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Original Article

Pathology Section

Extra Nodal Non Hodgkin's Lymphomas using Immunohistochemistry from a Tertiary Care Centre, Andhra Pradesh, India- A Retrospective Study

N RUKMANGADHA[1], SIVAKUMAR VULAVA[2], C SURESH PRAVEEN[3]

ABSTRACT

Introduction: Primary Extra nodal Lymphomas (pENL) are a group of lymphomas arising from tissues other than lymph nodes and even sites which normally contain no lymphoid tissue. Over the past 25 years pENLs have shown a rapid increase in incidence. The pENLs can originate from almost any anatomical site such as the gastrointestinal tract (most common), head and neck (waldeyer's ring, nose/paranasal sinuses/nasopharynx, salivary glands, etc.,), skin, Central Nervous System (CNS) etc. The definition of Extra nodal Lymphoma (ENL), particularly in the presence of both nodal and extra nodal disease remains a controversial issue. To categorise these entities, different criteria were proposed by different authors.

Aim: To study the occurrence of extra nodal non hodgkin's lymphomas in different sites/organs using Immunohistochemistry (IHC).

Materials and Methods: This was a retrospective study carried out in the Department of Pathology, Sri Venkateswara Institute of Medical Sciences (SVIMS), Tirupati, Andhra Pradesh, India, over a period of seven years six months starting from January 2010 to July 2017. Data was retrieved from the medical records and paraffin blocks were retrieved from the stores. All cases of extra nodal primary lymphomas were included in the study. All cases were classified based upon morphologic and immunophenotypic criteria according to World Health Organisation (WHO) 2008 classification. The distribution of primary extra nodal lymphomas among different age groups with mean age of occurrence was noted. Distribution of pENLs among male and female population with male to female ratio was calculated. The percentage of different sub-types of ENL and the Immunohistochemistry (IHC) markers in each subtype of ENL were calculated.

Results: In this retrospective study, a total of 317 newly diagnosed lymphoma cases were included. Among them, 79 cases were pENL. A total of 77 (24.29%) cases of primary extra nodal non hodgkin's lymphomas were included in the study. The age of the patients ranged from 4-81 years with mean age of 48.42 years, with male predominance (M:F-1.85:1). Gastrointestinal Tract (GIT) (35/77-45.45%) was the most common site, followed by head and neck (16/77-20.77%), mediastinum (4/77-5.19%), kidney (4/77-5.19%) were the common sites of occurrence of primary extra nodal NHLs. In the GIT, stomach was the most commonly involved site (24/35) followed by the small intestine (5/35), colon and anal canal (3/35) and caecum region (3/35). The Diffuse Large B-cell Lymphoma (DLBCL) was the most common histological type observed in 51/77 (66.23%), followed by Peripheral T-cell Lymphoma- Not Otherwise Specified (PTL-NOS) were observed in 14/77 (18.18%) cases, and extra nodal marginal zone lymphoma of Mucosa-associated Lymphoid Tissue (MALT) type seen in 9/77 (11.68%) cases.

Conclusion: pENLs are a diverse group of haemato-lymphoid malignancies, the incidence of which is low in India compared to rest of the world, the prognosis of which depends on the extra nodal site. The diagnosis is challenging due to the morphological mimics and varied clinical presentation and that is the reason, all should keep in mind the possibility of pENLs.

Keywords: Gastrointestinal tract, Head and neck, Lymphoma B-cell, Lymphoma T-cell, Mucosa associated lymphoid tissue

INTRODUCTION

The primary Extra nodal Lymphomas (pENL) are a group of lymphomas that arise in different extra nodal organs and tissues where lymphoid tissue is not seen. A 25% of lymphomas are extra nodal in origin [1]. The pENLs can be diagnostically challenging due to their morphological mimics, molecular alteration and clinical presentations. Over the past 25 years, pENLs have shown a rapid increase in incidence [2] especially in Central Nervous System (CNS) [3]. Gastrointestinal tract and skin, which can be attributed to immunosuppression due to Human Immunodeficiency Virus (HIV) infection or immunosuppressive treatments, infections such as *Helicobacter pylori, Chlamydia psittaci, Borrelia burgdorferi* and *Campylobacter jejuni,* autoimmune disorders and environmental factors. The trend of increase in incidence is seen particularly in developing countries like the middle-east and far-east [4].

