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A STUDY ON EXPRESSION OF LATENT MEMBRANE PROTEIN 1 OF EPSTEIN BARR VIRUS IN NASOPHARYNGEAL CARCINOMA IN SOUTH INDIA

Pathology

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ABSTRACT

Background- Nasopharyngeal carcinoma arises from the nasopharynx epithelial lining and is caused by Epstein Barr virus. Latent Membrane Protein 1(LMP 1) expressed on cytoplasmic portion of cell surface of Epstein Barr virus is an oncoprotein which triggers multiple cell signalling pathways and facilitates the expression of virus infected cell population at an early stage of development of nasopharyngeal carcinoma. **Aims and Objectives-** To study the age,sex distribution, clinical presentation, histologic spectrum and expression of LMP1 in the histologic types of nasopharyngeal carcinoma. **Material And Methods-** The present study is a cross sectional study done over a period of two years at a tertiary care hospital.Histologic typing of nasopharyngeal carcinoma done on light microscopy followed by expression of Latent Membrane Protein 1 by Immunohistochemistry. **Results-** The present study showed a male predominance (75%), bimodal age distribution in second and fifth decades of life, presented most commonly as nasal obstruction (82.5%), non keratinising undifferentiated type being the commonest histologic type (80%) and LMP 1 expression in 45% of cases. **Conclusion-** The detection of Latent Membrane Protein 1 by immunohistochemistry helps in early diagnosis of nasopharyngeal carcinoma and may aid in treatment as a therapeutic target.

KEYWORDS

Nasopharyngeal carcinoma, Epstein Barr virus, Latent Membrane Protein 1, Immunohistochemistry

INTRODUCTION

Nasopharyngeal carcinoma (NPC) is the most common malignant tumour of nasopharynx.

Globally 133,000 new cases were detected in 2020[1]. Nasopharyngeal carcinoma arises from nasopharyngeal epithelial lining and is caused by viral, environmental and hereditary factors. The viral factor is associated with Epstein-Barr virus infection.[2,3]

Nasopharyngeal carcinoma is rare world-wide but more commonly seen in China and Southeast Asia. The incidence rate is much higher in Asia, with 150 to 500 annual cases per 1 million in Southern China. The peak incidence of NPC in Asia is between 40 to 50 years of age. In India though rare, cases have been reported in Northeastern states of Nagaland, Manipur and Mizoram with incidence range of 0.5-2/100000.[4]

The presenting complaints of NPC are non-specific like neck mass, nasal obstruction, epistaxis and are related to the location of the primary tumour and degree of spread. The early clinical presentation is confusing until the disease has reached the advanced stage. There is delay in diagnosis as post nasal space is relatively inaccessible to examination and the presence of normal lymphoid epithelium makes an accurate diagnosis even more difficult.

Epstein-Barr virus (Human Herpes Virus-4) has been linked to development of cancers originating from lymphoid and epithelial cells. The first protein from EBV having its carcinogenic nature is Latent membrane protein 1, which is expressed on cell surface. EBV encoded LMP1 and LMP2 oncoproteins by Immunohistochemistry are widely used methods for identifying EBV in tumour cells. LMP1 is the major transforming protein of EBV and drive oncogen in NPC. It plays an important role in pathogenesis due to triggering of multiple cell signalling pathways and has been implicated in facilitating the expression of EBV infected cell population at an early stage of NPC development. Thus LMP1 may be a good therapeutic target in the treatment of EBV associated carcinoma.[5]

AIMS AND OBJECTIVES

1. To study the age, sex distribution and clinical presentation of nasopharyngeal carcinoma.
2. Histological typing of nasopharyngeal carcinoma according to WHO classification.
3. To study the LMP 1 expression in the histologic spectrum of NPC.

MATERIAL AND METHODS

The present study is a cross sectional study done over a period of two years from January 2020 to December 2022 in the Department of Pathology, Osmania Medical College, Hyderabad. A total of 40 cases were studied. Age, sex distribution, clinical presentation and histologic typing of nasopharyngeal carcinoma on Hematoxylin and Eosin sections of biopsies were done under light microscopy following WHO classification. Immunohistochemistry with LMP 1 was done on all cases following Diagnostic Biosystems protocol- Primary antibody LMP 1 protein, antigen retrieval with Montage Opus, detection methods by Avidin-biotin complex method, chromogen substrate used was 3,3'-Diaminobenzidine and counterstain with Meyer's Hematoxylin. LMP1 expression done based on positivity of tumour cells with cytoplasmic immune staining. The negative control was done on a case of chronic sinusitis. All the collected data was analysed by percentages, graphs, figures and tabular forms.

Inclusion Criteria

Biopsy confirmed nasopharyngeal carcinoma cases with adequate tumour tissue to perform IHC and availability of clinicopathological details.

Exclusion Criteria

Inadequate biopsy and insufficient tumour tissue to perform IHC.

