

CLINICOPATHOLOGICAL AND IMMUNOHISTOCHEMICAL PROFILE OF LYMPHOMA OF HEAD AND NECK REGION

Uzma Jabbar Khan¹, Wajiha Alamgir¹, Aroosa Ashraf¹, Adeel Haider¹, Salman Rashid², Faheem Abrar³

¹University College of Dentistry, University of Lahore, Lahore Pakistan

²Avicenna Medical and Dental College, Lahore Pakistan

³Punjab Health Care Commission, Lahore Pakistan

ABSTRACT

Objective: The study was intended to analyze the frequency distribution and relative sites of occurrence of extra nodal lymphoma in head and neck region. Moreover, it was also focused to set down the histological types and subtypes of the diagnosed lymphoma through various immunohistochemical panels.

Material and Methods: This cross-sectional observational study was conducted in the department of Oral and Maxillofacial Pathology of University Dental Hospital, University of Lahore, Lahore. Total 39 proven cases of head and neck lymphoma from period of June 2018 to July 2021 were included. Inclusion criteria consist of lymphoma of head and neck region in age group 25 years to 100 years. Exclusion criteria comprised of lymphoma in age less than 25 years and lymphoma of CNS. The immunohistochemical panel used included the antibodies against LCA, CD3, CD10, CD15, CD20, CD23, CD40, CD43, CK, Cyclin D1, Bcl2, GDFP-15, Kappa and Lambda. SPSS version 25 was used to analyze the data. Pearson chi square was applied and $p < 0.05$ was taken as significant.

Results: Out of 39 cases, 21 (54%) were males and 18 (46%) were females. The mean age was 57.89 ± 16.43 years. Out of the total, 38 cases were diagnosed as Non-Hodgkin lymphoma (NHL) and only one turned out to be Classical Hodgkin lymphoma of mixed cellularity type. Among NHL diffuse large B cell Lymphoma (DLBCL) was greatest in number ($n=29$, 76.31%), followed by 6 cases of marginal zone (15.3%) and one case each of small lymphocytic lymphoma, follicular lymphoma and unspecified peripheral T cell lymphoma. The most common extra nodal site involved was tonsil $n=18$ (46.5%), followed by parotid gland $n=6$ (15.38%), palate $n=3$ (7.69%), alveolar ridge ($n=2$, 5.12%), tongue $n=2$ (2.15%), submandibular region $n=2$ (5.12%), buccal mucosa $n=2$ (5.12%), upper jaw $n=1$ (2.56%), oropharynx $n=1$ (2.56%), gingiva $n=1$ (2.56%) and lip $n=1$ (2.56%)

Conclusion: Non-Hodgkin lymphoma comprise of 97.4% of total cases where as Hodgkin lymphoma only 2.5%. Most common site involved was tonsil with greatest frequency of diffuse large B cell lymphoma.

Key Words: Hodgkin Lymphoma, Non-Hodgkin Lymphoma, Immunohistochemistry, Diffuse Large B cell lymphoma.

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INTRODUCTION

Lymphomas are a group of malignant tumors of the hematopoietic system which are characterized by the neoplastic proliferation of lymphoid cells or their precursors [1]. They are the second commonly occurring malignancy of head and neck region and spotted as a major health issue globally [2,3]. Patients with primary and secondary immunodeficiency are found at an increased risk for lymphoma [3]. Lymphomas are categorized into two main entities; HL and NHL [4]. According to WHO, HL is further categorized into five sub categories and NHL is categorized into B cell and T cell types which are further classified into different subtypes [4]. NHL is more commonly occurring and its prevalence tends to increase with age [5]. HL comprise of 10% of all

lymphomas while NHL account for 90% with 30% of cases comprising of subtype Diffuse large B cell lymphoma [1,6].

