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HISTOMORPHOLOGICAL STUDY OF LESIONS OF NOSE AND PARANASAL SINUSES WITH SPECIAL REFERENCE TO PREVALENCE OF HPV IN NEOPLASTIC LESIONS

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ABSTRACT **Aims:** Present study has been done to study histomorphology of sinonasal lesions in detail and find out prevalence of human papilloma virus (HPV) in neoplastic lesions.

Materials & Methods: The study was conducted in the department of pathology from September 2017 to 2019. It was a combined study of prospective (70 cases) and retrospective (70 cases) type over last 5 years. After getting clinical detail, biopsy was done. 3-4 µ Section taken and stained with haematoxylin & eosin, additional sections were cut for IHC to study the HPV Antibody expression in neoplastic lesions.

Results: Out of 140 specimens, 80 Non-neoplastic (57%) and 60 neoplastic (43%) cases were reported. Among the neoplasms, 40 (29%) cases were benign and 20 (14%) cases were malignant. Total no. of cases that were positive for HPV were 9/26 (35%). Out of 26 neoplastic cases 10 cases (38%) were benign and 16 cases (62%) were malignant. Maximum cases showed mild grade (31%) HPV positivity followed by trace positivity (23%) and (4%) moderate positivity.

Conclusion: Sinonasal lesions are common lesions of head and neck region with higher prevalence of benign lesions, where nasal cavity is commonest site. Malignant lesions mostly occur in higher age group and prominently affect paranasal sinuses. Overall HPV prevalence was low and observed almost equally in benign and malignant cases. Thus, it can be inferred that HPV per se may not act as etiological factor but may play a supportive role along with other etiological factors in causation of sinonasal neoplastic lesions.

KEYWORDS : Benign, non-neoplastic, malignant, sinonasal, human papilloma virus.

INTRODUCTION:

Nose and nasopharyngeal areas have different type of lining epithelium and therefore can have inflammatory as well as neoplastic lesion of varied morphology in this area. Therefore it is essential to know histomorphological features in detail. The epithelial lining in the major part of the respiratory tract is composed of respiratory epithelium (columnar cells), whereas stratified squamous epithelium covers the mucosa of the pharynx and a part of the larynx. Because of this divergent histology of the mucosal lining, a wide variety of both benign and malignant tumours arises in the respiratory tract.

HPV has been found to be associated with various lesion of head and neck region. This coexistence of two different epithelia creates squamo–columnar junctions at multiple sites in the respiratory tract, entities that are thought to be a prerequisite for the spread of HPV infections in this region. Benign papillomas are preferentially associated with the low-risk HPV types 6 and 11 where as their malignant counterparts are exclusively positive for HPV 16 DNA. [1] However its role has not been studied in detail in neoplastic lesions of nose and paranasal sinuses. Therefore present study has been done to study histomorphology of these lesions in detail and find out prevalence of HPV in neoplastic lesions.

MATERIAL AND METHODS:

The study was conducted in the department of pathology in collaboration with ENT department from September 2017 to September 2019. It was a combined study of prospective (70cases) and retrospective (70cases) type over last 5 years and included 140 specimens. Slides and blocks of specimen (biopsy/surgical) received in department of pathology for last 3 years were taken out and re-evaluated. This was carried forward for 2 years including 70 prospective specimens. Relevant clinical and follow-up data was obtained from the clinical histories of the patients and special attention was paid to clinicopathological details including age, gender, risk factor, tumour site and size. Prospective specimens were fixed in 10% formalin, processed by conventional methods. 3-5µm section taken and stained with haematoxylin & eosin and addition 3µm sections were cut for Immunohistochemistry (IHC) to detect HPV Antibody expression.

Antigen retrieval carried out in Tinto Retriever Pressure Cooker (Tris buffer PH 10.0) for 20 minutes at 106-110°C. Mouse Monoclonal Anti- HPV monoclonal antibody, clone BSB-66 (SB24) (Tinto) was used in the study which reacts with an epitope of major capsid protein of HPV, which is broadly expressed among different HPV subtypes and raised against HPV type 16 antigen.

Cells showing positive expression of HPV antigen on IHC in the form of brown colouration of nucleus or nuclear-cytoplasm both were counted randomly in 10 high power fields by pin hole method and then percentage was calculated against the total number of nuclei recorded. Only cytoplasmic brown colouration was taken as negative. Grading was done according to n% of cells showing HPV antigen expression (Negative <1%, trace 1-10%, mild 11-30%, moderate 31-60% and sever >60%). **(Fig. 1a-d)** This study has been performed in accordance with the ethical standards.

