



Morphological Typing of Extranodal Lymphoma with Immunohistochemical Study

Authors

Dr K. S. Dhumure¹, Dr R. D. Hanmante^{2*}, Dr S. W. Chopade³, Dr R. S. Bindu⁴

¹Assistant Professor, MIMSR Latur, Maharashtra
Mob. No. 9545083197

²Associate Professor, GMC Akola, Maharashtra

³Assistant Professor, GMC Akola, Maharashtra

Mob. No. 9860064099, Email: chopade.shriram@gmail.com

⁴Professor & HOD, GMC Aurangabad, Mob. No. 9890987147

Name & Address of Department to which work should be attributed: Department of Pathology, Government Medical College, Aurangabad, India

*Corresponding Author

Dr Rajharsh Dattatrey Hanmante

Associate Professor, Department of Pathology, Govt. Medical College Akola, Maharashtra.444001, India

Email: drrajharshhanmante@gmail.com, Mob no. 9766251669

Abstract

Background: Lymphomas are neoplasms of the lymphoid tissue. It can affect the lymph nodes as well as extra-nodal tissue. A substantial percentage of non-Hodgkin's lymphomas arises from tissue other than lymph nodes. These are referred as primary extranodal lymphomas. The diagnosis of extranodal lymphoma requires clinico-pathological consideration and immunohistochemistry study. The present study includes the morphological typing of the Extranodal lymphoma with the help of Immunohistochemistry markers.

Methodology: This was the prospective study carried out in the pathology department at a tertiary care teaching hospital over a period of two years. Patients clinically suspected or diagnosed as extranodal lymphoma and those detected by histopathology were included in study. The results were confirmed with the help of Immunohistochemistry study.

Results: Total 35 cases of extranodal lymphoma were studied. The study population ranges from age group 06 to 76 years with mean age 46.7 years with M:F ratio 2.88:1. Gastrointestinal tract (60%) was the predominant site of primary extranodal lymphoma followed by Oral cavity (14.29%), Parotid (8.56%), Testis (5.71%) and 2.86% each of Ovary, Nose, Breast and Chest.

Histopathologically, all cases were of Non-Hodgkin's lymphoma type. DLBCL was the commonest variant of extranodal lymphoma found during the study.

Conclusion: Morphology forms the basis of diagnosis of extranodal Lymphoma. WHO classification mandates the use of immunohistochemistry. IHC has diagnostic as well as therapeutic benefits.

Keywords: DLBCL, Extra-nodal, IHC, Lymphoma, NHL etc.

Introduction

Lymphomas are neoplasms of the lymphoid tissue (lymphocyte). It can affect the lymph nodes as well as extra-nodal tissue. Lymphoma primarily affects the cells of the immune system. The term “lymphoma” was introduced and defined by Virchow in 1862-63.¹

Lymphomas are broadly divided into two major categories namely Hodgkin’s lymphoma (HL) and Non-Hodgkin’s lymphoma (NHL).¹

Different terminologies for different lymphomas used in the past for precise histopathological identification have led to various classifications by different authors. As a result, the classification of lymphoma has undergone significant reappraisal over the past 50 years. Currently, more than 40 distinct entities are recognized in the WHO classification of tumors of hematopoietic and lymphoid tissues (2008).² This classification uses all the available information- morphology, immunophenotype, genetic features and clinical features- to define diseases.

A substantial percentage of non-Hodgkin’s lymphoma (NHL) arises from tissue other than lymph nodes and even from sites which normally contain no lymphoid tissue.³ These forms are referred to as primary extranodal lymphomas. At least one-fourth of the lymphomas are probably of extranodal origin.^{3,4}

The term extranodal lymphoma encompasses a vast assortment of morphologies, molecular alterations, and clinical presentations. Correct diagnosis and appropriate treatment of extranodal lymphoma are often complicated by the variety of lymphoma types and the relative rarity of many of these tumor types. Moreover, in comparison with nodal presentation, B and T-cell lymphoma diagnosed at extranodal sites may have quite different outcomes and may frequently require different therapeutic approaches due to specific organ-related problems. Until very recently, the literature on many of the specific types and sites of extranodal lymphomas was scant and often contradictory, lacking uniformity in histopathological classification. The first attempt

to eliminate this problem was made only in 1994 with the proposal of the REAL classification⁵ and afterwards with the World Health Organization (WHO) classification,⁶ which definitely recognized the presence of specific extranodal entities.⁷

Over the years, application of immunohistochemistry (IHC) in surgical pathology has increased tremendously. Immunohistochemistry (IHC) has become an integral part of lymphoma diagnosis.

Although in the early 1980s a routine lymphoma panel included less than 10 antibodies, the current diagnostic armamentarium includes more than 50 antibodies. IHC must be interpreted in the context of a panel to avoid errors in assignment of cell lineage or of an abnormal phenotype.

Thus diagnosis of extranodal lymphoma is multistep process requiring clinico-pathological consideration and immunohistochemistry study with battery of antibodies.