Commonly involved sites affected by lymphomas include gastrointestinal tract, head, neck and chest. Second most frequently affected site is head and neck [5]. Lymph nodes of neck and mediastinum are most commonly involved extranodal sites that account for 5% cases of HL whereas approximately 30% lesions of NHL [7]. Frequently affected sites in the head and neck region are paranasal sinuses, salivary glands, mandible, maxilla and Waldeyer's ring [7]. Extra nodal lymphomas of oral cavity are rare with 3% of total in general population and 4% in patients with AIDS. In oral cavity, lymphoma generally occurs as an asymptomatic soft swelling in lips, tongue, buccal mucosa, upper lower gingiva, hard palate and retromolar trigone [8,9]. Extranodal lymphoma occurring intra orally look similar to tumors, dental abscess and osteoradionecrosis leading to delay in treatment and poor prognosis [1,9]. Hence it is

Correspondence: Dr Uzma Jabbar Khan, Department of Oral and Maxillofacial Pathology, University Dental Hospital, University College of Dentistry, University of Lahore, Lahore, Pakistan.

Email: uzma.jabbar@ucd.uol.edu.pk

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essential for the Oral and Maxillofacial Pathologists as well Surgeons to have an unambiguous understanding regarding the presentation of lymphoma and their characterization which in turn is achieved by the application of Immunohistochemistry (IHC). The former will exert profound effect on diagnosis and management of the patients along the course of the disease as well as the clinical outcome.

Histology is considered as a gold standard in diagnosis of lymphoma. However, IHC has become an essential part in identifying the specific lineage of lymphomas. IHC panel is selected based on the morphological findings in light microscopy. Commonly used markers include CD45, CD20, and CD3 to differentiate T- and B-cell lymphomas and CD15 and CD30 for HLs. Other markers used for subtyping of lymphoma are CD10, CD5, CD23, BCL2, Cyclin D1 and proliferation marker Ki67 [10]. IHC thus helps in the subtyping of lymphomas into different categories which have therapeutic and prognostic importance.

This study is intended to analyze the frequency distribution of extranodal lymphoma and their relative sites of occurrence in head and neck region. In addition, it is also focused to set down the histological types and subtypes of the diagnosed lymphoma through various immunohistochemical panels

MATERIAL AND METHODS

This cross-sectional observational study was conducted in the department of Oral and Maxillofacial Pathology, University College of Dentistry, University of Lahore. Sampling was done with non-probability purposive sampling technique. The research was conducted following the approval by the institutional ethical committee, University of Lahore. Total 39 histopathologically diagnosed cases of head and neck lymphoma from the period of June 2018 to July 2021 were included in the study. Inclusion criteria consist of lymphoma cases in age group 25-100 years and lymphoma of head and neck region. Exclusion criteria comprised of cases in age less than 25 years and lymphoma of CNS. Blocks were retrieved from the records. Patient's demographic details and site of involvement were also recorded. Fresh slides were prepared and histopathological examination was conducted by one histopathologist and one oral pathologist for re-confirmation of initial diagnosis as well as for minimizing inter-observer bias. For accurate typing of lymphoma, immunohistochemical panels were advised by the

examining consultants on the marked sections showing well preserved and major tumor area.

Selected blocks corresponding to the marked sections were sent to a well reputed outsource laboratory which was ISO certified and registered with Punjab Health Care Commission for immunohistochemistry due to lack of this facility at institutional histopathology laboratory. Various panels of immunohistochemical stains were applied on the block.

Basic IHC panel was used to identify specific lineage of lymphoma. To rule out HL and NHL, CD15, CD20 and LCA were used. CD3, CD5, CD10, CD20, CD23, CD43, Cyclin D1 and Bcl2 used to rule out B cell and T cell lymphoma. For identification of Plasma cell dyscrasias, Kappa and Lambda light chains were used.

To differentiate lymphoma of salivary gland from breast metastatic tumor, a specific marker GCDP-15 was used. Antibodies were used manually to stain the blocks, using 20 minutes heat induced epitope retrieval in target retrieval solution. The slides were incubated with the primary antibody for 30 minutes followed by detection of streptavidin – biotin immunoenzymatic antigen system after washing of endogenous peroxidase. Statistical Package for Social Sciences (SPSS) version 25 was used for data analysis. Results were stated as mean \pm standard deviation, numbers and percentages. Pearson chi-square test was used to compare the categorical data, p-value of < 0.05 was considered significant.

