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The Diagnostic And Prognostic Role Of Immunohistochemistry In Head & Neck Tumours Along With Radiological & Histopathological Study

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Abstract:
Background: *Head & neck tumors are defined as benign, premalignant & malignant tumors above the clavicles, except the tumors of brain, spinal cord and esophagus. This includes tumors of paranasal sinus, nasal cavity, salivary glands, thyroid and upper aerodigestive tract, lymph nodes of neck, skin & its appendages, soft tissue tumors, midline carcinomas & metastatic tumors. Ultrasonography, computed tomography, magnetic resonance imaging were the modalities for initial evaluation. Histopathology was the GOLD standard for diagnosis followed by IHC for confirmation because correct diagnosis & cancer subtyping of the neoplasia is essential for successful therapy and prognosis.*
Materials & Methods: *Study was conducted for 2 years with 100 specimens of head & neck tumors. Thorough Radiological investigation was done followed by Histopathological examination including biopsy & whole resected specimens. IHC markers appropriate to the histopathological diagnosis was done.*
Results*: Among 100 patients of head & neck tumors, most belonged to 5th decade with male: female ratio of 2.3:1. Squamous cell carcinoma (66%) followed by Lymphoma(11%), tumors of salivary glands(8%), metastatic lymphadenopathy(7%) were the lesions present. SCC was most common carcinoma in oral cavity, larynx, nasopharynx and ear with positive p63 marker. 1 case of Basal cell carcinoma with positive BerEP4 & in NHL, salivary gland tumors & Metastatic lymphadenopathy panel of IHC were included for confirmation.*
Conclusion: *Immunohistochemistry has a diagnostic & prognostic role in Head & Neck tumors along with Radiological & histopathological examination.*
Keywords: *Head & Neck tumors, Radiology, Histopathology, Immunohistochemistry, Diagnostic, Prognostic*

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I. Introduction:

Head & neck tumors are defined as benign, premalignant & malignant tumors above the clavicles, with exception of tumors of brain and spinal cord and esophagus.[1] This includes tumors of the paranasal sinus, the nasal cavity, the salivary glands, the thyroid and upper aerodigestive tract (oral cavity, pharynx and larynx)[2], lymph nodes of neck, skin & it's appendages, soft tissue tumors, midline carcinomas & metastatic tumors.

Ultrasonography (USG), computed tomography (CT), magnetic resonance imaging (MRI) are the various modalities available for imaging of head and neck cancers. USG is a better modality to differentiate between solid and cystic lesions as well as for superficial lesions. While CT scan and MRI are used for deep seated and penetrating lesion & is also useful to determine the extent of the lesion.

The histopathology of the cancers differs from site to site, but the most common ones are squamous cell carcinomas, accounting for more than 85% of the head and neck neoplasms[3].

The introduction of the immunohistochemical method has become a powerful complementary tool in tumor analysis. It has increased the possibilities for histogenetic diagnosis of tumors. Through the identification of specific cellular components of cell patterns, using a specific cellular panel of monoclonal or polyclonal antibodies, the immunohistochemical method has transformed the diagnosis of these tumors. The immunohistochemical technique has revolutionized surgical pathology knowledge because the correct diagnosis & cancer subtyping of neoplasia is essential for successful therapy and prognosis.

Radiological, Histopathology and IHC correlation of head and neck tumors are less explored topics hence the study was undertaken to analyze the diagnostic and prognostic utility of IHC in head and neck tumors

and to outline an approach to the evaluation of these lesions by using immunohistochemistry for targeted therapy.

II. Materials & Methods:

Study Design & Location: This study was conducted at a tertiary care teaching hospital for 2 years.

Sample size and subject selection method: 100 specimens of patients with head & neck tumors using non-probability sampling and consecutive sampling method, those who met our inclusion criteria.

Inclusion criteria:

1. Patients of all ages and both sexes were included.
2. Tissue biopsy and dissected tumors of head and neck region above the clavicles; including the paranasal sinus, the nasal cavity, the salivary glands, the thyroid and the upper aerodigestive tract (oral cavity, pharynx and larynx) were included in the study.
3. Any biopsy material received for the histopathological diagnosis of head and neck tumors which was sufficient for IHC.

