Evaluation of the Predictive Role of Human Papilloma Virus in Patients with Benign Sinonasal Lesions

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ABSTRACT

Background:

Benign lesions of the nasal cavity represent a diverse group of pathologies, starting from simple inflammatory polyp to end with benign nasal tumor with potential malignant transformation. Viral infections have been blamed for tumorigenesis in many area of the body. Many evidences implicated HPV in aetiology of genital area and skin tumor.

However, its role in sinonasal tumor still debate. advanced technique of immunohistochemistry and PCR open the door to study the exact relation between this virus and tumor development.

Aim of the study:

Highlighting the malignant potential role of human papilloma virus in benign nasal lesions and in potential development of malignant nasal lesions.

Materials and Methods:

64 cases of benign nasal mass are incorporated within this study. Tissue was saved with formaldehyde and embedded in paraffin blocks. We used Immunohistochemical panel in order to
prove the correlation between HPV infection and etiology of benign nasal mass. Furthermore, PCR study as gold standard test used to confirm reliability of immunohistochemistry markers

Results:

64 of benign nasal mass, 35 of them were inverted papilloma and 29 cases were inflammatory nasal polyp. 13 cases were positive for HPV (9 inverted papilloma 4 nasal polyp) and 7 cases positive for P53 (6 inverted papilloma and 1 nasal polyp). For 13 cases which was positive by immunohistochemistry 3 of them were positive by PCR and the remaining 54 cases confirmed negative for HPV by PCR. The majority of age group included in this study was between 60-69 years (21.9) Majority of patients included in this study were male (70.3%) 54.7% of nasal mass incorporated in our study confirmed by 2 histopathological reading to be inverted papilloma, and the remaining diagnosed as inflammatory polyps.

In Immunohistochemical panel for all masses 2.1% were positive for both markers (HPV and P53) and both of them diagnosed as inverted papilloma 71.9% of cases were negative for both previously mentioned markers (22 cases diagnosed as inverted papilloma)

Conclusion:

1. There is role for human papilloma virus in development of chronic inflammatory polyp.

2. Though the role of HPV in tumorigenesis in other area of the body (e.g., genital area) is well established, its role in sinonasal inverted papilloma is controversy.

INTRODUCTION:

The nose is the most prominent part of the face with substantial aesthetic and functional significance. It is one of the few organs of body invested with an aura of emotional and cultural importance. Anatomical location of the nose and it passage have been regarded as the direct avenue to the brain, man's source of intelligence and spirituality.
Patients presenting with ENT (Ear, Nose, and Throat) outpatient departments report nasal obstructions, nasal discharge, epistaxis and changes in scent. Sinonasal masses have a wide range of differential diagnoses\(^{(2)}\).

Associated with congenital, inflammatory, neoplastic (benign or malignant), or traumatic conditions, congenital nasal masses can present intranasally, extranasally, or as external nasal masses. Dermoids, gliomas, and encephaloceles are examples of congenital masses. Polyps are the leading cause of nasal obstruction in adults, with about 4\% of the general population carrying them.\(^{(3)}\)

Polyps are masses of tissue that bulge or project downwards from the surface of the body, and which are easily visible macroscopically.\(^{(4)}\)

It is known that this condition will be treated with little improvement despite its common occurrence and unknown etiopathologic correlation; however, there are many factors involved in this condition\(^{(5)}\).

In the period 460-370 B.C., Hippocrates offered a graphic description of nasal polypoidal masses, establishing him as the "Father of Rhinology.". Forrestus (1522-1597 A.D.) wrote about a woman with nasal polyps who carried heavy weights, causing mucous membranes to force their way into the nose, leading to the growth of polyps\(^{(6)}\).

It is a common assumption that unilateral sinonasal masses in adults are either inverted papillomas or malignant tumors, even though most cases of sinonasal mass are thought to be inflammatory polyps. Although some researchers reported squamous cell carcinoma and simple nasal polyp as prevalent sinonasal lesions, the cause of nasal polyp remains unknown. Certain conditions such as allergy, asthma, and atopy may cause nasal polyp. It has been associated with infection, aspirin hypersensitivity, and cystic fibrosis.\(^{(7)}\)

As a result of the complex anatomy of the sinuses and nasal cavity, the tumor can grow through a specific sinus or nasal cavity before penetrating the periosteum or perichondrium or invading the bone.\(^{(8)}\)
There is potential for late disease in patients with sinonasal tumors due to this reason. Sinonasal masses can be diagnosed by endoscopic evaluation, radiological examination, and pathological examination. (9)