RESULTS

The number of cases analyzed were 39 in duration of three years. Out of 39 lymphoma cases, 21 (54%) were males and 18 (46%) were females. Male to female ratio was 1.2:1. Age range at diagnosis ranged from 25 years to 96 years, the mean age was 57.89 \pm 16.43 years (Table-I). Out of 39 cases, 38 were diagnosed as NHL while only 1 case turned out as HL (Figure-I). Commonly involved extra nodal site in n=39 cases of head and neck region was tonsils n=18 (46.15%) (Table-II).

Table-I: Age and Gender wise distribution of cases of lymphoma in (n=39) cases of lymphoma

Age Groups (Years)	No. of Cases n (%)	Gender	
		Male	Female
25 -54	13 (33.33%)	07	06
55 -84	24 (61.5%)	12	12
85 -105	02 (5.1%)	02	00

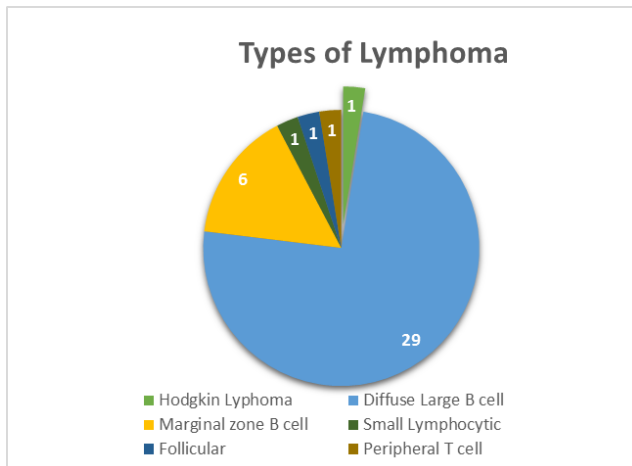


Figure-I: Frequency of type of lymphoma in head and neck region (n= 39).

Table-II: Frequency of involved sites in cases of lymphoma(n= 39).

Site	No. of cases n (%)
Upper Jaw	02 (5.1%)
Gingiva	01 (2.5%)
Oropharynx	01 (2.5%)
Lip	01 (2.5%)
Buccal Mucosa	02 (5.1%)
Submandibular Region	02 (5.1%)
Alveolar Ridge	01 (2.5%)
Tongue	02 (5.1%)
Palate	03 (7.6%)
Parotid	06 (15.3%)
Tonsils	18 (46.1%)

Association of 39 cases of lymphoma was tested with immunohistochemical markers by Pearson chi-square test. Cluster of differentiation (CD) 5 ($p=0.000$), 10 ($p=0.001$), 15 ($p=0.000$), 20 ($p=0.046$), 23 ($p=0.000$), 30 ($p=0.000$), Cyclin D1 ($p=0.000$) and Bcl2 ($p=0.000$) were significantly associated with various types of histologically diagnosed lymphoma.

DISCUSSION

Lymphoma is a group of lymphoid malignancies representing approximately 4% of malignancy worldwide [7]. It is the third most frequently occurring malignancy in head and neck region after squamous cell carcinoma [6]. Lymphoma occurring at sites other than lymph nodes is referred as extra nodal and at least one fourth of the lymphomas are of extra nodal origin [11].

The statistics of Surveillance, Epidemiology and End result (SEER) program of United State showed that the total lymphoid malignancies increase with age in both sexes, which is possibly attributed to an increased susceptibility of infections because of weak immune system [12,13]. According to American Cancer Society the median age of lymphoma in United States was estimated to be 60 years at the

time of diagnosis [12]. In the current study, the mean age was 57.89 ± 16.43 years which is consistent with the findings of Hung Sheng chi *et al*, Walter *et al* and Bukhari *et al* [3,1,13]. In current study, there is a slight predominance of male patients with male to female ratio of 1.2:1 which is in agreement with the findings of previous studies [4,14,5,13]. In current study maximum incidence of cases including both genders were seen in age group 55 years to 65 years.

Rarely HL and around 25% of NHL arise from extra nodal sites [8]. This is evident in our study where frequency of NHL is far greater than HL, similar to the findings of Shahid *et al* and Storck *et al* [4,6]. Extra nodal lymphomas can arise from nearly every anatomic site of the body including head and neck [15]. Most common site involved in the current study was tonsil (n=18, 46.5%) , consistent with the finding of Chi *et al* and Akbari *et al* [3,16] .Parotid gland was the second common affected site (n= 6,15.38%) which is in line with the results of Walter *et al* [1]. Most common intra oral site of extra nodal NHL in the present study was oral mucosa comprising of alveolar ridge, gingiva and upper jaw (n=4,10.2%) and second affected site was palate (n=6,15.3%) that is similar to the findings of Natalia *et al* and Akbari *et al* [17,16]. However, the finding of the current study was in contrast to the result of Chi *et al* where the most common intra oral site of extra nodal NHL was tongue base [3].