Exclusion Criteria:

1. Dissected tumors of brain and spinal cord & esophagus.
2. Tumors with extensive necrosis without sufficient viable tumor cells for accurate evaluation of the IHC results.

Written informed consent for investigations and use of the resected specimen and biopsy material for the study was obtained from all the patients upon admission.

Methodology:

Thorough Radiological investigation report of cases where needed was obtained from Radiology department of the hospital along with pictures.

For resected specimens:

The representative areas from the specimen were submitted for histopathological examinations. Tissue blocks of 2cm x 2cm x 0.5cm were collected and fixed in 10% neutral buffered formalin solution for 24 hours and subsequently subjected to histological processing and paraffin embedding. Histological paraffin sections of 3-5µm thick were taken. H & E staining was performed and examined by light microscopy.

Biopsy specimen:

The whole of the specimen was submitted for the histological processing and paraffin embedding. Histological paraffin sections, 3- 5µm thick, were taken. H & E staining was performed and examined by light microscopy.

Immunohistochemistry (IHC):

Serial sections from the specimen containing the lesion of interest were used for IHC staining. Immunohistochemistry protocols were developed in the settings, using antibodies tagged with chromogens to identify specific markers.

IHC staining was performed using standard IHC protocol. The primary monoclonal ready to use antibodies were of DAKO (p63, PANCK, CD3, CD5, CD10, CD34, CYCLIN D1, PAX5, Vimentin, SMA, S100, PR), THERMOFISCHER (CK7, CK20, CD15, CD30, CD45) and BIOGENEX (CK5/6, EMA, CD19, CD20, CD23, CD31, CD43, TTF-1, Napsin-A, ER, CDX2, Synaptophysin, Chromogranin, BCL2, BerEP4) company.
Appropriate positive & negative controls were used.

III. Result:

The present study was conducted at a tertiary care teaching hospital for 2 years. This study consisted of total 100 specimens of patients with head & neck tumors according to the inclusion criteria.

- We observed that majority(59%) of the patients had ulcer, while 41% of the patients had mass(swelling) on head & neck region.
- The majority(92%) of patients were from ENT ward, followed by 06% patients from Surgery ward, 01% from Ophthalmology and Odontology.
- Most of the patients (62%) had undergone radiological investigations(USG, CT, MRI) as needed for the case.
- Few patients had undergone laryngoscopy(16%).
- Majority(79%) of specimens were biopsy, while 21% of specimens were excised tissue from Head& neck tumors.

Table 1: Distribution of Age (n=100)

Age group (years)	Frequency (%)
<21	04 (04%)
21-30	06 (06%)
31-40	16 (16%)
41-50	25 (25%)
51-60	23 (23%)
61-70	18 (18%)
> 70	08 (08%)
Total	**100 (100%)**

Out of 100 patients of head & neck tumors, 25% of the patients belonged to 5th decade, followed by 6th decade (23%).

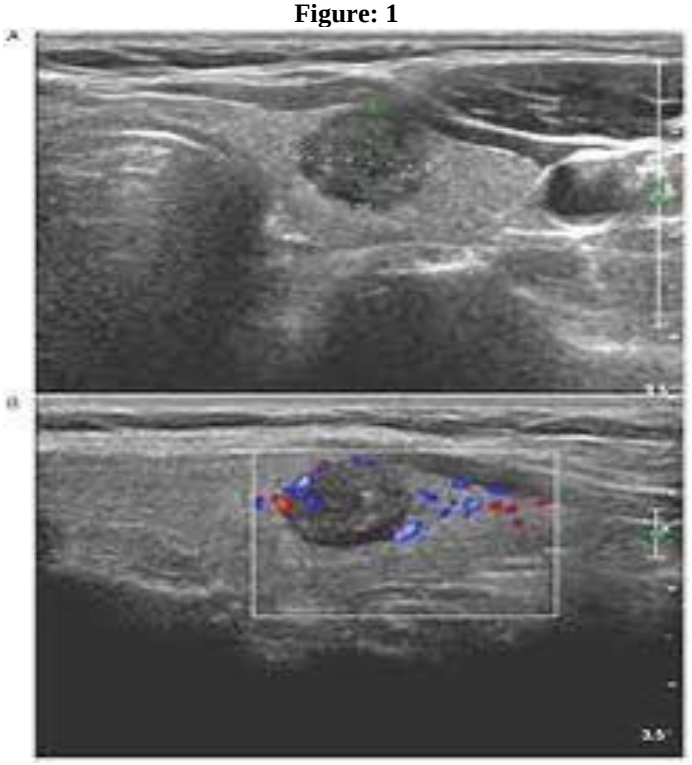
The majority (70%) of the patients were male, while only 30% of the female patients were affected. Overall male: female ratio was 2.3:1 in patients with head & neck tumors.