The type of ENL varies from one site to another. They are classified into primary and secondary. Lymphomas originating from the non lymphnodal tissue are termed as pENL, whereas secondary lymphomas present first in the nodes and subsequently involves the extra nodal sites [5,6].

The pENLs can originate from almost any anatomical site such as the gastrointestinal tract (most common), head and neck (waldeyer's ring, nose/paranasal sinuses/nasopharynx, salivary glands, etc.,), skin, CNS, bone, thyroid, breast, rarely adrenal, pancreas and genitourinary tract [7]. The definition of ENL, particularly in the presence of both nodal and extra nodal disease remains a controversial issue. To categorise these entities, different criteria were proposed by different authors [6,8,9].

According to Dawson's criteria lymphoma is said to be primarily extra nodal if: 1) Absence of palpable superficial lymph nodes on first physical examination; 2) Absence of mediastinal lymphadenopathy detected by plain chest x-ray; 3) Dominant lesion at the extra nodal site; 4) Involvement of lymph nodes in the vicinity of the primary lesion; and 5) White Blood Cell count (WBC) count within the normal range [8]. Literature on extra nodal NHLs originating in almost every organ of the body is widely available, reports on pENLs as a group are limited [6].

The aim of the present study was to study the occurrence of extra nodal non Hodgkin's lymphomas in different sites/organs and to subtype the extra nodal NHL using IHC markers.

MATERIALS AND METHODS

This was a retrospective study carried out in the Department of Pathology, Sri Venkateswara Institute of Medical sciences (SVIMS), Tirupati, Andhra Pradesh, India, over a period of seven years six months starting from January 2010 to July 2017 approved by Ethical Committee with SVIMS (IEC No.794). Analysis was done in 2019. A total of 317 newly diagnosed lymphoma cases were retrieved, among them 79 were pENLs.

Inclusion criteria: The liberal definition of primary extra nodal NHLs proposed by Krol ADG et al., was adopted that includes all patients who present with NHL that apparently originated at an extra nodal site, even in the presence of disseminated disease, as long as the extra nodal component was clinically dominant [6]. The tonsil and Waldeyer's ring were included as an extra nodal site although many respects, they could be considered of nodal origin. There has been debate on whether they should be considered as nodal or extra nodal lymphoma sites but they have historically been included among the extra nodal types [10,11].

Exclusion criteria: All the histopathologically diagnosed nodal lymphomas and nodal lymphomas with extra nodal involvement and plasmacytomas were excluded from the study.

Study Procedure

Data was retrieved from the medical r ecords and paraffin blocks were retrieved from the stores. Paraffin embedded Haematoxylin and Eosin (H&E) stained tissue sections were analysed to reach at a morphological diagnosis. The IHC analysis was performed manually on the paraffin embedded tissue sections by using a panel of monoclonal antibodies (peroxidase-antiperoxidase method). Antigen retrieval was done by pretreatment of paraffin sections by heating in micro Owen in 0.01 M citrate buffer (pH 6.0) [12]. The panel of antibodies used for IHC included Pan Cytokeratin (pan CK), Leukocyte Common Antigen (LCA), Cluster of Differentiation cells (CD)3, CD5, CD19, CD20, CD23, CD10, CD43, CD15, CD30, CD99, CD56, CD138, B-cell lymphoma (Bcl) 2, Bcl6, Terminal deoxynucleotidyl transferase (Tdt), Endomysial Antibodies (EMA) and Anaplastic Lymphoma Kinase-1 (ALK-1). Paraffin tissue blocks, H&E stained section slides and IHC slides were retrieved and reviewed by three pathologists in double blinded manner. All cases were classified based upon morphologic and immunophenotypic criteria according to World Health Organisation (WHO) 2008 classification [13].

STATISTICAL ANALYSIS

All the details were recorded in the study proforma. Data was entered in Microsoft Excel 2010. All the entries were double-checked for accuracy. Continuous variables were summarised as mean±standard deviation, median (range) as appropriate. The frequency distribution of different sub-types of ENL was calculated as percentages. The frequency distribution of IHC markers in each subtype of ENL were calculated as percentages.

RESULTS

In this retrospective study, a total of 317 newly diagnosed lymphoma cases were retrieved, among them pENLs constituted (79, 24.92%), of which extra nodal NHL accounted for (77, 24.29%) were included in the study. Hodgkin lymphomas accounted for two cases which were not included. Majority of the patients were from higher age group with peak incidence seen in fifth decade of life with age range of 4-81 years with a mean age of 48.42 years [Table/Fig-1]. The study included 50 males and 27 females, with a male to female ratio of 1.85:1.