The understanding of characterization and immunophenotyping of lymphoma is possible with immunohistochemistry [12]. Lymphoma of B cell lineage outnumbered T cell lymphoma in the present study as diagnosed on the basis of panel of IHC. In present study, DLBCL occur in greatest number (n=29, 76.9%) which is in agreement to statistics reported in Pakistan [4,13,18,19].

Second common subtype of B cell lymphoma in current study was extra nodal Marginal Zone B cell lymphoma of MALT type (n=6, 15.3%) which is similar to the findings of Padhi *et al* and Kin *et al* [11, 20]. This finding was in contrast to the result of Bukhari *et al* [14] where small lymphocytic lymphoma was the second after DLBCL in occurrence.

Based on the result of histologically diagnosed cases of lymphoma in the present study, basic screening panel was proposed for immunophenotypic analysis. For B cell lymphoma, cluster of differentiation 10 & 20 (CD 10 and CD20) were used. CD20 is the most commonly used marker for B cells and is expressed from the immature B cell until the final phases of B cell development [21]. CD20 positivity was noted in 94.8% of B cell

lymphomas whereas CD3, a T cell marker was focally positive in two cases of DLBCL. Gross cystic disease fluid protein 15 (GCD FP-15) was first identified as a main constituent of breast cyst fluid and is also present in saliva. It is an excellent tissue marker for the apocrine epithelium however some non-apocrine tissues such as serous cells in salivary gland also display positive immunostaining with GCDFP-15. It is useful in differentiating salivary gland lymphoma from breast metastatic tumor and other high-grade salivary gland tumors with glandular differentiation [22]. One case of DLBCL of parotid gland in the existing study show positive staining with GCDFP-15.

Peripheral T cell lymphoma in this study was uncommon with only one case which is in line with the finding of Kim JM *et al* [20].

CONCLUSION

The present study highlighted the frequency and distribution pattern of various types of lymphoma in head and neck region. Extra nodal Non-Hodgkin lymphoma is more prevalent type with male predominance. DLBCL is the most frequent subtype. Most commonly involved site in head and neck region is tonsil which is in agreement with worldwide statistics.

LIMITATIONS

The study was not a multicenter study and some vital information, such as medical history, signs and symptoms and lymphoma size at time of presentation were missing.

RECOMMENDATIONS:

Larger scale study with increased sample size must be conducted in order to have in-depth analysis of incidence and typing of lymphomas in head and neck region. Multicenter studies are needed in order to highlight the significance of typing of lymphoma to make a sound clinical decision, and design the most efficacious management protocol.

AUTHOR CONTRIBUTION

Uzma Jabbar Khan: Article writeup.

Wajiha Alamgir: Date collection.

Aroosa Ashraf and Adeel Haider: Result compilation.

Salman Rashid: Statistical analysis.

Faheem Abrar: Outsource laboratory.