The present study includes the morphological typing of the Extranodal lymphoma with the help of Immunohistochemistry markers.

Methodology

This was the prospective study carried out in the pathology department at a tertiary care teaching hospital over a period of two years. Total 35 cases of extranodal lymphoma were studied. Patients clinically suspected or diagnosed as extranodal lymphoma and those detected by histopathology were included in study. Patients with leukemia or with previous history of leukemia were excluded. This was followed by a complete clinical evaluation of the patient which included accounting of complete clinical details and various relevant investigations. Following histopathological examination, Immunohistochemistry was performed on each case. An attempt was made in each case to classify it according to WHO 2008 classification.

Histopathological diagnosis was achieved using the microscopic examination of slides stained with Haematoxyline and eosin stains. The results were

confirmed with the help of Immunohistochemistry study.

Results

During this study, total 35 cases, first diagnosed by histopathological examination of the specimen and finally confirmed by immunohistochemical study were studied. The study population ranges from age group 06 years to 76 years with mean age 46.7 years. Predominant age group affected was the 6th decade of life contributing for 26% of cases. During this study, 26 were male 09 were female with M:F ratio 2.88:1. The distribution of cases according to the age group is shown in Table No. 1.

During this study, gastrointestinal tract (60%) was the predominant site of primary extranodal lymphoma followed by oral cavity (14.29%). Site wise distribution of primary extranodal lymphoma is shown in Table No. 2. Among the 21 cases (60%) of extranodal lymphoma of gastrointestinal tract, stomach 06 cases [Fig. 1] (28.57%) constitute the major site followed by caecum 04 cases (19.05%) and colon 04 cases (19.05%) and then ileocaecal region 03 cases (14.29%), 02 cases (09.52%) each of ileum [Fig. 2] and jejunum. Among the 05 (14.29%) cases of primary oral cavity lymphoma, 02 (40%) cases found at alveolus, 02 (40%) cases at floor of mouth [Fig. 3] and 01 (20%) case occurred at tonsil [Fig. 4]. The primary gastrointestinal tract lymphoma cases were presented with variable clinical features.

Among these 21 cases of primary gastrointestinal tract lymphoma cases, pain in abdomen was the predominant complain in 19 cases (90.48%) followed by abdominal lump in 07 cases (33.33%) and increased bowel habit in 06 cases (28.57%). The constitutional symptom like fever was present in 06 cases (28.57%), vomiting in 05 cases (23.81%) and weight loss in 03 cases (14.29%). The abdominal ultrasound and CECT examination of these cases revealed the bowel wall thickening and mass lesion in the 15 cases.

Of the 5 primary oral cavity lymphoma cases, 2 cases (40%) presented with solid growth on right

alveolus and 2 cases (40%) presented with solid growth over the floor of mouth and 01 case (20%) presented with growth over right tonsil.

Among the 03 primary parotid lymphoma cases [Fig.5], 02 cases presented with right sided parotid swelling and 01 case as left sided parotid swelling. Primary testicular lymphoma [Fig.6], of these 02 cases was presented as testicular swelling, one on each side.

Primary ovarian lymphoma (01 case) [Fig.7] was presented with features of abdominal pain. On ultrasound examination, evidence of bilateral ovarian masses seen. Primary breast lymphoma (01 case) [Fig.8] was presented with lump in both breasts. The lump was not associated with nipple discharge, pain, nipple retraction and fixity to chest wall or underlying structures. These bilateral breasts swelling present in the lower outer quadrants.

The primary nasal lymphoma (01 case) [Fig.9] was presented with swelling and large ulcerated, destructive lesion involving nasal cavity. Primary Mediastinal T cell Lymphoma [Fig.10] was presented with complaints of breathlessness. On radiological examination, chest X ray showed the mediastinal widening and CECT Chest findings showed heterogeneously enhancing anterior mediastinal mass ?malignant thymoma? Germ cell tumor.

There were 21 cases of primary gastrointestinal tract lymphoma of which majority cases i.e. 6 cases (28.57%) were in the age group of 51 – 60 years. M:F ratio was 4.25:1.

All the 35 cases were first assessed by histopathological examination and finally examined for the Immunohistochemistry study using the panel of antibody markers according to the lesion. Histopathologically, all these cases were of Non-Hodgkin's lymphoma type. After Immunohistochemistry study, these lymphoma cases were classified according to their site of occurrence as shown in Table 3.

Thus, DLBCL was the commonest variant of extranodal lymphoma found during the study. Of these 20 (57.14%) cases of DLBCL, 01 case

showed immunoblastic variant and another (01) case showed centroblastic variant of high grade type with T cell rich B cell lymphoma.