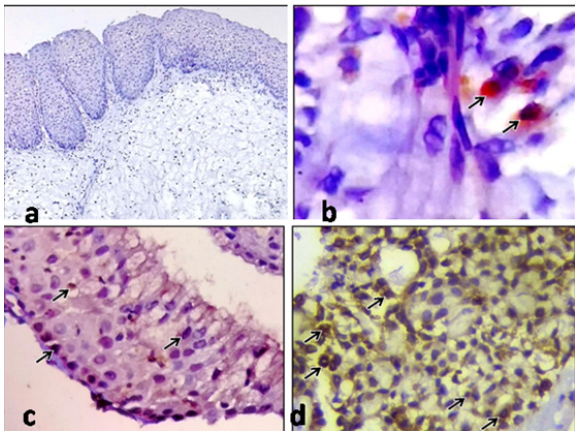

Figure 1(a) Slide showing negative HPV expression. (IHC-100X) (b) Section showing trace positivity for HPV.(just 2 cells positive for HPV)(IHC- 400X) (c) Slide showing mild grade positivity for HPV (IHC-400X) (d) Slide showing moderate grade positivity for HPV. (IHC- 400X)

RESULTS:

A total of 140 specimens from sinonasal region were included in the

present study. Of these 80 were non-neoplastic (57%) and 60 were neoplastic (43%). Of the neoplasms, 40 (29%) cases were benign and 20 (14%) cases were malignant.

The age of the patients included in the study was 3 to 76 years with mean (SD) age of 33 (18) years. Majority of non-neoplastic lesions were seen in 2nd and 3rd decade with mean (SD) age of 31 (17) years, while benign neoplasm seen in 2nd decade with mean (SD) age of 28 (17) years. Malignant neoplasm was mostly seen in 6th to 7th decade with mean (SD) age of 47 (18) years, which was higher than benign neoplasm. However the difference was not significant (p>0.05). Male preponderance was more than female with ratio of 1.5:1.

The commonest site for non-neoplastic lesion was nasal cavity, where as paranasal sinuses (PNS) were the commonest site for malignant lesions. Benign neoplastic lesions were equally distributed among all the three sites. Maximum lesions were seen in nasal cavity (NC) (62%) followed by PNS (24%) and nasopharynx (14%). No non-neoplastic lesion was found in Nasopharynx. (Table 1)

Table 1 - Shows The Distribution Of Lesion As Per The Site.

Site	Nasal cavity	Paranasal sinus	Nasopharynx
Non neoplastic (n=80)	69 (86%)	11 (14%)	-
Benign (n= 40)	14 (35%)	12 (30%)	14 (35%)
Malignant (n=20)	04 (20%)	10 (50%)	06 (30%)
Total (n= 140)	**87(62%)**	**33 (24%)**	**20 (14%)**

Non-neoplastic lesions were the most common lesion involving this region. NC was most common site (86%) followed by PNS (14%). Nasal polyps (80%) were the commonest non-neoplastic lesion followed by choanal polyp 14%, nasolabial cyst (2.5%), rhinosporidiosis (1.25%), rhinophyma (1.25%) and fungal sinusitis (1.25%). **(Table 2) (Fig. 2a, 2b, 2c)**

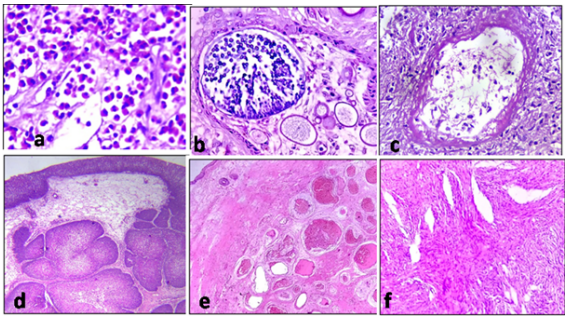

Figure 2 (a) Slide from case of allergic polyp showing numerous eosinophils. (H&E-400x) (b) Rhinosporidiosis showing sporangium containing numerous spores. (H & E – 400X) (c) Fungal sinusitis showing numerous fungal hyphae. (H&E-400x) (d) Inverted papilloma showing lobules of papillomatous squamous epithelium growing downward in stroma. (H & E – 100X) (e) Angiofibroma showing numerous proliferating capillariesof various size, shape and thickness along with dense fibrocollagenous stroma.(H & E – 100X) (f) Schwannoma showing showing verocay bodies and proliferation of blood vessels.(H & E – 400X)

Table 2- Shows Details Of Different Non-neoplastic Lesions Included In Study.