Infection with HPV plays a vital role in the development of clinical symptoms of inverted papilloma, while its role in other lesions is still not well understood. (10)

HPVs are a large group of related viruses. Each virus in the group is given a number, which is called an HPV type. (11)

The HPV virus is a double-stranded DNA virus that does not have an envelope. Seventeen hundred and ninety-two base pairs are homologous in 90% of the types. It has two key proteins coded for as L1 and L2, each of which is arranged in a circle. "Immunogenes," responsible for self-assembling and interacting with the virus, act as infectious proteins. (12)

Breaks in the epidermis of the skin are the route by which the virus is transmitted between humans. Viral attachment to cells of the skin stem called tissue-specific heparin sulfate proteoglycans occurs once the virus has entered the skin proteoglycans. (13)

Once within the squamous cell, the virus separates, duplicates, and proliferates, moving to the next cell. Among the greatest risks for malignant degeneration is the persistence of the HPV inside squamous cells. Local self-limiting infections, chronic infections, or rare malignancies may result from the invasion of infected host cells. (14)

Studies have extensively examined and described the oncogenic mechanisms of HPV infection on head and neck cancers.

Inverted Papilloma are still not understood to be related to these mechanisms. The presence of HPV E6 transcriptions is strongly suggested in inverted papilloma. (15)

E6 cause cellular transformation by different mechanisms. One of these mechanisms is the ability of p53 to be ubiquitinated.

Based on the following observations, it appears that HPVs are a causal factor in human cancers:
1) Cancer biopsies have been found to contain HPV DNA

2) E6 and E7 viruses are known viral oncogenes that are present in tumor tissue

3) Genes such as E6 and E7 are responsible for regulating growth in host cells

4) Several genes involved in E6 and E7 expression have been identified in immortalized cervical cancer cell lines

5) Cervical cancer, according to epidemiological studies, is most often caused by HPV

6) In research models, such as rabbits and cattle, there is a risk of cancer caused by species-specific papillomaviruses; and human neonatal foreskin infected with HPV-16 and placed in severe combined immunodeficient mice form intraepithelial neoplasms (Mandel). (16)

   On a pathogenetic level, the HPV-E6 gene product has been linked to the HPV-induced development of human cancer by binding to the human p53 tumor suppressor protein.

   Cell cycle transformation occurs between the G0 phase and phase 1 phase when the p53 protein is activated. Degradation of the p53 protein directly affects cell growth.

   Several studies have found that mutations in p53, associated with E7, inhibit cell death (apoptosis) by preventing G1 or arrest phases of the cell cycle. Hence, both genes adversely impact normal cell cycles leading to unrestrained, unhindered cell growth, including tumorigenesis. (17)

Patient and Methods:

Study design and setting:

The current study was accomplished by the Middle Euphrates Cancer Research Unit, (faculty of medicine in university of Kufa).

Data collection started at November 2020 and study had been finished at July 2021.
All cases incorporated in current study are collected in random way from different private pathological laboratories in Al Najaf governate.

Clinicopathological data were obtained from the relevant histopathological reports available with the tissue specimens, which consisted of patient’s age, gender, type of biopsy and final diagnosis.

**Selection of Samples:**

A: sample collection:

We have 64 cases of benign nasal mass, 35 of them were inverted papilloma and 29 cases were nasal polyp.

All samples were taken in period between 2015-2020

Tissue was embedded in paraffin blocks.

B) Control:

We used formaldehyde conserved submersed block of malignant specimen from cervix as guidance for assessment of Human papilloma virus in nasal mass tissue.

For P53 one tissue block of breast carcinoma was used.

**Materials and Chemicals:**

**Immunohistochemical detection system:**

**Dako monoclonal mouse anti-human papillomavirus (HPV)**

Clone: K1H8

Code: M3528

Isotype: IgG, kappa
Dilution: 1:50 Dako antibody Diluent. (57)

**Dako monoclonal mouse antihuman P53 protein**

Clone: DO-7

Code number: M7001

Immunogen: recombinant human wild-type P53 protein

Isotype: IgG2b, kappa

Dilution: Ready to use (58)