REFERENCES

1. Walter C, Ziebart T, Sagheb K, Nedjat R, Manz A, Hess G. Malignant lymphomas in the head and neck region- A retrospective, single center study over 41 years. *Int J Med Sci.* 2015; 12(2): 141-45.
2. Cabecades J, Martinez D, Andreassen S, Mikkelsen LH, Uraa RM, Hall D, *et al.* Lymphomas of the head and neck region: An update. *Virchows Arch.* 2019; 474(6): 649-65.
3. Chi HS, Lee KW, Chiang FU, Tai CF, Wang LF, Yang SF, *et al.* Head and neck extra nodal lymphoma in a single institute: A 17-year retrospective analysis. *Kaohsiung J Med Sci.* 2012; 28: 435-441.
4. Shahid R, Gulzar R, Avesi L, Hassan S, Danish F, Mirza T. Immunohistochemical profile of Hodgkin and non-Hodgkin Lymphoma. *J Coll Physicians Surg Pak.* 2015; 26(2): 103-107.
5. Tsabedze VN. Clinical profile and histological characteristics of head and neck lymphomas in patients seen at two referral centres in Nairobi Kenya. [Thesis]. University of Nairobi.2019.
6. Mahmood H, Habib M, Aslam W, Khursheed S, Fatima S, Aziz S. *et al.* Clinicopathological spectrum of Diffuse large B cell lymphoma: A study targeting population yet unexplored in Pakistan. *BMC Res Notes.* 2021; 14: 354.
7. Shabbir S, Ahmed KN, Marri M, Mengal M, Jan MH, Jamali MS, *et al.* Epidemiological features of lymphoma in Pakistan. *Pure Appl Biol.* 2019; 8(1): 977-994.
8. Rathore A, Ranjan S, Kapoor R, Singh J, Arvind P, Pandya T, *et al.* Clinicoepidemiological profile of extra nodal lymphoma: The experience of a tertiary care center in India. *J Clin Invest.* 2020; 9(2): 42-48.
9. Sirsath NT, Lakshmaiah KC, Das U, Lokanatha D, Chennagiri SP, Ramarao C. Primary extranodal Non-Hodgkin's lymphoma of oral cavity. *J Cancer Res Ther.* 2014; 10(14): 945-50.
10. Rao IS, Role of immunohistochemistry in lymphoma. *Indian J Med Paediatr Oncol.* 2010; 31(4): 145-147.
11. Padhi S, Paul TR, Challa S, Prayaga AK, Rajappa S, Raghunadharao D, *et al.* Primary extra nodal non-Hodgkin lymphoma: A 5-year retrospective analysis. *Asian Pac J Cancer Prev.* 2012; 13(10): 4889-4895.
12. Basharat S, Iqtidar BH, Naeem S, Batool Z, Ali N, Ahmad Z. Clinicopathological and immunohistochemical profile of patients with Non-Hodgkin's Lymphoma. *Rawal Med J.* 2019; 44(3): 472-76.
13. Bukhari U, Jamal S, Lateef F. Non-Hodgkin's lymphoma - A study. *PODJ.*2015;35(3):412-415.
14. Kuo CY, Shih CP, Cheng LH, Liu SC, Chiu FH, Lin YY. *et al.* Head and neck lymphomas: Review of 151 cases. *J Med Sci.* 2020; 40(5): 215-223.
15. Silva TDB, Ferreira CB, Leite GB, Pontes JR, Antunes HS. Oral manifestations of lymphoma: A systematic review. *E cancer.* 2016; 10: 665.
16. Akbari ME, Bastani Z, Mokhtari S, Moghadam SA. Oral lymphoma prevalence in Iranian population: A multicenter retrospective study. *Iran J Cancer Prev.* 2015; 8(6): 4142.
17. Kasuke N, Custodio M, De souza SC. Oral lesions as the primary diagnosis of no Hodgkin's lymphoma: a 20-year experience from an oral pathology service and review of the literature. *Eur Arch Oto-Rhino-L.* 2019; 276: 2873-79.
18. Mushtaq S, Akhtar N, Jamal S, Mamoon N, Khadim T, Sarfaraz T. Malignant lymphoma in Pakistan according to the WHO classification of lymphoid neoplasms. *Asian Pac J Cancer Prev.* 2008; 9: 229-32.
19. Abid MB, Nasim F, Anwar K, Pervez S. Diffuse large B cell Lymphoma (DLBCL) in Pakistan: An emerging epidemic? *Asian Pac J Cancer Prev.* 2005; 6: 531-4.

20. Kim JM, Ko YH, Lee SS, Huh J, Kim CW, Kang YK, *et al.* WHO Classification of malignant lymphomas in Korea: Report of the third nationwide study. *Korean J Pathol.* 2011; 45(3): 254-60.
21. Higgins RA, Blankenship JE, Kinney MC. Application of immunohistochemistry in the diagnosis of non-Hodgkin and Hodgkin lymphoma. *Arch Pathol Lab Med.* 2008; 132(3): 441-61.
22. Stains and CD markers. Gross cystic disease fluid protein 15(GCDFP-15). Pernick N. *Pathology outlines* 2021.