Table 2: Distribution of lesion by location (n=100)

Location	Male (%)	Female (%)	Total (%)
Oral cavity	34 (34%)	12 (12%)	46 (46%)
Nasopharynx	03 (03%)	02 (02%)	05 (05%)
Neck	12 (12%)	08 (08%)	20 (20%)
Nose	01 (01%)	01 (01%)	02 (02%)
Cheek	07 (07%)	02 (02%)	09 (09%)
Larynx	09 (09%)	02 (02%)	11 (11%)
Ear	02 (02%)	02 (02%)	04 (04%)
Scalp	01 (01%)	01 (01%)	02 (02%)
Eyelid	01 (01%)	00 (00%)	01 (01%)
Total	**70 (70%)**	**30 (30%)**	**100 (100%)**

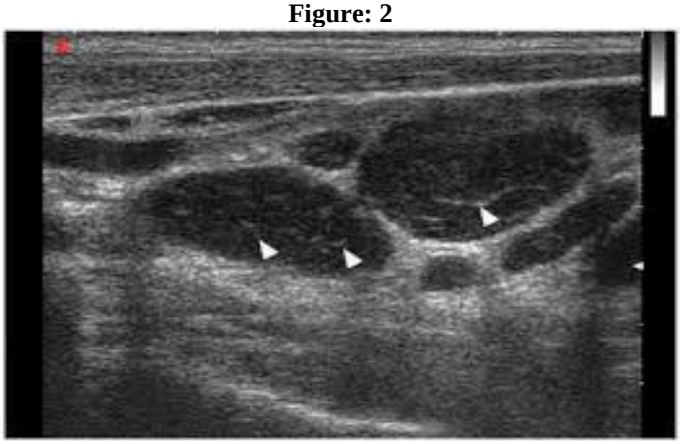
A majority (46%) of patients had ulcer in oral cavity, followed by mass in neck, larynx, cheek, nasopharynx, ear, nose, scalp and eyelid.

Figure: 1



USG: A well-defined solid hypoechoic nodular lesion with lobulated margins in left lobe of thyroid showing moderate internal vascularity suggestive of **Papillary carcinoma**.

Figure: 2



USG: Enlarged lymph nodes at right level V with diffusely reduced parenchymal attenuation and loss of hilar definition suggestive of **Metastatic cervical lymph nodes.**

Table 3: Categorization of Head & Neck Lesions (n=100)

Lesions	Frequency (%)
Squamous cell carcinoma (SCC)	66 (66%)
Basal cell carcinoma (BCC)	01 (01%)
Adnexal tumors	03 (03%)
Soft tissue tumor	02 (02%)
Salivary gland tumors	08 (08%)
Lymphoma and related lesions	11 (11%)
Metastatic lymphadenopathy	07 (07%)
Thyroid tumors	02 (02%)
Total	**100 (100%)**

Majority of lesions were Squamous cell carcinoma, followed by Lymphoma, tumours of salivary glands and metastatic lymphadenopathy.