Age (years)	Number of cases (n)
1-10	1
11-20	3
21-30	8
31-40	10
41-50	18
51-60	16
61-70	12
71-80	7
81-90	2

[Table/Fig-1]: Age distribution of the study participants; N=77; mean age: 48.42 years.

Gastrointestinal tract (35/77, 45.45%) was the most common site, followed by head and neck (16/77-20.77%), Mediastinum (4/77-5.19%), kidney (4/77-5.19%), mesenteric mass, testis (3/77-3.89%) in each, ovary and skin (2/77-2.59%) in each, bone, brain, breast, paraspinal region, liver, lung, uterus and ankle (1/77-1.29%) in each were the rare anatomic sites of pENLs observed [Table/Fig-2].

Other sites	No. of cases	DLBCL	PTL-NOS	Mantle cell lymphoma	Plasmablastic lymphoma
Mediastinum	4	3	1	-	-
Breast	1	1	-	-	-
Lung	1	1	-	-	-
Liver	1	-	1	-	-
Skin	2	-	2	-	-
Paravertebral mass	1	1	-	-	-
Kidney	4	3	-	1	-
Testis	3	3	-	-	-
Ovary	2	1	1	-	-
Mesenteric mass	3	3	-	-	-
Brain	1	1	-	-	-
Ankle	1	-	1	-	-
Tibia	1	1	-	-	-
Uterus	1	1	-	-	-

[Table/Fig-2]: Distribution of different histological types of primary extra nodal NHLs in uncommon anatomical sites (n=26).
DLBCL: Diffuse large B-cell lymphoma; PTL-NOS: Peripheral T-cell lymphoma-not otherwise specified; MALT: Extra nodal marginal zone lymphoma of mucosa associated lymphoid tissue type

In the GIT, stomach was the most commonly involved site (24/35) followed by the small intestine (5/35), colon and anal canal (3/35) and caecum region (3/35) [Table/Fig-3]. In the head and neck region, nasopharynx (6/16) was most common site involved followed by tonsil and oral cavity (3/16 in each), orbit, base of tongue, postcricoid, and scalp (1/16 in each) [Table/Fig-4].

Gastrointestinal tract	No. of cases	DLBCL	PTL-NOS	MALT lymphoma	Mantle cell lymphoma
Oesophagus	0	-	-	-	-
Stomach	24	12	4	8	-
Duodenum	1	1	-	-	-
Jejunum	0	-	-	-	-
Ileum	4	3	-	1	-
Caecum	3	2	1	-	-
Colon	2	2	-	-	-
Anal canal	1	-	1	-	-

[Table/Fig-3]: Anatomical distribution of different histological types of primary extra nodal NHLs in gastrointestinal tract (n=35).
DLBCL: Diffuse large B-cell lymphoma; PTL-NOS: Peripheral T-cell lymphoma not otherwise specified; MALT: Extra nodal marginal zone lymphoma of mucosa associated lymphoid tissue type

Head and neck	No. of cases	DLBCL	Anaplastic large cell lymphoma	PTL-NOS	Plasmablastic lymphoma
Nasopharynx	6	5	1	-	-
Oral cavity	3	2	-	1	-
Tongue	1	-	-	1	-
Tonsil	3	3	-	-	-
Postcricoid	1	1	-	-	-
Orbit	1	1	-	-	-
Scalp	1				1

[Table/Fig-4]: Anatomical distribution of different histological types of primary extra nodal NHLs in head and neck n=16.
DLBCL: Diffuse large B-cell lymphoma; PTL-NOS: Peripheral T-cell lymphoma-not otherwise specified

On IHC, 62/77 (80.51%) of pENLs had B immunophenotype whereas T-cell phenotype was observed in 15/77 (19.48%) patients. Diffuse large B-cell lymphoma, not otherwise specified (DLBCL) was the most common histological type observed in 51/77 (66.23%), followed by PTL NOS were observed in 14/77 (18.18%) cases, and Extra nodal marginal zone lymphoma of MALT type seen in 9/77 (11.68%) cases [Table/Fig-2-4], among them, two cases were associated with *H.pylori* in gastric biopsy. Seropositivity for HIV was seen in a case of plasmablastic lymphoma involving the scalp. One case of T-cell lymphoma was observed in small intestine in which the patient had history of renal transplant. Mantle cell lymphoma and anaplastic large cell lymphoma involving the kidney and nasopharynx were observed in one patient each, respectively [Table/Fig-5].