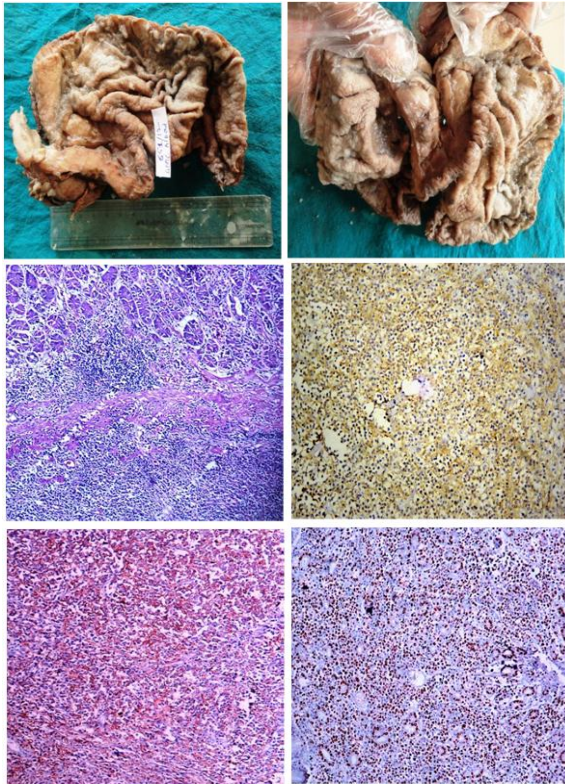


Fig. 1. Primary Gastric DLBCL

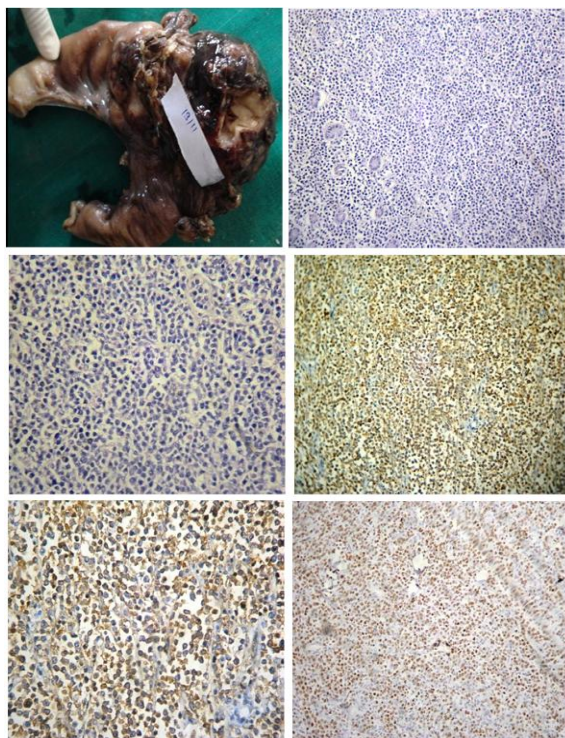


Fig. 2 Burkitt's Lymphoma of Ileum

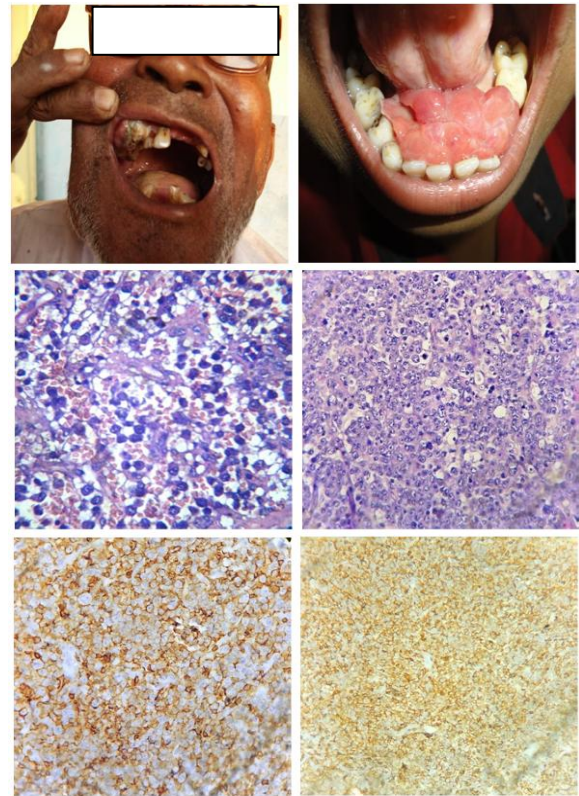


Fig.3. Plasmablastic Lymphoma of Oral Cavity

