesions have identified low and high-risk types. High-risk types include types 16, 18, 31, 33 and 35. Types 39, 45, 51, 52, 56 – 59 and 61 may be found in dysplastic and malignant lesions. Low risk types include 6, 11, 32, 34, 40, 42, 44, 53 – 55 and 63. Defining these types amongst non-head and neck lesions has spurred interest in identifying types and their role in various head and neck lesions, given there similar epithelial histologic and biologic constructs.

Various HPV types are associated with both benign and malignant diseases in the head and neck. Typically, HPV 6/11 have been associated with benign disease, such as laryngeal papillomatosis, oral cavity papillomas, condyloma acuminata, focal epithelial hyperplasia and leukoplakia. HPV associated with malignancy, such as in
squamous cell carcinoma of the tonsil, oral cavity and larynx, are more associated with types 16 and 18. A similar distribution of types is found amongst benign and malignant variants of Schneiderian papillomas. The current study did find a statistically significant difference between high and low-risk types amongst MSP, BSP, CS and normal nasal mucosa. None of the specimens, either malignant or benign, had detectable HPV-18, thereby possibly defining a less important role for this subtype in head and neck pathology. Furthermore, other previously defined HPV types were not identified in any samples.

In detecting the presence of HPV, initial investigations centered on the use of light microscopy, electron microscopy and immunohistochemistry. These, however, were fraught with inconsistent results. More recent advances center on the use of hybridization techniques and polymerase chain reaction.

Most often, tissue samples are derived from archival material, embedded in paraffin. The process of formalin fixation and paraffin embedding makes it more difficult to elaborate DNA. Several authors have noted that the increasing age of the tissue blocks makes DNA amplification more difficult. Furthermore, they attribute the decrease in positive detection of HPV to increased DNA degradation over time. These results are consistent with the results achieved in the current study, in which only 41.7% of the specimens had detectable DNA. Those specimens in which DNA was detected were consistently younger with respect to the year of surgery, with DNA not being detected in any tissue samples older than 12 years.
Hybridization techniques include Southern blot, dot blot and in-situ hybridization. These can identify specific genetic sequences in cells. Southern blot is the gold standard amongst hybridization techniques, as it only requires a fraction of the DNA to detect a given HPV type. However, it is expensive, complex and subject to laboratory variability. Dot blot hybridization is a technique whereby multiple specimens can be examined with small quantities of DNA. This technique is not as sensitive as Southern blot. Using in-situ hybridization, tissue sections are directly probed with HPV probes, therefore preserving the tissue morphology. This technique is expensive and labor intensive, and prone to cross hybridization with other cellular sequences under low stringency conditions.

In order to increase the sensitivity of HPV DNA detection, methods for signal amplification and gene amplification have been developed, with polymerase chain reaction (PCR) being the most sensitive. PCR is a method by which specific target sequences form a DNA specimen may be amplified, thereby increasing sensitivity and specificity of detection. Although very sensitive, this study is subject to potential contamination by other extraneous foreign specific DNA, and extreme care is needed to avoid false results. Furthermore, HPV genotype determination still relies on several hybridization reactions in order to identify HPV types, thereby increasing the technical difficulty of the technique.

In order to decrease the degree of technical difficulty and risk of contamination, the line blot assay utilizes a reverse hybridization reaction of biotin-labeled amplified DNA with oligomer probes that are fixed on a strip. Due to the fact that the amplified DNA is hybridized with all the relevant probes in a single reaction, the processing time is
greatly shortened. Although there is no literature comparing traditional PCR to line blot assay with respect to HPV analysis in SP, in anogenital lesions, equivalent sensitivity and specificity results were obtained. This current study is the first to utilize this technology in detecting HPV in SP. From this, it can be concluded that line blot assay offers a more rapid and equally sensitive and specific means of detecting HPV in SP when compared to traditional PCR techniques.

The mucosa lining the nasal cavity consists of a pseudo-stratified columnar epithelium. In an attempt to further define the role of HPV in the development of SP, the current study examined the presence of HPV in normal nasal mucosa as well as non-neoplastic benign nasal pathology, namely chronic sinusitis. This study confirmed the presence of HPV DNA in only 1 of 20 (5%) patients in the control group of normal nasal mucosa. This supports previous works which failed to detect HPV in normal mucosa. This current study also detected the presence of HPV in only one patient with CS. As HPV is not found in the overwhelming majority of normal mucosal specimens, this further supports the premise that HPV infection is involved at least in part in the development of SP. This is further supported by the fact that HPV is not found in the overwhelming majority of patients with other nasal pathologies. As well, the role of normal nasal mucosa as the ideal control for investigating HPV infection in the nose and paranasal sinuses was confirmed.

The exact role of HPV in SP remains to be elucidated. This current study revealed the presence of HPV in 7/28 patients with either MSP or BSP. As such the mechanism of development of SP is at least partially explained in these patients by infection with HPV. This is further supported by the statistically significant difference of
HPV presence between SP, CS and normal mucosa. However, because only 24% of SP patients had detectable HPV, it appears unlikely that HPV is the primary method by which SP develops, and it more likely that HPV plays a co-etiologic or synergistic role with an as of yet undefined mechanism. Furthermore, the fact that all of the SP patients that tested positive for HPV were of the inverting histologic type, it is possible that HPV is more adept at causing this form of SP as compared to fungiform or cylindrical variants. The reason for this remains to be determined.


Study Limitations:

During the course of the investigation, several limitations were noted. The first of these centered on data acquisition. The current study is a retrospective case-controlled investigation. Ideally, a prospective study should be done, however, there are time constraints associated with this approach. Another limitation is with regards to population size. Due to the rarity of this clinical entity, it is difficult to obtain large numbers of patients, in particular if examining specific types, be it malignant versus benign, or specific histologic types.

Other limitations center on the analysis of the tissue samples. The use of archival material adversely affects the results, as indicated earlier. It not only affects the quality of retrieved material due to the difficult task of removing the paraffin, but also limits the ability to retrieve material in older samples due to DNA degeneration. Ideally, fresh samples could be evaluated, again limited by the small number of patients. The use of a smaller base pair segment in identifying HPV types is currently being undertaken in order to attempt to relieve the issue of DNA fragmentation.
Future Directions:

In hopes of eliminating the problem of DNA fragmentation, our current investigations are targeting an 88 base-pair segment, instead of the currently used 450 base-pair probe. By doing so, it is hoped that the sensitivity of the line blot assay will be increased. In doing so, the aim would be two fold. Firstly, to identify those patients at potential risk for the development of Schneiderian papilloma or other HPV related neoplasms (ie high-risk HPV types). Secondly, having identified those with a potential risk, institute a comprehensive screening / monitoring protocol for these patients. Finally, identification of the exact mechanism of transformation of the mucosa by HPV alone or in conjunction with other mediators remains to be determined, and is the focus of future experiments.
Summary:

SP are uncommon nasal tumors. HPV is believed to play at least a partial but significant role in their etiology. This is the first study using line blot assay to identify various HPV types in SP. Statistically significant differences of HPV infection were found amongst SP, CS and normal mucosa. As well, differences amongst high and low-risk types were detected between the various investigation groups. The exact role of HPV and the mechanism of SP transformation remain to be fully explained.

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