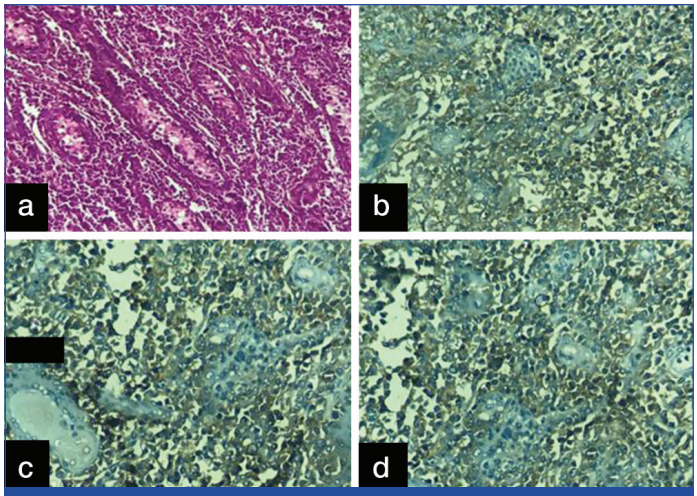
The histopathological features of primary diffuse large B-cell lymphoma of testis, uterus endometrium and of the uterus ovary are shown in [Table/Fig-6-8], respectively. Primary Anaplastic large cell lymphoma of Nasopharynx has been depicted in [Table/Fig-9]. The features of Primary Peripheral T-cell lymphoma NOS of liver, primary diffuse large B-cell lymphoma of colon, MALT Lymphoma ileum, primary peripheral T-cell lymphoma NOS of stomach and primary mantle cell lymphoma of kidney are illustrated in [Table/Fig-10-14].

DISCUSSION

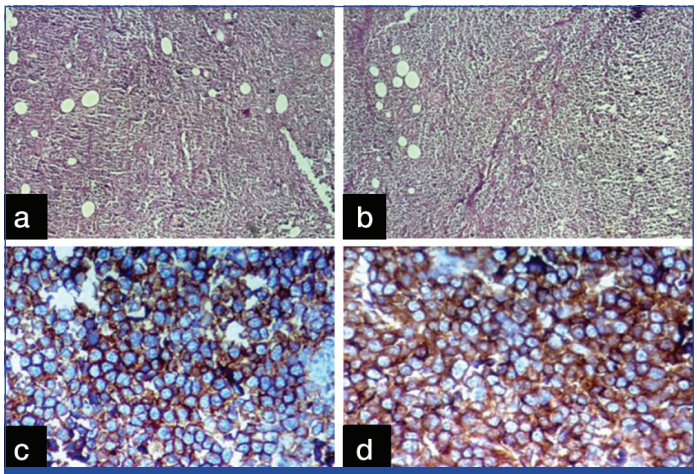
Extra nodal Non Hodgkin Lymphomas (NHLs) are heterogeneous disorders in relation to geography, ethnicity, anatomic, aetiological