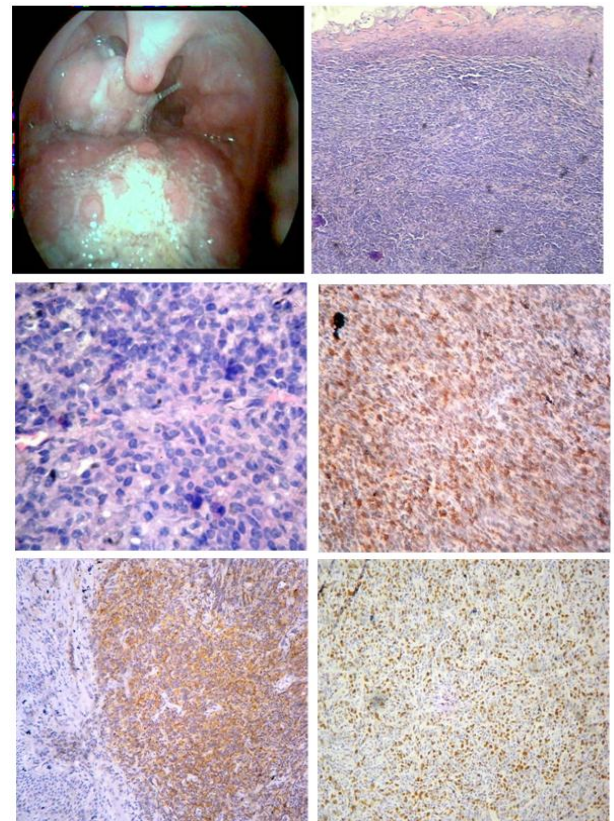


Fig. 4 Mantle cell Lymphoma of Tonsil

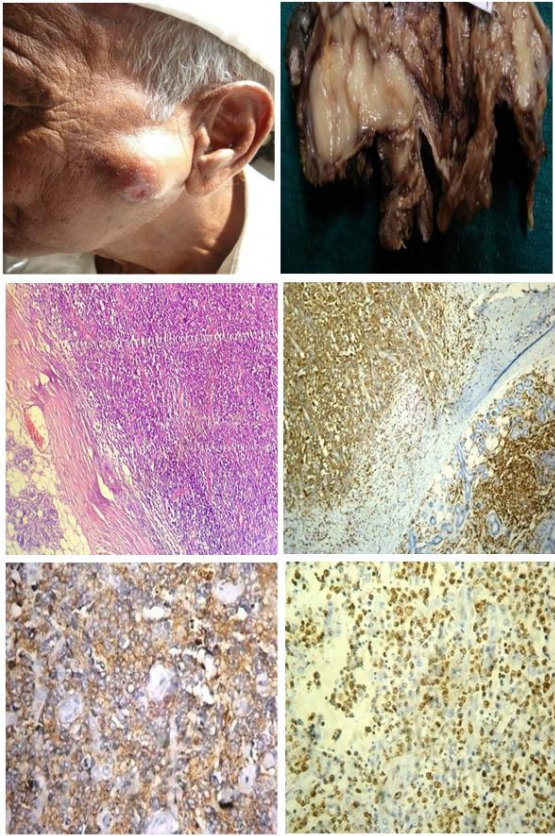


Fig. 5 Primary Parotid DLBCL

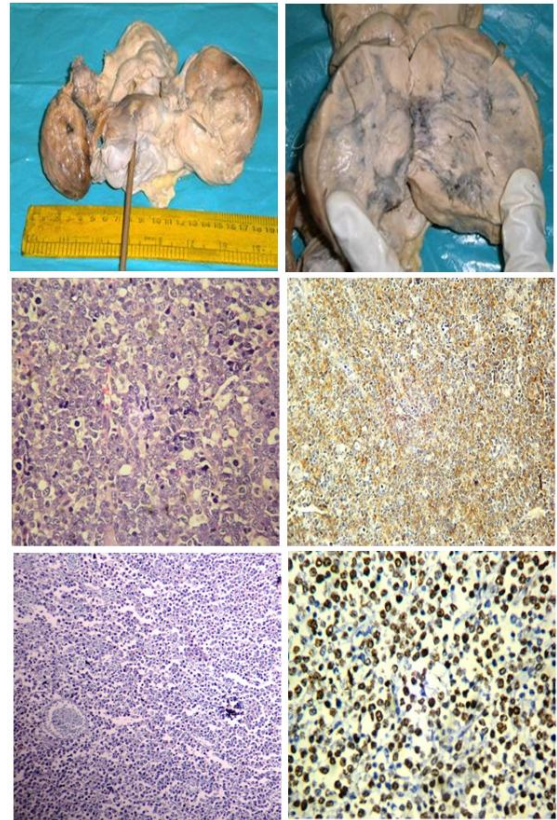


Fig.7. Primary Ovarian DLBCL.