Table 4: Histological Distribution according to lesion location

Location (n=)	SCC	Lymphoma and related lesions	Salivary gland tumours	Metastatic lymphadenopathy	Adnexal tumors	Soft tissue tumors	Thyroid tumors	BCC
Oral cavity (46)	45 (97.8%)	01 (02.0%)	-	-	-	-	-	-
Nasopharynx (05)	05 (100%)	-	-	-	-	-	-	-
Neck (20)	-	09 (45.0%)	02 (10.0%)	07 (35.0%)	-	-	02 (10.0%)	-
Nose (02)	01 (50.0%)	-	-	-	-	-	-	01 (50.0%)
Cheek (09)	01 (11.1%)	01 (11.1%)	06 (66.7%)	-	01 (11.1%)	-	-	-
Larynx (11)	11 (100%)	-	-	-	-	-	-	-
Ear (04)	03 (75.0%)	-	-	-	-	01 (25.0%)	-	-
Scalp (02)	-	-	-	-	01 (50.0%)	01 (50.0%)	-	-
Eyelid (01)	-	-	-	-	01 (100%)	-	-	-
Total (100)	**66 (66%)**	**11 (11%)**	**08 (08%)**	**07 (07%)**	**03 (03%)**	**02 (02%)**	**02 (02%)**	**01 (01%)**

We observed that HNSCC was **most common carcinoma** in oral cavity (97.8%), larynx (100%), nasopharynx (100%) and ear (75%).

Out of 66 cases of HNSCC, majority (31.9%) of cases were in age group of 41-50 years, followed by 51-60 years(22.8%).

Overall male to female ratio of HNSCC cases was 2.9:1.

Majority (86.5%) of cases were conventional type of SCC, followed by Nasopharyngeal carcinoma (7.5%); Basaloid variant of SCC (3%) and Spindle cell variant of SCC (3%).

All 66 cases of HNSCC (100%) were positive for p63 marker.

In neck region, 45% cases were of lymphoma, 35% cases of metastatic lymphadenopathy and 10% each case of salivary gland and thyroid tumors.

Out of 11 cases of Lymphoma and related lesions, majority (45.4%) of cases were in age group of 51-60 years. Overall male to female ratio was 10:1.

Majority (54.5%) of cases were diagnosed as non-Hodgkin's lymphoma followed by Classic Hodgkin's lymphoma (27.3%).

NHL- T cell type- ALCL was positive for CD45, CD30, CD3, CD5, CD43, BCL2 & negative CD15, CD19, CD20.

NHL- T cell type showed diffuse positivity of CD3, CD5 and focal positivity of BCL2 and negativity of Cyclin D1, CD19, CD20, CD23.

NHL- B cell type showed diffuse positivity of CD20, CD5 and focal positivity of CD19 and negativity of CD3, CyclinD1, CD23.

NHL- Mantle cell lymphoma showed diffusepositivity of CD45, CD19, CD20, CD5, CyclinD1, PAX5, BCL2 and negativity of CD23, CD3.

NHL- DLBCL (T cell/ Histiocytic rich variant) showed diffuse positivity of CD45, PAX5, BCL 2, CD20, CD3, CD5 and focal positivity of CD30 in large cells and negativity of CyclinD1, CD19, CD23, CD10.

Classic HL showed positivity of CD30, weak nuclearpositivity of PAX5 in RS cells and negativity of CD45, CD19, CD20.

Cutaneous T- cell Hyperplasia showed positivity of CD45, CD3 and negativity of CD20.

Angio lymphoid hyperplasia with eosinophilia showedCD31, CD34 positivity and vimentin negativity.

01 case of Basal cell carcinoma in nose region, 2 cases of benign adnexal tumours in cheek and scalp region, 2 cases of benign soft tissue tumours in scalp and ear region and 2 cases of thyroid tumors were observed. Basal cell carcinoma showed p63, CK 5/6, BerEP4 positivity.

Apocrine tubular adenoma showed CK 7 positivity,EMA positivity in luminal surface of tubules, SMA positivityin outer tubules and S100 positivity in myoepithelial cells.

Trichoepithelioma showed p63 positivity and CD10 positivity in stromal cells.