Site	Histopathological diagnosis	CD3 and CD5	CD 19 and CD20	CD 10	CD 79a	CD 43	BCL 2	BCL 6	CD 15	CD 30	ALK-1
Stomach	DLBCL-12 cases	1-ve	12+ve	4+ve	12+ve	12-ve	1+ve	3+ve	12-ve	12-ve	12-ve
	PTL-NOS-4 cases	4+ve	1-ve	1-ve	1-ve	1-ve	1-ve	1-ve	1-ve	1-ve	1-ve
	MALT lymphoma-8 cases	8-ve	8+ve	4+ve	8+ve	8+ve	1+ve	8-ve	8-ve	8-ve	1-ve
Duodenum	DLBCL-1 case	1-ve	+ve	1-ve	+ve	1-ve	1-ve	1-ve	1-ve	1-ve	1-ve
Ileum	DLBCL-3 cases	1-ve	3+ve	2+ve	3+ve	3-ve	1+ve	3-ve	3-ve	3-ve	1-ve
	MALT lymphoma-1 case	1-ve	1+ve	+ve	1-ve	+ve	1-ve	1-ve	1-ve	1-ve	1-ve
Colon	DLBCL-2 cases	2-ve	2+ve	1+ve	2+ve	2-ve	2+ve	1-ve	2-ve	2-ve	1-ve
Anal canal	PTL-NOS-1 case	1+ve	1-ve	1-ve	1-ve	1-ve	1-ve	1-ve	1-ve	1-ve	1-ve
Nasopharynx	DLBCL-5 cases	5-ve	5+ve	5+ve	5+ve	5-ve	3+ve	5+ve	5-ve	5-ve	1-ve
	Anaplastic large cell lymphoma-1 case	1+ve	1-ve	1-ve	1-ve	1-ve	1-ve	1-ve	1-ve	1-ve	1+ve
Oral cavity	DLBCL-2 cases	2-ve	2+ve	2+ve	2+ve	2-ve	2-ve	2+ve	2-ve	2-ve	1-ve
	PTL-NOS-1 case	1+ve	1-ve	1-ve	1-ve	1-ve	1-ve	1-ve	1-ve	1-ve	1-ve
Tongue	PTL-NOS-1 case	1+ve	1-ve	1-ve	1-ve	1-ve	1-ve	1-ve	1-ve	1-ve	1-ve
Tonsil	DLBCL-3 cases	3-ve	3+ve	3+ve	3+ve	3-ve	1+ve	3+ve	3-ve	3-ve	1-ve
Post cricoid	DLBCL-1 case	1-ve	1+ve	1+ve	1+ve	1-ve	1-ve	1+ve	1-ve	1-ve	1-ve
Orbit	DLBCL-1 case	1-ve	1+ve	1+ve	1+ve	1-ve	1-ve	1+ve	1-ve	1-ve	1-ve
Scalp	Plasmablastic lymphoma-1 case	1-ve	1-ve	1+ve	1+ve	1+ve	1-ve	1-ve	1-ve	1-ve	1-ve
Mediastinum	DLBCL-3 cases	3-ve	3+ve	3+ve	3+ve	3-ve	3-ve	3+ve	3-ve	3-ve	1-ve
	PTL-NOS-1 case	1+ve	1-ve	1-ve	1-ve	1-ve	1-ve	1-ve	1-ve	1-ve	1-ve
Breast	DLBCL-1 case	1-ve	1+ve	1+ve	1+ve	1-ve	1-ve	1+ve	1-ve	1-ve	1-ve
Lung	DLBCL-1 case	1-ve	1+ve	1+ve	1+ve	1-ve	1-ve	1+ve	1-ve	1-ve	1-ve
Liver	PTL-NOS -1 case	1+ve	1-ve	1-ve	1-ve	1-ve	1-ve	1-ve	1-ve	1-ve	1-ve
Skin	PTL-NOS -2 cases	2+ve	2-ve	2-ve	2-ve	2-ve	2-ve	2-ve	2-ve	2-ve	1-ve
Paravertebral mass	DLBCL-2 cases	2-ve	2+ve	2+ve	2+ve	2-ve	2+ve	2+ve	2-ve	2-ve	1-ve
Kidney	DLBCL-3 cases	2-ve	2+ve	2+ve	2+ve	2-ve	1+ve	1+ve	2-ve	2-ve	1-ve
	Mantle cell lymphoma-1 case	1-ve	1+ve	1-ve	1+ve	1+ve	1+ve	1-ve	1-ve	1-ve	-ve
Testis	DLBCL-3 cases	3-ve	3+ve	3+ve	3+ve	3-ve	2+ve	3+ve	3-ve	3-ve	1-ve
Ovary	DLBCL-1 case	1-ve	1+ve	1+ve	1+ve	1-ve	1-ve	1+ve	1-ve	1-ve	1-ve
	PTL-NOS-1 case	1+ve	1-ve	1-ve	1-ve	1-ve	1-ve	1-ve	1-ve	1-ve	1-ve
Mesenteric mass	DLBCL-3 cases	3-ve	3+ve	3+ve	3+ve	3-ve	1+ve	2+ve	3-ve	3-ve	1-ve
Brain	DLBCL-1 case	1-ve	1+ve	1+ve	1+ve	1-ve	1+ve	1+ve	1-ve	1-ve	1-ve
Ankle	PTL-NOS-1 case	1+ve	1-ve	1-ve	1-ve	1-ve	1-ve	1-ve	1-ve	1-ve	1-ve
Tibia	DLBCL-1 case	1-ve	1+ve	1+ve	1+ve	1-ve	1-ve	1+ve	1-ve	1-ve	1-ve
Uterus	DLBCL-1 case	1-ve	1+ve	1+ve	1+ve	1-ve	1-ve	1+ve	1-ve	1-ve	1-ve