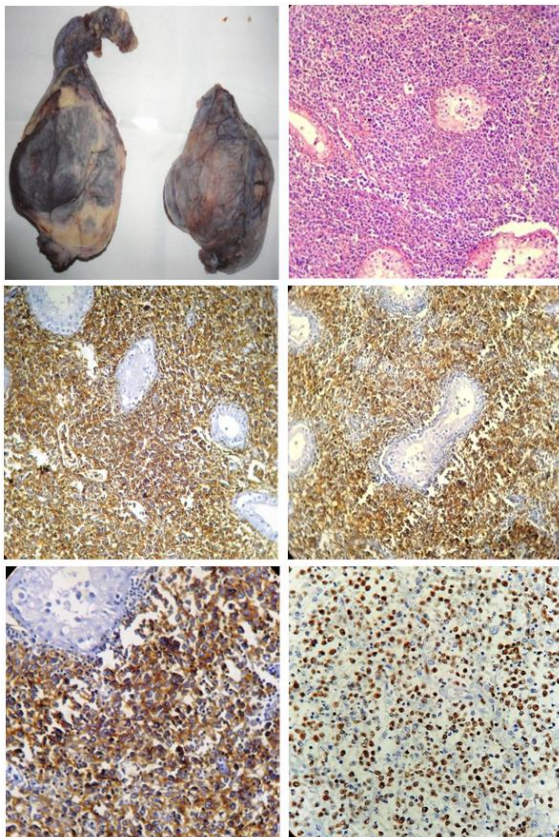


Fig. 6. Primary Testicular Diffuse Large B cell Lymphoma

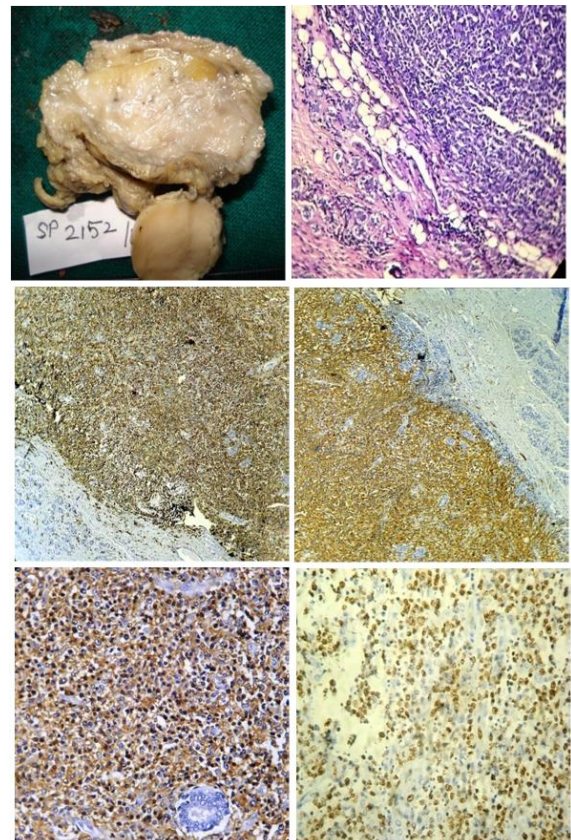


Fig. 8. Primary Breast Diffuse Large B cell Lymphoma.

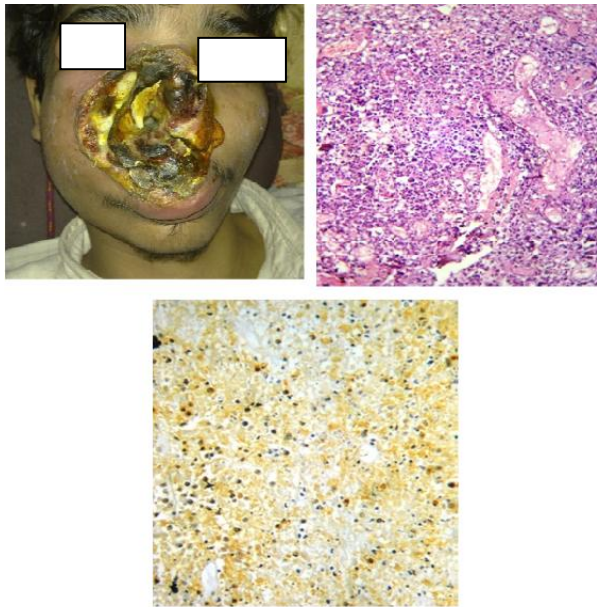


Fig. 9 Primary Nasal NK/T Cell Lymphoma

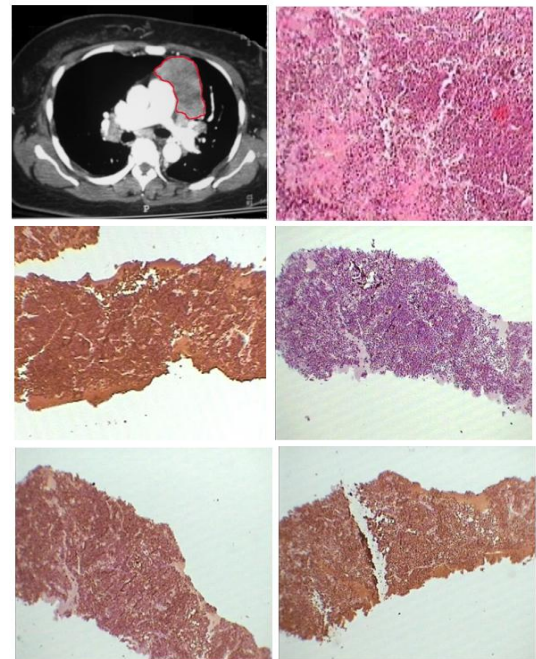


Fig. 10 Primary Mediastinal T cell Lymphoma

Table No. 1 Distribution of cases according to the age group. (n=35)