Table 5: Distribution of Metastatic lymphadenopathy in Head & Neck Region (n=07)

Metastasis from	Total (%)	PANCK	p63	CK5/6	TTF	CK7	CK20	ER	PR	CD45	Napsin
Squamous cell Carcinoma	04 (57.1%)	+ve	+ve	+ve	-ve	-ve	-ve	-ve	-ve	-	-
Breast carcinoma	01 (14.3%)	+ve	-ve	-ve	-ve	+ve	-ve	+ve	-ve	-	-
Adenocarcinoma	01 (14.3%)	+ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve	-ve
Thyroid carcinoma	01 (14.3%)	+ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve	-	-ve
Total	**07 (100%)**										

- In 4 cases PANCK, p63, CK5/6 was positive suggestive of Metastatic lymphadenopathy from Squamous cell carcinoma.
- In 1 case PANCK, CK7 and ER was positive suggestive of Metastatic lymphadenopathy from breast carcinoma.
- One case positive PANCK, CK7 and negative TTF, p63, CK20, ER suggestive of Metastatic lymphadenopathy from Adenocarcinoma.
- One case showed positivity of PANCK, TTF and negativity of Napsin, ER, p63, CK7 suggestive of Metastatic lymphadenopathy from thyroid carcinoma.

Table 6: Pattern of IHC staining of salivary gland tumors

Sr No.	Lesion	PANCK	CK7	CK5/6	p63	S100	Vimentin	SMA	EMA	CD10	P16
1.	Pleomorphic Adenoma	+ve inductal cells	+ve in ductal cells	+ve in myo-epi. Cells	+ve in myo-epi. cells	+ve in myo-epi. cells	+ve	+ve inductal cells	-ve	-ve	-ve
2.	Warthin Tumor	+ve	+ve	Focal +ve	Focal +ve	-ve	-ve	-ve	-ve	-ve	-ve
3.	Oncocytoma	+ve	+ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve	-ve
4.	Mucoepidermoid Carcinoma	+ve	+ve	+ve	+ve	-ve	+ve	-ve	+ve	-ve	-ve

- All 3 cases of pleomorphic adenoma showed positivity of PANCK, CK7 and SMA in ductal cells; positivity of p63, CK5/6 and S100 in myoepithelial cells and Vimentin positivity in both cells.
- A case of Warthin's tumor showed positivity of PANCK, CK7 and focal positivity of CK5/6 and p63.
- A case of oncocytoma showed positivity of PANCK, CK7 and vimentin.
- All 3 cases of mucoepidermoid carcinoma showed positivity of PANCK, CK7, CK5/6, p63, Vimentin and EMA.

Majority (73%) of cases has confirmatory role of IHC as compared to diagnostic role (27%).
In majority (57%) of cases we had used single IHCmarker in compared to IHC panel (43%).

Out of 100 cases, 98 cases showed concordance and only 02 cases showed discordance with histopathological diagnosis of head & neck tumors.

IV. Discussion:

The present study is an effort to evaluate immunohistochemical expression in head and Neck tumors along with their histopathological finding & Radiological findings.

Various authors studied head and neck lesions and data has been published under various headings including site specific and diagnoses specific headings.
The incidence of head and neck lesions is high in old patients.

Mean age of other studies was quite comparable with our study. Specially in Massa et al.[4] & **Larizadeh at al.[5] study.**

Male to female ratio had a wide range among head and neck lesions (from 4.5:1 for larynx to 1:1 for nose and ear) which is quite comparable with **Larizadeh at al.[5] study.**

Most common involved site was oral cavity (46%) and disease was squamous cell carcinoma. This finding was in agreement with **Bhattacharjee et al.[6] (2006) study.** In **Larizadeh et al.[5] (2014) study** most common involved site was larynx (46.8%) and in present study 11% cases had involved larynx.

45% cases were of lymphoma and 35% cases were of metastatic lymphadenopathy in neck region. These findings were in agreement with **Larizadeh et al.[5] (2014) study** in which 50% cases of lymphoma and 38.6% cases of metastatic lymphadenopathy was observed.

In present study, all 66 cases of HNSCC (100%) were positive for p63. Similar observation was seen in **Patel et al.[7] (2017) study**.

Out of 9 cases of lymphoma 33.3% cases were diagnosed as HL and 66.7% cases were diagnosed as NHL. Similar observations were seen in various study.

In present study, all 3 cases of HL were subtyped as mixed cellularity type which was quite comparable with **Chakrabarti et al.[8] (2010) study** and **Mozaheb et al.[9] (2011) study**.