Type of lesion	Total No. of cases	(%)	M : F	NC	PNS	Mean age (In years)
Nasal polyps • Inflammatory (52) • Angiomatous (05) • Allergic (07)	64	80%	1.29 : 1	64	-	31
Choanal polyp (11)	11	14%	4:7	-	11	29
Nasolabial cyst	02	2.5 %	female	2	-	52
Rhinosporidiosis	01	1.25 %	Male	1	-	10
Rhinophyma	01	1.25 %	Male	1	-	47
Fungal sinusitis	01	1.25%	female	1		13
Total	**80**	**100%**	**1.1:1**	**69 (86%)**	**11 (14%)**	**31**

Of the 40 benign cases, angiofibroma was the most common with 14/40 (35%) cases followed by capillary haemangioma with 12/40 (30%) cases and inverted papilloma (IP) with 12/40 (30%) cases. (Table 3) (Fig. 2d, 2e, 2f) NC and nasopharynx were equally involved (35% each) followed by PNS (30%).

Table 3- Shows Details Of Different Non-neoplastic Lesions Included In Study.

Type of lesion	Total No. of cases	(%)	M : F	NC	PNS	Mean age (In years)
Nasal polyps • Inflammatory (52) • Angiomatous (05) • Allergic (07)	64	80%	1.29 : 1	64	-	31
Choanal polyp (11)	11	14%	4:7	-	11	29
Nasolabial cyst	02	2.5 %	female	2	-	52
Rhinosporidiosis	01	1.25 %	Male	1	-	10
Rhinophyma	01	1.25 %	Male	1	-	47
Fungal sinusitis	01	1.25%	female	1		13
Total	**80**	**100%**	**1.1:1**	**69 (86%)**	**11 (14%)**	**31**

This study included 20 malignant cases and the most common lesion was squamous cell carcinoma (SCC) 10/20 (50%) followed by adenoid cystic carcinoma (ACC) 4/20 (20%) small round blue cell tumour (SRBCT) 4/20 (20%) cases and nasopharyngeal carcinoma (NPC) 2/20 cases (10%). **(Table 4) (Fig. 3a to 3f)** Out of the total 20 cases the PNS (50%) was most commonest site for malignant neoplasm followed by nasopharynx (30%) and NC (20%).

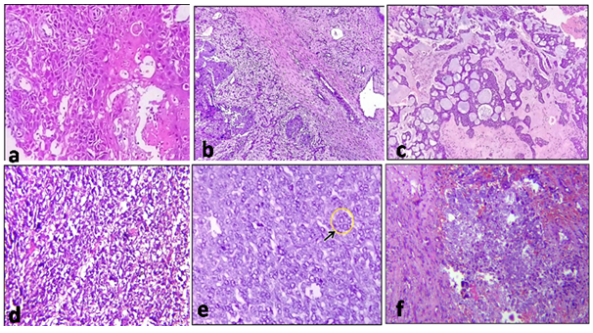

Figure 3 (a)Slide of WDSCC showing tumour cells in nest along with keratin flakes.(H & E- 400X) (b) NKSCC show lining epithelium showing squamous metaplasia and deeper area show island of dysplastic non keratinizing squamous cells. (H & E- 100X) (c) ACC showing Small basaloid cells arranged in cribriform pattern.(H & E-100X) (d) SCC-spindle type showing mixture of round polygonal cells admixed with spindle shaped cells.(H & E- 400X) (e) Olfactory neuroblastoma showing poorly formed rosettes.(H & E- 400X) (f) NK-NPC showing markedly pleomorphic, hyperchromatic cells in irregular island along with few atypical mitoses. (H & E- 100X)

Table 4–Shows Details Of Different Malignant Lesion Included In Study.

Diagnosis	No. of case	Percentage	M:F	NC	PNS	Naso-pharynx	Mean age (years)
SCC -Keratinizing - Well diff. -Moderately -Poorly -Non- Keratinizing -Spindle cell SCC	(10) 1 5 1 2 1	50 %	4:1	4	6	 - - - - -	55
ACC	4	20 %	1:1	-	-	4	29
SRBCT	4	20 %	1:3	-	4	-	36
NPC	2	10%	1:1	-	-	2	46
Total	**20**	**100 %**	**1.5 : 1**	**4 (20 %)**	**10 (50 %)**	**6 (30 %)**	**47**

Expression of HPV in neoplastic sinonasal lesins:
Expression of HPV was done in neoplastic lesion only. No. of positive nucleus/ nucleus with brown cytoplasm were counted and percentage was calculated.