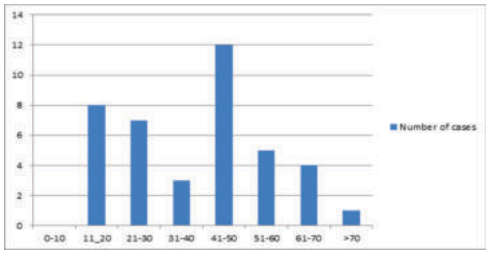
RESULTS

A total of 40 cases of nasopharyngeal carcinoma are included in the present study. Clinicopathological findings and immunohistochemical results are described below.

Table 1: Demographic Details

Total number of cases	40
Males	30 (75%)
Females	10 (25%)
Male:Female Ratio	3:1
Age Range	13-75 years
Mean Age	39.7 years

Of the total 40 cases studied, male predominance was observed constituiting 75% of cases and female cases constituited to 25% of cases with a male to female ratio of 3:1. Age range was 13-75 years with a mean age of 39.7 years and median age of 45 years.



Bar Graph 1: Age Range And Distribution Of Cases

The predominant number of cases occurred in the age range of 41-50 years constituting 12 cases (30%) followed by 8 cases (20%) in the age range of 11-20 years. Bimodal age distribution of cases was observed with predominant cases in second and fifth decades of life.

Table 2 : Clinical Presentation

Presenting complaint	Number of cases(percentage)
Nasal obstruction	25(62.5%)
Epistaxis	8(20%)
Neck mass	7(17.5%)

In the present study cases presented with more than one clinical presentation, out of 40 cases, 25 cases (62.5%) presented predominantly with nasal obstruction followed by 8 cases (20%) with epistaxis and 7 cases (17.5%) with neck mass.

Table 3: Distribution Of Cases According To WHO Classification

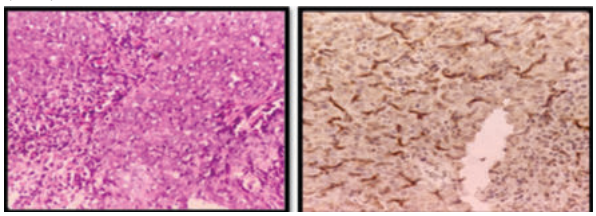
Histologic Type	Number of cases (percentage)
NPC, Keratinising-Moderately differentiated	01(2.5%)
NPC,Non- Keratinising differentiated	07(17.5%)
NPC Non-keratinising undifferentiated	32(80%)

Of the total 40 cases of NPC, 32 (80%) cases were Non-Keratinizing Undifferentiated type, followed by 07 (17.5%) cases of Non - Keratinizing differentiated type and 01 (2.5%) case of Keratinizing moderately differentiated type.

Table 4: Expression of Latent membrane protein 1(LMP1) Immunohistochemistry

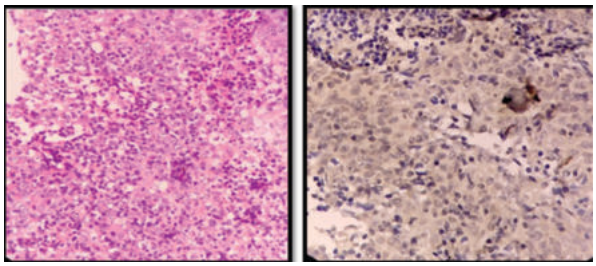
LMP1- immunohistochemistry	Number of Cases(Percentage)
Positive	18 (45%)
Negative	22 (55%)

Of the total 40 cases, LMP1 was positive in 18 (45 %) cases with cytoplasmic staining pattern. No expression of LMP1 was seen in 22 (55%) cases.



A B

Figure 1: A case of NPC non-keratinising undifferentiated H and E (A) and IHC showing cytoplasmic immune reactivity with LMP1 (B)



C D

Figure 2: A case of NPC non-keratinising undifferentiated type Hematoxylin and Eosin staining (C) and Immunohistochemistry showing absent cytoplasmic immune reactivity with LMP 1(D)

DISCUSSION

The present study is conducted in the Department of Pathology, Osmania Medical College ,Hyderabad over a period of two years from 2020-2022 and included 40 cases of nasopharyngeal carcinoma.All the clinical details were recorded followed by histopathological diagnosis, LMP 1 immunohistochemistry and comparison with other studies was done.

There is a male preponderance of NPC with a male to female ratio of 3:1.This finding was comparable to studies done by Parikhit Borthakr et al[6] and Xiao et al[7] .Male preponderance may be attributed to increased addictions and occupational exposure. Age range of the cases was from 13 to 75 years with mean age of 39.7 years and median of 45 years. The mean age among men was 39.2 and median was 44.5 years. The mean age among women was 41.2 and median was 45 years. There is bimodal age distribution with majority of the cases seen in second and fifth decades of life with 8(20%) and 12 (30%) cases respectively in the present study. Similar observation was made by studies done by Balakrishnan et al[8] and Freddie Bray et al[9].Bimodal age distribution is seen in non-endemic regions. In endemic areas, NPC shows unimodal age distribution mainly affecting young to middle age group.[9]

In the present study, majority of the cases 62.5% presented with nasal obstruction, 20% with epistaxis and 17.5% of cases presented with neck mass. These findings were comparable to study done by Suzina SA et al [10]. Owing to the hidden site of nasopharynx, cases present late and the presentation of neck mass indicates tumour metastasis to regional lymph nodes.