Age group (years)	0 - 10	11 - 20	21 - 30	31 - 40	41 - 50	51 - 60	61 - 70	71 - 80
No. of cases	02 (5.71%)	04 (11.43%)	03 (8.57%)	02 (5.71%)	06 (17.15%)	09 (25.72%)	07 (20%)	02 (5.71%)

Table No. 2 Site wise Distribution of Primary Extra Nodal Lymphoma. (n=35)

Site	GIT	Oral cavity	Parotid	Testis	Ovary	Breast	Nose	Chest	Total
No. of cases	21	05	03	02	01	01	01	01	35
%	60	14.29	8.56	5.71	2.86	2.86	2.86	2.86	100%

Table No. 3. Distribution of Cases according to the Lymphoma variant and it's site of occurrence

Site		No. of cases according to variant						
		DLBCL	BL	MZBL	PBL	ML	MT LL	NK/T cell L
GIT	Stomach	05	--	01	--	--	--	--
	Caecum	02	02	--	--	--	--	--
	Colon	04	--	--	--	--	--	--
	IleoCaecum	02	01	--	--	--	--	--
	Illeum	01	01	--	--	--	--	--
	Jejunum	01	01	--	--	--	--	--
Oral Cavity	Alveolus	--	--	--	02	--	--	--
	Floor of mouth	--	--	--	02	--	--	--
	Tonsil	--	--	--	--	01	--	--
Parotid		01	--	02	--	--	--	--
Testis		02	--	--	--	--	--	--
Ovary		01	--	--	--	--	--	--
Breast		01	--	--	--	--	--	--
Chest		--	--	--	--	--	01	--
Nose		--	--	--	--	--	--	01
Total (35)		20	05	03	04	01	01	01
Percentage 100%		57.14	14.28	8.6	11.43	02.86	02.86	02.86

DLBCL: Diffuse Large B Cell Lymphoma. BL: Burkitt's Lymphoma.

MZBL: Marginal Zone B Cell Lymphoma. PBL: Plasmablastic Lymphoma.

ML: Mantle Cell Lymphoma. MTLL: Mediastinal T Cell Lymphoblastic Lymphoma.

NK/T Cell L: NK/T Cell Lymphoma of Nasal Type.

Discussion

The term extranodal lymphoma encompasses a vast assortment of morphologies, molecular alterations, and clinical presentations. The overview of extranodal lymphoma regarding their clinical presentation, diagnostic modalities and clinical behavior taken in to consideration during this study. The findings of the present study were compared with the previous literature data. The mean age group in the present study was 47.6 year which was similar to findings of Vural F.et al.⁸ (47 year) and Lee J-D.et al.⁹(48 year).

The studies on the extranodal lymphoma showed that occurrence of extranodal lymphoma were more common in male as compared to female. The present study also shows the male predominancy with M:F ratio 2.88:1 which is similar to the results of Lee J-D.et al.⁹ (3:1) and Wang GB et al.¹⁰ (2.52:1).

It was found that GIT was the most common site for primary extra nodal lymphoma in our study which is fairly correlated with the findings of Kroll A.D. G. et al.¹¹ and Economo-poulosa T. et al.¹² Among the primary GIT extra nodal lymphoma, stomach was the predominant site of affection found during the present study. Similar findings were noted by Lee JD. et al.¹⁰ (32%), Kroll A.D.G.et al.¹¹ (60%). Abdominal pain was the main presenting feature of primary gastrointestinal tract lymphoma. Our findings are consistent with findings of Abbondanzo S.L. & Sobin L.H.¹³ and Zinzani P.L. et al.¹⁴

Abbondanzo S.L. & Sobin L.H.¹³ and Yoon S.et al.¹⁵ studies showed that Marginal zone B cell mucosa associated lymphoid tissue (MALT) lymphoma were the majority group of cases among primary gastrointestinal tract lymphoma.

Ibrahim E.M. et al.¹⁶, Lee J-D.et al.¹⁰, Zinzani P.L. et al.¹⁴, Wang GB et al.¹⁰ and Al Diab A.R. et al.¹⁷ showed that, diffuse large B cell lymphoma (DLBCL) was the predominant histological variety among the primary gastrointestinal tract lymphoma followed by MALT lymphoma, Burkitt lymphoma and Mantle cell lymphoma. The other histological variety includes Immunoblastic,

Plasmablastic and Follicular lymphoma. Present study also showed that, DLBCL was the predominant histological variety among primary gastrointestinal tract lymphoma. It accounts for 71.43%.