Total no. of cases that were positive for HPV were 9/26 (35%). Out of 26 neoplastic cases, 10 cases (38%) were benign (IP) and 16 cases (62%) were malignant [SCC (n=10), ACC (n=4) & NPC (n=2)]. Maximum cases showed mild grade (31%) HPV positivity followed by trace positivity (23%). One case (4%) of moderate grade positivity was noted. Four out of 10 (40%) benign cases (IP) showed mild grade positivity for HPV antigen and one case (10%) was in trace grade. Among malignant lesions 2/10 (20%) cases of SCC showed mild positivity and 4 cases showed trace positivity for HPV. 3 out of 4 Cases (75%) of ACC were positive for HPV, out of 3 positive cases 2 (50%) were of mild grade and 1 case (25%) was of moderate grade. None of the NPC case (2) showed positive HPV expression. **(Table 5)**

Table 5 HPV Expression In Different Neoplastic Sinonasal Lesions.

Grading		IP (n=10)	SCC (n=10)	ACC (n=4)	NPC (n=2)
Negative (<1%)	(42%)	50%	40%	25%	50%
Trace (1-10%)	(23%)	10%	40%	-	50%
Mild (11-30%)	(31%)	40%	20%	50%	-
Moderate (31-60%)	(04%)	-	-	25%	-
Severe (>60%)	(0%)	-	-	-	-
Total Positivity (9/26)	**(35%)**	**40%**	**20%**	**75%**	**0%**

DISCUSSION:
Incidence of sinonasal lesions in our study was 28 cases per year, almost similar incidence (34.3 cases/year) was noted by Khan *et al* [2]. Out of 140 cases, 80 (57%) cases were non-neoplastic and 60 (43%) cases were neoplastic lesions, that was consistent with finding of Zafar *et al* [3]. In contrast higher non-neoplastic lesions was noted by (80%) Parmar *et al* [4]. The most affected age group in this study was 11-20 years (29%) and 21-30 years (19%). This observation was in accordance Shaila *et al* [5] but higher age group (31-40 years) was reported by Singh *et al* [6]. Overall mean age (SD) occurrence of sinonasal lesions was 33 (18) years in current study, which was greater than study (22.5years) conducted by M Kulkarni *et al* [7]. Male preponderance was more than female with ratio of 1.5:1, which was concordance with finding of (1.3:1) Singh *et al* [6].

Overall most common site for sinonasal lesion in our study was nasal cavity (64%) followed by PNS (22%) and nasopharynx (14%), which was close to study reported by Shaila *et al* [5]. According to M kulkarni *et al* [7] 90% cases were seen in NC which was higher than our study.
Most common age group for non-neoplastic lesions in present study was 11-20 years (30%), which was lesser than observation of Maru *et al* [8]. Mean age (SD) of non-neoplastic lesion was 31 (17) years in present study that was greater (22.5yrs) than mean age observed by Khan *et al* [2]. M:F ratio in our study was 1.1:1, which was close (1.2:1) to finding of Jyothi et al [9]. In present study most common site for non-neoplastic lesion was NC (86%) followed by PNS (14%). Shaila *et al* [5] reported almost similar findings. In our 80 (57%) non-neoplastic cases, nasal polyps were the most common (80%), that was close to observation reported by Chandra *et al* [10].

Out of 140 cases of sinonasal lesion, 40 (29%) cases were benign, which concordance with study (28.7%) conducted by Agarwal *et al* [11], however lower incidence (16%) was observed by Bijjaragi *et al* [12]. Most common age group for benign neoplasm in our study was 11-20years (40%), which was close to finding of Jyothi *et al* [9]. The mean age (SD) was 28 (17) years in this study, while mean age was 33 years according to Singh *et al* [6]. M:F ratio in present study was 3:1, which was same as reported by Khan *et al* [3]. NC and nasopharynx was equally (35%) involved in current study followed by PNS (30%), while according to Khan *et al* [2] NC (50%) was most common site for benign neoplasm followed by nasopharynx (43%) and PNS (7%). In present study angiofibroma was the most common benign neoplasm (33%). Jyothi *et al* [9] also found angiofibroma as most common benign neoplasm.