The predominant histologic type of NPC included Non keratinising undifferentiated type-32 cases(80%) followed by Non- keratinizing differentiated type 07 cases(17.5%).This finding was comparable to studies done by Hong and Guo et al[11] and Luo et al[12] with non keratinising undifferentiated type as the most common histologic type of NPC.LMP-1 was positive in 18 cases (45%) which was comparable to study done by Chan et al[13] .When compared to studies done by Parikhit Borthakur (P) et al [6]andGuo et al [14] ,LMP 1 positivity is low in the present study as the present study is done in a non endemic area for Epstein Barr virus associated NPC.

CONCLUSION

The Nasopharyngeal carcinoma is a silent malignancy with varied clinical presentation. Early diagnosis of NPC is important as the stage and extent of disease at the time of diagnosis and initiation of treatment is an important prognostic factor. Though the present study is done in a non endemic area EBV association with NPC was found to be high. LMP1 has been implicated in facilitating the expression of EBV infected cell population at an early stage of NPC development. Thus, LMP-1 detection could be useful for prognostic specifications and development of new therapeutic strategies .

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Conflicts of Interest: None

REFERENCES

1. Cancer.Net .Nasopharyngeal Cancer: Statistics June/2022.
2. Initiative for Vaccine Research (IVR): Viral cancers. World Health organization. Archieved from the original on 1 April 2019. Retrieved 2 October 2012.
3. Lo,Kwok-Wai; Chung, Grace Tin-Yun; To, Ka-Fai (2012). Deciphering the molecular genetic basis of NPC through molecular, cytogenetic, and epigenetic approaches. Seminars in Cancer Biology. 22 (2): 79-86. Doi:10.1016/j.semcancer. 2011.12.011. PMID 22245473.
4. Kataki AC, Simons MJ, Das AK, Sharma K, Mehra NK. Nasopharyngeal carcinoma in the Northeastern states of India. Chin J Cancer. 2011 Feb;30(2):106-13. doi: 10.5732/cjc.010.10607. PMID: 21272442; PMCID: PMC4013339.
5. Tsao SW, Tramoutanis G, Dawson CW, Lo AK, Huang DP. The significance of LMP1 expression in nasopharyngeal carcinoma. Semin Cancer Biol. 2002 Dec;12(6):473-87 dol:10.1016/s1044579x02000901.PMID:12450733
6. Borthakur P, Kataki K, Keppen C, Khamo V, Medhi S, Deka M. Expression of Epstein Barr Virus Encoded EBNA1 and LMP1. Oncoproteins in Nasopharyngeal Carcinomas from Northeast India. Asian Pac J Cancer Prev.2016;17(7):3411-6. 27509984.
7. Guangli Xiao, Yabing Cao, Xibin Qiu, Weihua Wang and Yufeng Wang. Influence of gender and age on the survival of patients with nasopharyngeal carcinoma. BMC Cancer 2013,13:226.
8. Balakrishnan, V., Gangadharan, P. And Nagaraj Rao, D.Some epidemiological aspects of nasopharyngeal cancer. In : Liver Cancer: Cancer problems in Asian Countries. Eds. K.Shanmugarathnam, R.Nambiar, K.K. Tan and L.K.C. Chab. Singapore Cancer Society, Somga Pre. P 268, 1976.
9. Freddie Bray, Marion Haugen, Tron A.Moger, Sternar Tretli. Age-Incidence Curves of Nasopharyngeal Carcinoma World Wide:Bimodality in Low-Risk Populations and Aetiologic Implications. Cancer Epidemiology, Biomarkers and Prevention .September 2008.DOI:10.1158/1055-9965.EPI-08-0461.
10. Suzina SA, Hamzah M.Clinical presentation of patients with nasopharyngeal carcinoma. Med J Malaysia. 2003;58(4):539-45.
11. Hong M-H, Guo X. Nasopharyngeal Carcinoma.Chinese Medical Science and Technology Press. 2003,11,115.
12. R-ZH, Zhong B-L, Zong Y-SH, et al. The Constitutional features of nasopharyngeal carcinoma Histopathological Types in High-Risk Area.Zhong Guo Zhong Liu. 2001, 10,473-5.
13. Chan AT, Teo PM, Johnson PJ (2002). Nasopharyngeal carcinoma. Annals of Oncology 13: 1007–1015, 2002 Review DOI: 10.1093/annonc/mdf17980.
14. Guo Q, Tham IWK, Lin S, et al (2012). Prognostic significance of pre-treatment latent membrane protein 1 from nasopharyngeal swabs for stage III-IVA nasopharyngeal carcinoma. Head Neck Oncol, 4, 81.