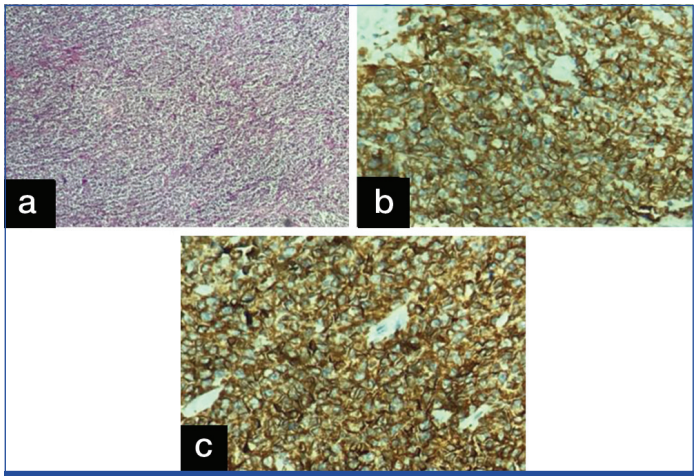
[Table/Fig-5]: Immunohistochemical (IHC) markers in various primary extra nodal NHLs.
†CD138-Positive in Plasmablastic lymphoma; ‡Cyclin -D1- Positive in mantle cell lymphoma; §ALK-1-Positive in Anaplastic large cell lymphoma. 3 positive, 5 positive are the number of cases showing positive staining with the particular IHC marker. NHLs: Non Hodgkin's lymphomas*; DLBCL: Diffuse large B-cell lymphoma; PTL-NOS: Peripheral T-cell lymphoma not otherwise specified; MALT: Extra nodal marginal zone lymphoma of mucosa associated lymphoid tissue type; CD: Cluster of differentiation; BCL: B-cell lymphoma; ALK-1: Anaplastic lymphoma kinase-1



[Table/Fig-6]: Primary Diffuse Large B-cell lymphoma of testis; a) showing diffuse sheets of medium to large lymphoid cells infiltrating the testicular parenchyma encasing the seminiferous tubules (H&E 100X); b) the cells show intense cytoplasmic and membranous positivity for CD79a (IHC 100X); c) the cells show intense cytoplasmic and membranous positivity for CD19 (IHC 100X); d) the cells show intense cytoplasmic and membranous positivity for CD20 (IHC 100X).

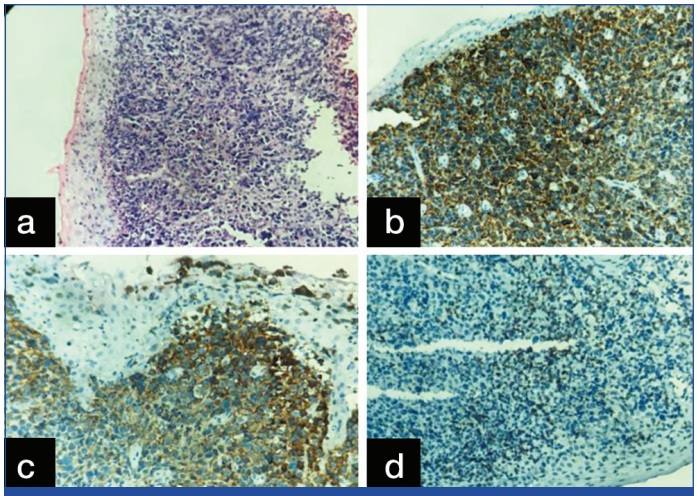


[Table/Fig-7]: Primary Diffuse Large B-cell lymphoma of uterus endometrium; a,b) Showing diffuse sheets of medium to large lymphoid cells (H&E 40X); c) The cells show moderate cytoplasmic and membranous positivity for CD19 (IHC 400X); d) The cells show diffuse intense cytoplasmic and membranous positivity for CD20 (IHC 200X).