Incidence of malignant neoplasm in our study was 14% of total sinonasal lesions, which was close to (10.6%) finding of Singh *et al* [6]. While (2.6%) M Kulkarni *et al* [7] reported lower incidence of malignant lesions. Most common age group in present study was 6th & 7th decade, that was consistent with finding of Maru *et al* [8]. As compared to finding of (35 year) Khan *et al* [3] mean age (SD) of malignant lesions in current study was 47 (17) years. M:F ratio in our study was 1.5:1, while Singh *et al* [6] reported (0.75:1). Most common site for benign neoplasm in present study was PNS (50%) followed by nasopharynx (30%) and NC (20%). In contrast according to Shaila *et al* [5] nasal cavity was most common site for malignant lesions.

SCC was most common malignancy in present study and most of earlier studies mention except according to Maru *et al* [8] NPC was most common malignancy.

Association Of HPV In Neoplastic Lesions:
To determine the role of HPV in etiology of sinonasal neoplasm, 26 cases of neoplastic lesions were examined for presence of HPV antigen by IHC.

4/10 cases (40%) of inverted papilloma were positive for HPV, which was close to study observed by (46%) Masahiro et al [13] and (33%) Syrjanen et al [14]. As compared to present study higher positivity for HPV was seen by Yi Zhang et al [15] and Dsouza et al [16], while lower positivity was seen by Hoffmann et al [17].

Among malignant neoplasm, 20% cases of SCC were positive for HPV and 40% cases showed trace positivity. Similar incidence was reported by Hoffmann et al [17], while Paul et al [18] (33%) and Bishop et al [19] observed higher HPV positivity in SCC. Kraft et al [20] reported lower HPV positivity in SCC. No HPV expression was seen for NPC in our study which was differ from Dsouza et al [16] and Paul et al [18], who reported 50% HPV positivity each. 3/4 Cases (75%) of ACC of present study was positive for HPV. None of the earlier studies showed HPV association with ACC. **(Table 6)**

Table 6 - Shows Comparison Of Prevalence Of HPV In Various Sinonasal Studies.

S. No.	Study	Polyp	papilloma	SCC	NPC	ACC	Method
1	Kraft et al (2001)	-	-	11%	-	-	IHC, PCR
2	Syrjanen et al (2003)	-	33%	22%	-	-	IHC,PCR, ISH
3	Hoffmann et al (2006)	0/20	12%	20%	-	-	ISH (insitu hybridization)
4	Shah et al (2010)	-	59%	-	-	-	PCR,ISH, IHC
5	Masahiro et al (2012)	7.6 %	46%	27.3%	-	-	PCR (polymerase chain reaction)
6	Bishop et al (2013)	-	-	31%	-	-	IHC,PCR,ISH,TMA(tissue microarray)
7	Dsouza et al (2013)	-	86%	-	50%	-	IHC, koilocytic change
8	Paul et al (2017)	6%	18%	33%	50%	-	IHC
9	Yi Zhang et al (2017)	-	65%	-	-	-	PCR, ISH
10	**Our study**	-	**40%**	**20%**	**0/2**	**75%**	**IHC**

None of the lesions in present study demonstrated severe positivity for HPV and most of cases showed mild or trace positivity. This was probably due to primary antibodies which reacts with an epitope of a major capsid protein of HPV & is broadly expressed among different HPV subtypes and raised against HPV type 16. Therefore probably this antibody detected high risk type HPV cases only than low risk HPV infected cases. Earlier IHC for HPV was done in our department in oesophagus lesions which showed severe HPV positivity but in that study we used monoclonal mouse anti HPV clone K1H8 (DAKO Denmark). This anti HPV clone K1H8 reacts with a non-conformational internal linear epitopes of major capsid protein of HPV-1, which is broadly expressed among different HPV subtypes. [21]

Short coming: Major short coming of the present study was
1. Smaller number of cases in which HPV prevalence could be studied.
2. Only IHC method was used for demonstrating HPV expression.

Larger number of cases and addition of PCR method could further help in finding the role of HPV in neoplastic sinonasal lesions.

To conclude, Sinonasal lesions are common lesions of head and neck region with higher prevalence of non-neoplastic lesions, where commonest site of origin is nasal cavity. Malignant neoplastic lesions predominantly occur in higher age group and prominently affect paranasal sinuses.

HPV was demonstrated in benign as well as malignant lesions. Overall HPV prevalence was low and observed almost equally in benign and malignant cases. Highest HPV prevalence was found in inverted papilloma (40%) followed by squamous cell carcinoma (20%). Maximum cases showed mild or trace positivity only. Thus it can be inferred that HPV per se may not act as etiological factor but may play a supportive role along with other etiological factors in causation of sinonasal neoplastic lesions.

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