Yin H-F.et al.¹⁸ found that DLBCL accounts for 73.53% among primary oral cavity lymphoma. Delecluse H.J.et al.¹⁹ found that Plasmablastic lymphoma accounts for 100% cases among primary oral cavity lymphoma. Present study also showed that Plasmablastic lymphoma accounting for 80%, was the major histological variant among primary oral cavity lymphoma

Gleeson M.J. et al.²⁰ found that diffuse small cell lymphoma (DSCL) comprises the predominant histological variety among primary parotid lymphoma. DSCL was followed by DLBCL and Follicular lymphoma. Barnes L et al.²¹ found that, follicular lymphoma accounting for 48.48%, was the predominant histological variety among the primary parotid lymphoma. Follicular lymphoma followed by DLBCL (27.27%). None of the case of MALT lymphoma found during the study of Gleeson M.J. et al.²⁰ and Barnes L et al.²¹ Present study showed that MALT lymphoma (02) was the most common variety among the 03 cases of primary parotid lymphoma. It was followed by DLBCL in 01 case. The findings of the present study are not consistent with the above mentioned studies, related to parotid lymphoma. It might be due to low sample size of parotid lymphoma in present study as compared to previous study.

Primary testicular lymphoma most commonly occurred in age group of 50 - 70 year. Testicular swelling was the predominant presenting feature of all the testicular lymphoma cases. Hasselblom S. et al.²², Vural F.et al.⁸, found the DLBCL was the predominant histological variety among the primary testicular lymphoma. Similar findings were obtained in the present study.

Primary breast lymphoma involves most commonly the right breast as per the Loughrey M.B. et al.²³ and Al Diab A.R. et al.¹⁷ studies but the present study had bilateral breast involvement. They were presented with palpable breast mass.

Male breast involvement, though rare was seen in studies of Al Diab A.R. et al.¹⁷, Loughrey M.B. et al.²³ Present study showed that Diffuse large B-cell lymphoma involved bilateral breast.

Primary ovarian lymphoma is a rare entity. Ray S. et al.²⁴ and Dantkale S. S. et al.²⁵ presented the case report of primary ovarian lymphoma. We also found one case of primary extranodal lymphoma of ovary. Histologically, all cases were Non-Hodgkin's type and immunohistochemically, diffuse large B cell type.

Ko Y.H. et al.²⁶ found the 10 cases of primary nasal cavity lymphoma. There was one case of primary nasal cavity lymphoma in our study. Primary nasal cavity lymphoma showed the T cell phenotype in the studies of Ko Y.H. et al.²⁶ and Li Y. X. et al.²⁷ Present study shows the NK/T cell phenotype of primary nasal cavity lymphoma.

Primary mediastinal lymphoma usually presented with the B cell phenotype. In the present study, it was found that primary mediastinal lymphoma presented with T cell phenotype. It was very rare presentation. In the literature, Yang C.G. et al.²⁸ presented the first case report of large T cell lymphoma of mediastinum.

Conclusion

Proper and adequate tissue preservation and processing is the cornerstone of good morphology and immunohistochemistry (IHC). Though, morphological diagnosis forms the basis of diagnosis of extranodal Lymphoma, its classification according to WHO classification mandates the use of immunohistochemistry at the very least. Judicious use of panel of antibodies in the light of cyto-architectural features helps to determine the lineage in cases of extranodal lymphoma. Not to be forgotten is the role of IHC in identifying antigens which can be targeted for therapy. Thus, IHC has diagnostic as well as therapeutic benefits.

Source of Support: Nil

References

1. Rosai J. lymph nodes. In: Rosai and Ackerman's surgical pathology. Volume 2, 9th ed. St. Louis, Missouri: Mosby; 2004. p.1917.
2. Swerdlow S.H., Campo E, Harris N.L., Jaffe E.S., Pileri S.A., et al.: Introduction to WHO classification of tumors of hematopoietic and lymphoid tissue. In: to WHO classification of tumors of hematopoietic and lymphoid tissue. 4th ed. IARC: Lyon, 2008. P.14 - 5.
3. Zucca E., Roggero E., Bertoni F. & Cavalli F. Primary extranodal non Hodgkin's lymphomas. Part I: Gastrointestinal, cutaneous and genitourinary Lymphomas. *Annals of Oncology*, 1997;8: 727-37.
4. Freeman C, Berg JW, Cutler SJ. Occurrence and prognosis of extranodal lymphomas. *Cancer* 1972; 29: 253-60.
5. Harris NL, Jaffe ES, Stein H, et al. A revised European – American classification of lymphoid neoplasms: a proposal from the International Lymphoma Study Group. *Blood* 1994; 84: 1361–92.
6. Jaffe ES, Harris NL, Stein H, Vardiman JW. Pathology and genetics of tumours of haematopoietic and lymphoid tissues. World Health Organization Classification of Tumours, 3rd edn. Lyon: IARC Press, 2001:1–351.
7. Zucca E. and Cavalli F. Challenging issues in the management of extranodal lymphomas. In: Cavalli F. Stein H. Zucca E. Extranodal Lymphomas Pathology and Management, 1st ed. London: Informa Healthcare; 2008. p.3.
8. Vural F, Cagircan S, Saydam G, Hekimgil M, Soyer NA, Tombuloglu M. et al. Primary testicular lymphoma. *Journal of the national medical association*. November 2007; vol. 99 (11), 1277-82.
9. Lee J-D, Ko Y-H, Park J-H, Min K-R, Park K-N. Histological and Immunohisto-