**Materials**

**Accessory solution**

The following solutions used in current study

Table (1) solutions

<table>
<thead>
<tr>
<th>Material</th>
<th>Company/origin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Absolute ethanol, analytical grade</td>
<td>Scharlau, Spain</td>
</tr>
<tr>
<td>Distilled water</td>
<td>Iraq</td>
</tr>
<tr>
<td>Harris hematoxylin</td>
<td>Germany</td>
</tr>
<tr>
<td>Mounting medium: Distyrene- Plasticizer- Xylene (DPX)</td>
<td>Germany</td>
</tr>
<tr>
<td>TBS solution, PH=7.4</td>
<td>Syrbio, Syria</td>
</tr>
<tr>
<td>Retrieving solution (tries buffer PH=6)</td>
<td>Dako</td>
</tr>
<tr>
<td>Xylene</td>
<td>Riedel – de haen, Germany</td>
</tr>
</tbody>
</table>
Agarose | Germany
---|---
DNA loading dye | USA
Ethidium bromide | USA
Proteinase K | Intron biotechnology, South Korea
Viral DNA /RNA extraction kit | Intron biotechnology, South Korea

**Apparatus**

The following apparatus used in study

**Table (2) apparatus**

<table>
<thead>
<tr>
<th>Apparatus</th>
<th>Company/country</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cover slips</td>
<td>Dako</td>
</tr>
<tr>
<td>Digital timer</td>
<td>China</td>
</tr>
<tr>
<td>Eppendorfs</td>
<td>China</td>
</tr>
<tr>
<td>Glass and plastic staining jars</td>
<td>China</td>
</tr>
<tr>
<td>Gloves</td>
<td>China</td>
</tr>
<tr>
<td>Hot air oven</td>
<td>Memert</td>
</tr>
<tr>
<td>Water bath</td>
<td>Memert/Germany</td>
</tr>
<tr>
<td>Light microscope</td>
<td>Leica</td>
</tr>
<tr>
<td>Locally made humid chamber to preserve moisture</td>
<td>Iraq</td>
</tr>
<tr>
<td>Metal plate with heat source</td>
<td>Memert</td>
</tr>
<tr>
<td>Micropipettes: 0.5-10μl and its tips</td>
<td>Eppendorf/Germany</td>
</tr>
<tr>
<td>100μl and its tips</td>
<td></td>
</tr>
<tr>
<td>-------------------------</td>
<td>--------</td>
</tr>
<tr>
<td>Microscope normal glass slides</td>
<td>Dako</td>
</tr>
<tr>
<td>Microtome</td>
<td>Leica</td>
</tr>
<tr>
<td>Plastic kaplan jars</td>
<td>China</td>
</tr>
<tr>
<td>Sensitive balance</td>
<td>Denever</td>
</tr>
<tr>
<td>Polymerase chain reaction (PCR)</td>
<td>Eppendorf/Germany</td>
</tr>
<tr>
<td>Vortex</td>
<td>VELP</td>
</tr>
<tr>
<td>Freezer (-20)</td>
<td>Samsung/Korea</td>
</tr>
<tr>
<td>Microwave</td>
<td>Concord</td>
</tr>
<tr>
<td>Electrophoresis apparatus</td>
<td>Biometra</td>
</tr>
<tr>
<td>Gel documentation system</td>
<td>USA</td>
</tr>
<tr>
<td>UV- Trans illuminator</td>
<td>UVP/USA</td>
</tr>
</tbody>
</table>

Methods

Pretreatment Steps

Tissue section: The formaldehyde conserved and paraffin immersed specimen were exposed to thin section instrument to (four micron) slices in density, and assorted on charged slides.

Hematoxylin and eosin staining procedures

Immunohistochemical staining method

Quantitative Scoring Methods

<table>
<thead>
<tr>
<th>Score</th>
<th>0</th>
<th>+1</th>
<th>+2</th>
<th>+3</th>
<th>+4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive cells</td>
<td>&lt;10%</td>
<td>10-25%</td>
<td>25-50%</td>
<td>50-75%</td>
<td>&gt;75%</td>
</tr>
<tr>
<td>Score</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-------</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intensity of staining</td>
<td>week</td>
<td>Moderate</td>
<td>Strong</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Quick score (Q): Results are scored by multiplying the percentage of positive cells (P) by the intensity (I). Formula: \( Q = P \times I \); Maximum = 300.(60)(61)

**DNA extraction protocol according to INtRON biotechnology**

**Electrophoresis**

**Pouring a Standard 0.6% Agarose Gel:**

**Analyzing Your Gel:**

Using the DNA ladder in the first lane as a guide (the manufacturer's instruction will tell you the size of each band), you can infer the size of the DNA in your sample lanes.