Out of 8 cases of salivary gland tumors, 62.5%cases were benign and 37.5% cases were malignant epithelial tumors which were quite comparable with various studies.

In present study, pleomorphic adenoma was the most common benign epithelial tumour of salivary gland and mucoepidermoid carcinoma was the most common malignant epithelial tumour of salivary gland.

V. Conclusion:

Most of the head & neck tumors can be treated effectively if diagnosed properly at an early stage. Radiology followed by Histopathology is **the gold standard** for most of the head & neck lesions. Immunohistochemistry is useful for diagnosis especially in those tumors which could not be recognized by routine histopathology. IHC is very useful in further evaluation & in differential diagnosis of head and neck tumors. The most commonly used IHC markers in head and neck region are p63 (for SCC), cytokeratin (PAN CK, CK7) and basal/myoepithelial markers (S100, p63, CK5/6). Application of basic required panel of IHC markers for lymphoma aids in the sub-classification and could modify the therapeutic modalities & thereby prognosis. IHC has been very useful in diagnosis of metastatic lymphadenopathy and thereby tumor staging and its consequent management. IHC marker study has been most commonly used in salivary gland tumours for identification of myoepithelial cells.

Our study helped many patients in better overall outcomes in their disease process decreasing their overall morbidity by targeted therapy & thereby avoiding unnecessary disfiguring surgeries.

Further studies can be aided by using cytogenetics and Fluorescence In Situ Hybridization (FISH) as it can be helpful for the oncologists to correlate it with response to targeted therapy.

References:

[1] Konings H, Stappers S, Geens M, De Winter By, Lamote K, VanMeerbeeck Jp, Specenier P, Vanderveken Om, Ledeganck Kj. A Literature Review Of The Potential Diagnostic Biomarkers OfHead And Neck Neoplasms. Front Oncol. 2020 Jun 26;10:1020.
[2] Mehanna H, Paleri V, West Cm, Nutting C. Head And Neck Cancer--Part 1: Epidemiology, Presentation, And Prevention. Bmj.

2010 Sep 20;341:C4684.
[3] De Vries N, Van De Heyning Ph, Leemans Cr. Leerboek Keel- Neus-Oorheelkundeenhoofd-Halschirurgie. Houten: BohnStafleu Van Loghum; (2013). 10.1007.
[4] Sean T Massa, Lauren Cass, Sai Challapalli, Zisanshazahirsha, Matt Simpson, Nosayabaosazuwa-Peters, Gregory Ward. Age Differences In Demographic Predictors Of Head And Neck Cancer Survival. Cancer Epidemiology, Biomarkers & Prevention 27 (7_Supplement), A88-A88, 2018.
[5] Larizadeh Mh, Damghani Ma, Shabani M. EpidemiologicalCharacteristics Of Head And Neck Cancers In Southeast Of Iran. Iran J Cancer Prev. 2014; 7(2):80-6.
[6] Bhattacharjee A, Chakraborty A, Purkaystha P. Prevalence Of Head And Neck Cancers In The North East-An Institutional Study. Indian J Otolaryngol Head Neck Surg. 2006 Jan;58(1):15-9. Doi: 10.1007/Bf02907731. Pmid: 23120228; Pmcid: Pmc 3450618.
[7] Patel Sb, Manjunatha Bs, Shah V, Soni N, Sutariya R. Immunohistochemical Evaluation Of P63 And Cyclin D1 In Oral Squamous Cell Carcinoma And Leukoplakia. J Korean Assoc Oral Maxillofac Surg. 2017 Oct;43(5):324-330. Doi: 10.5125/Jkaoms.2017.43.5.324. Epub 2017 Oct 26. Pmid:29142867; Pmcid: Pmc5685862.
[8] Chakrabarti S, Sarkar S, Goswami Bk Et Al.Hodgkin_S And Non-Hodgkin_S Lymphomas In An Indian Rural Medical Institution: Comparative Clinicopathologic Analysis. Asian Pacific J CancerPrevention.2010;11:1605-1608.
[9] Mozaheb Z, Aledavood A, Farzad F. Distributions Of Major Sub- Types Of Lymphoid Malignancies Among Adults In Mashhad, Iran. Cancer Epidemiology,2011;35(1):26-29.