- chemical studies on primary gastrointestinal lymphoma. *Journal of Korean Medical Sciences*, 1993, 8; 1:17-23.
10. Wang GB, Xu G-L, Luo G-Y, Shan H-B, Li Y, Gao X-Y. et al. Primary intestinal non-Hodgkin's lymphoma: A clinicopathologic analysis of 81 patients. *World J Gastroenterol* 2011 November 7; 17(41): 4625-31.
 11. Krol A.D, G. le Cessie.S., Snijder S. Kluin-Nelemans J.C. Kluin P.M. Noordijk. E.M. et al. Primary extranodal non-Hodgkin's lymphoma (NHL). *Annals of Oncology*. 2003; 14: 131-9.
 12. Economopoulos T, Papageorgiou S, Rontogianni D, Kaloutsi V, Fountzilias G, Tsatalas C, et al. Multifocal Extranodal Non-Hodgkin Lymphoma. *The Oncologist* 2005; 10:734-8.
 13. Abbondanzo S.L. Sobin L.H. Gastric "Pseudolymphoma" A Retrospective Morphologic and Immunophenotypic Study of 97 Cases. *CANCER*. 1997; 79: 9, 1656 - 63.
 14. Zinzani P.I., Magagnoli M., Pagliani G., Bendandi M., Gherlinzoni F, Merla E. et al. Primary Intestinal Lymphoma: Clinical and therapeutic features of 32 Patients. *Haematologica*. 1997; 82:305-8.
 15. Yoon SO, Suh C, Lee DH, Chi H-S, Park CJ, Jang S-S. et al. Distribution of lymphoid neoplasms in the Republic of Korea: Analysis of 5318 cases according to the World Health Organization classification. *Am. J. Hematol*. 2010; 85:760-4.
 16. Ibrahim E.M, Ezzat AA, Raja MA, Rahal MM, Ajarim DS, Mann B et al. Primary gastric non-Hodgkin's lymphoma: Clinical features, management, and prognosis of 185 patients with diffuse large B-cell lymphoma: *Annals of Oncology*, 1999; 10: 1441-9.
 17. Al Diab AR, Aleem A, Qayum A, Al Askar AS, Ajarim DS. Clinico-Pathological Pattern of Extranodal Non-Hodgkin's Lymphoma in Saudi Arabia, *Asian Pacific Journal of Cancer Prevention* 2011; Vol 12, 3277- 82.
 18. Yin H-F, Jia L, Okada N and Takagi M. comparative study of clinico- pathological features of extranodal oral NHL and maxillofacial nodal NHL. *Oral med pathol*, 2000; 5:15-23.
 19. Delecluse H.J. Anagnostopoulos I. Dallenbach F. Hummel M. Marafioti T. Schneider.U. et al. Plasmablastic Lymphomas of the Oral Cavity: A New Entity Associated With the Human Immunodeficiency Virus Infection. *Blood*, Vol 89, No 4 (February 15), 1997:1413-20.
 20. Gleeson M. J, Bennett M. H. Cawson R. A. Lymphomas of Salivary Glands. *Cancer*, 1986; 58:699-704.
 21. Barnes L, Myers E and Prokopakis E. Primary Malignant Lymphoma of the Parotid Gland. *Arch Otolaryngol Head Neck Surg*. 1998; 124:573-7.
 22. Hasselblom S, Ridell B, Wedel H, Norrby K, Baum MS, Ekman T. Testicular Lymphoma. *Acta Oncologica*, 2004; 43: 8, 758-65.
 23. Loughrey M.B, Windrum P, Catherwood MA, Alexander HD, Markey GM, McManus DT. et al. WHO reclassification of breast lymphomas. *J Clin Pathol* 2004; 57:1213-4.
 24. Ray S. Mallick MG, Pal PB, Choudhury MK, Bandopadhyay A, Guha D. Extranodal Non-Hodgkin's lymphoma presenting as ovarian mass. *IJPM*. 2008; 51(4):528-30.
 25. Dantkale S.S, Gopal A. Pandit G.A, Joshi S.S, Pudale S.S. Primary Bilateral Non Hodgkin.s Ovarian Lymphoma A Case Report. *JKIMSU*. 2012; 1(2):155-8.
 26. Ko Y.H. Lee J.D. Kim C.M. Kim I.S. Lee M.L. et al. Malignant lymphoma of nasal cavity. *Journal of Korean medical science*, 1992; 7(4): 314-24.

27. Li Y.X, Coucke P.A, Li J-Y, Gu D-Z, Liu X-F, Zhou L-Q et al. Primary Non-Hodgkin's Lymphoma of the Nasal Cavity. *Cancer* August 1, 1998 / Vol 83 / No.(3) p.449-56.
28. Yang GC, Yee HT, Wu CD, Aye LM, Chachoua A. TIA-1+ cytotoxic large T-cell lymphoma of the mediastinum: *Diagn Cytopathol.* 2002 Mar; 26(3):154-7.