**Polymerase chain reaction amplification of HPV gene**

**primer preparation:**

**Amplification by Polymerase Chain Reaction**

As a first step, PCR optimization was performed with a gradient temperature ranging from 45 C to 53 C. After the determination of optimum annealing temperature (50.7 0 C), the PCR reaction mixture consisted of < 250 ng template DNA, 400 µM of each dNTP, 12.5 µl buffer of 1 U GoTaq DNA polymerase (Promega), 10 µM of each primer and 3 mM MgCl2 in 25 µl of total reaction volume.

The GTC Series thermocycler was used for amplification reactions (Eppendorf/Germany) apparatus.
For amplifying the target DNA fragments, the following program was set up in the thermocycler after determining the optimum annealing temperature.

Table (3) the program used for HPV: 6/11, 16, 18 sub types amplification sequence

<table>
<thead>
<tr>
<th>Stage</th>
<th>Temp.(°C)</th>
<th>Time(min)</th>
<th>Function</th>
<th>Cycles</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>94</td>
<td>5:0</td>
<td>Initial denaturation</td>
<td></td>
</tr>
<tr>
<td></td>
<td>94</td>
<td>0:30</td>
<td>denaturation</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>50.7</td>
<td>0.40</td>
<td>Primer annealing</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>72</td>
<td>0.35</td>
<td>Template elongation</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>72</td>
<td>10</td>
<td>Final elongation</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>-</td>
<td>Incubation</td>
<td>Hold</td>
</tr>
</tbody>
</table>

Amplified DNA fragments were electrophoresed on 0.6% agarose, (0.5x) TBE buffer for (40 min at 70 V).

RESULTS:

this cross-sectional study includes 64 of benign nasal mass, 35 of them were inverted papilloma and 29 cases were inflammatory nasal polyp.

Immunohistochemistry study by P53 and HPV had been done for all slides.

13 cases were positive for HPV (9 inverted papilloma 4 nasal polyp) and 7 cases positive for P53(6 inverted papilloma and 1 nasal polyp).
PCR study done to all 64 slides as gold standard to detect human papilloma virus in nasal lesions

For 13 cases which was positive by immunohistochemistry 3 of them were positive by PCR and the remaining 54 cases confirmed negative for HPV by PCR

4.2 Sociodemographic and Clinical Variables of Studied Samples.

Table 4-1 shows the majority of age group included in this study was between 60-69 years (21.9) Majority of patients included in this study were male (70.3%) 54.7% of nasal mass incorporated in our study confirmed by 2 histopathological reading to be inverted papilloma, and the remaining diagnosed as inflammatory polyps.

In Immunohistochemical panel for all masses 2.1% were positive for both markers (HPV and P53) and both of them diagnosed as inverted papilloma 71.9% of cases were negative for both previously mentioned markers (22 cases diagnosed as inverted papilloma) PCR study use kits specific for these subtypes (6/11,16 and 18) which considered as high risk for malignancy.

Only 3 cases were positive (3.1% for subtype HPV 16 and 1.6 % for HPV 18).

Of these 3 cases 2 histopathological diagnosis was (2 inverted papilloma and one inflammatory polyp).

Table 4:1 clinicopathological variables

<table>
<thead>
<tr>
<th>Clinico-pathological variables</th>
<th>NO.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age group</td>
<td></td>
<td></td>
</tr>
<tr>
<td>10-19</td>
<td>8</td>
<td>12.5</td>
</tr>
<tr>
<td>20-29</td>
<td>4</td>
<td>6.3</td>
</tr>
<tr>
<td>30-39</td>
<td>9</td>
<td>14.1</td>
</tr>
<tr>
<td>40-49</td>
<td>13</td>
<td>20.3</td>
</tr>
<tr>
<td>50-59</td>
<td>11</td>
<td>17.2</td>
</tr>
<tr>
<td>60-69</td>
<td>14</td>
<td>21.9</td>
</tr>
</tbody>
</table>
4:3: **Immunohistochemical expression of P53 and HPV in relation to clinico-pathological variables:**

No significance had been noticed between immunohistochemical expression of P53 and HPV and clinicopathological variants (age, gender and histopathological diagnosis).

Table (4.2) Immunohistochemical expression of P53 and HPV in relation to clinico-pathological variables:

<table>
<thead>
<tr>
<th>Age groups</th>
<th>P53 Positive (n=7)</th>
<th>P53 Negative (n=57)</th>
<th>HPV Positive (n=13)</th>
<th>HPV Negative (n=51)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. (%)</td>
<td>No. (%)</td>
<td>No. (%)</td>
<td>No. (%)</td>
</tr>
<tr>
<td>70-79</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gender</td>
<td>Male</td>
<td></td>
<td>Male</td>
<td></td>
</tr>
<tr>
<td></td>
<td>45</td>
<td>70.3</td>
<td>19</td>
<td>29.7</td>
</tr>
<tr>
<td>Diagnosis</td>
<td>IP</td>
<td>35</td>
<td>54.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>NP</td>
<td>29</td>
<td>45.3</td>
<td></td>
</tr>
<tr>
<td>Immunohistochemical panel</td>
<td>HPV +/P53 +</td>
<td>2</td>
<td>2.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HPV +/P53 -</td>
<td>11</td>
<td>17.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HPV -/P53 +</td>
<td>5</td>
<td>7.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>HPV -/P53 -</td>
<td>46</td>
<td>71.9</td>
<td></td>
</tr>
<tr>
<td>PCR (HPV types)</td>
<td>6/11</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>16</td>
<td>2</td>
<td>3.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>1</td>
<td>1.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>61</td>
<td>95.3</td>
<td></td>
</tr>
</tbody>
</table>
4-4: immune-histochemical co-expression of HPV and P53 in benign nasal lesions:

No significant correlation found between all 4 patterns of HPV and P53(+/+,+/-, -/+ and -/-)

Table (4.4) : immune-histochemical co-expression of HPV and P53 in benign nasal lesions
4:5: **Validity of HPV compared to PCR in detection of benign nasal lesion:**

Immunohistochemistry marker HPV compared to PCR in detection of HPV has sensitivity of 100%, specificity 83.6%, positive predictive value 23.1%, NNP 100% and accuracy of 84.4%. Validity of this marker in detection of HPV in nasal mass was not significant.

<table>
<thead>
<tr>
<th>Immune-histochemical</th>
<th>PCR</th>
<th>Total</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive No. (%)</td>
<td>Negative No. (%)</td>
<td></td>
</tr>
<tr>
<td>HPV</td>
<td>3 (23.1%)</td>
<td>10 (76.9%)</td>
<td>13 (100.0%)</td>
</tr>
<tr>
<td></td>
<td>0 (0.0%)</td>
<td>51 (100.0%)</td>
<td>51 (100.0%)</td>
</tr>
<tr>
<td>Total</td>
<td>3 (4.7%)</td>
<td>61 (95.3%)</td>
<td>64 (100.0%)</td>
</tr>
</tbody>
</table>

4:6: **HPV detection by PCR in benign nasal lesion:**

PCR consider as gold standard tool for detection of HPV in human tissue. In our study PCR appear to be not significant as tool for detection of human Papilloma virus in benign nasal mass (P value 0.99%).

<table>
<thead>
<tr>
<th>PCR HPV type</th>
<th>IP No.(%)</th>
<th>NP No.(%)</th>
<th>Total</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>6/11</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0.99</td>
</tr>
</tbody>
</table>
### Table

<p>| | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>16</td>
<td>1 (50%)</td>
<td>1 (50%)</td>
<td>2 (100%)</td>
</tr>
<tr>
<td>18</td>
<td>1 (100%)</td>
<td>0 (0%)</td>
<td>1 (100%)</td>
</tr>
<tr>
<td>Total</td>
<td>2 (66.7%)</td>
<td>1 (33.3%)</td>
<td>3 (100%)</td>
</tr>
</tbody>
</table>

### Discussion:

#### Age and gender distribution:

Old age group (60-69) was the predominant group in this study, and this not correlate with most of other studies in other studies. Development of sinonasal masses is most likely to occur in the in the 2nd and 4th decade of life (6).

According to Zafar et al. the mean age of presentation was 22.5 years. (64)

Bhattacharya et al., majority of non-neoplastic masses occurred in the age group 11–30 years (65).

This difference may be related to many causes like this may be due to few numbers of patient included in this study.

The differences in etiology of nasal mass may affect age of presentation, keeping in mind inflammatory nasal polyp, allergic nasal polyp has different incidence in different area of the world. (66)

70.3% in this study were male, and this similar to many studies that has overall male ratio higher than female. male-tofemale ratio of 1.7:1 in the study by Zafar et al. from India. (64)

#### A British review of nasal polyposis reported a ratio at 2:1 (M:F) (67)

Differences in anatomic size, tobacco susceptibility, and hormonal factors have been speculated to this difference.
Histopathological diagnosis:

54.7% of cases incorporated in this study diagnosed as inverted papilloma, and this differ from many studies in which the inflammatory polyp was reported to be more common.

In Turkish study, Kucur et al. 25.2% of the cases with unilateral sinonasal masses were found to be related to neoplastic and 74.8% of them to inflammatory causes. (68)

A study in Hawler medical university in Iraq (69) the inflammatory causes of unilateral nasal diseases were 73 (81.1%) compared with the neoplastic group, which was 17 (18.8%), another study in Babil university, Non-neoplastic conditions were the most common in unilateral nasal mass and include (36.2%) cases of chronic rhinosinusitis and (21.3%) cases of allergic fungal rhinosinusitis. (70)

This difference from these study may be of wrong practice of sending only the clinically and grossly suspicious lesion for histopathological study Similar to many studies, our results stated inverted papilloma as most common neoplastic causes of nasal mass Inverted papilloma was the commonest benign neoplastic condition (46.2%) in study in Karbala (71).

Immunohistochemical panel for nasal mass

We use 2 makers for IHC assay for patient included in this study.

These markers arte HPV and P53 2.1% of cases were positive for both of these markers. Both of these cases confirmed histopathologically as inverted papilloma. This result go with many recent studies have pointed out the increasing prevalence of the human papilloma virus in the area of head and neck tumor.

Study in Czech republic confirmed HPV positivity in 8% of patients with OSCC. (72)

Based on calculations, 60 (17.8%) of 336 inverted papillomas with absent or mild dysplasia were HPV-positive. (73)

However this correlation between virus and inverted papilloma in our study is not significant.

This may be due to few number of patient enrolled in this study.

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In addition to that the The “hit and run theory” of HPV tumorigenesis for inverted papilloma is now believed not be acceptable. On the basis of this theory, the virus invades tissues, causes tumors, and then is cleared/shed from the tissue. Yet, known HPV-related neoplasia (eg. common warts, condylomata acuminata, respiratory papillomatosis, everted papilloma, and oropharyngeal squamous cell carcinoma) do not demonstrate this behavior.\(^{(74)}\)

Due to the cellular host's dependence on viruses for viability, viruses have developed various strategies to recruit and utilize the host's machinery for their own purposes. As a transcription factor, p53 plays an important role in how cells undergo cellular differentiation. Therefore, it is not surprising that Viral targets involving p53 are prominent.\(^{(75)}\)

17.2% of our specimens were positive for HPV marker and negative for P53, this can be explained by the viral infection not always overexpress P53.

Routine screening for HPV in inverted papilloma, despite the relatively low rate of HPV transmission in early inverted papilloma, remains clinically valuable to identify patients' risk for cancer.\(^{(76)}\)

7.8% of our cases, however, showed positive P53 results. Although HPV testing was negative, this may suggest that nasal mass can host a variety of neoplasms due to non-viral factors like smoking or metaplasia from exposure to irritant.

**validity of immunohistochemistry in detection of HPV in nasal mass:**

Although immunohistochemistry marker is considered as highly sensitive for detection of HPV in nasal mass with sensitivity reach 100% in our study but its validity is not significant. This may be due to superimposed other viral infection.

Other explanation, we used only PCR kit for detect of high-risk group HPV (6/11, 16 and 18)

This finding similar to other studies like one that published in 2015 \(^{(77)}\) which stated that, There are many differences among studies reporting the prevalence of HPV in sinonasal polyposis, including different techniques, tissue fixation methods, numbers of samples, and
selection of controls that make comparisons challenging. The results presented in this study.
There is a distinct association between HPV and nasal polyps, although it is less pronounced than
in relation to antral polyps. This association does not appear to initiate proliferation or an
oncogene transformation; it is not thought to be a contributing factor to cancer development in
either case. They propose that it rather is matter of coincidental transient infection.

Other study reaches to same conclusion, they stated that recent reports have highlighted
SNSCCs which arise from inverted papillomas and are associated with low-risk HPV types.\(^{(78)}\)

**validity of PCR to detect of HPV in nasal mass**

Although it is considered as gold standard for detection of HPV in human tissue, our
results show no significance in use it as tool for identification of virus in nasal mass.

This may be attributed to many factors like small size of sample in this study, viral load and
anatomical site from which biopsy obtained.

A reliable HPV test has not yet been approved by the FDA for detecting head and neck cancers.
The detection of HPV in solid tumors is currently achieved by different techniques, such as
specific PCR methods, real-time PCR tests, in situ DNA hybridization (ISH) and
immunohistochemical methods (protein p16INK4a). There is a need for a multitest approach that
can help unravel discordant results since none of these methods provides optimal levels of
sensitivity
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