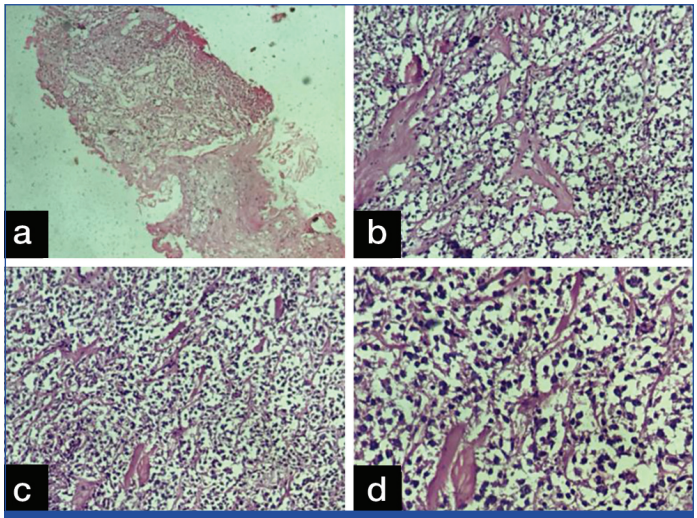


[Table/Fig-8]: Primary Diffuse Large B-cell lymphoma of uterus; a,b) Showing diffuse sheets of medium to large lymphoid cells (H&E 100X); c) The cells show diffuse intense cytoplasmic and membranous positivity for CD19 (IHC 200X).

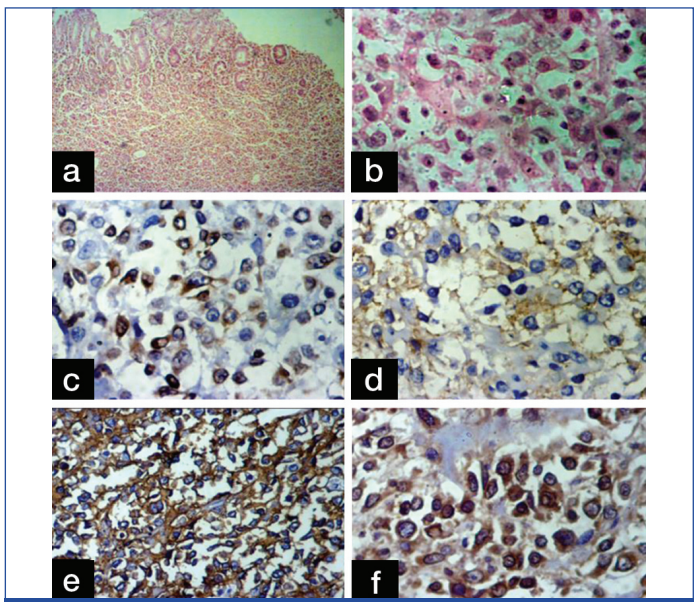
and morphological diversities [14]. The male to female ratio in the present study was 1.85:1, majority of the studies reported male predominance [7,15-18]. The peak incidence was seen in the fourth and fifth decades which was similar in some studies [15,16], while other studies reported younger age incidence in fourth and fifth decades [17,18]. The incidence of ENL tends to be high when the



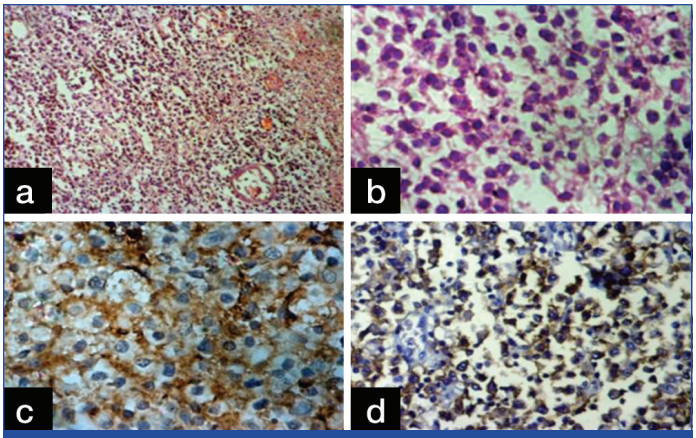
[Table/Fig-9]: Primary Anaplastic large cell lymphoma of Nasopharynx; a) Showing pleomorphic neoplastic lymphoid cells displaying nuclear hyperchromasia, infiltrating the lining squamous epithelium (H&E 100X); b) Cells show diffuse intense cytoplasmic and membranous positivity for CD3 (IHC 200X); c) Cells showing diffuse intense cytoplasmic and membranous positivity for CD5 (IHC 200X); d) Cells showing cytoplasmic positivity for ALK-1 (IHC 200X).



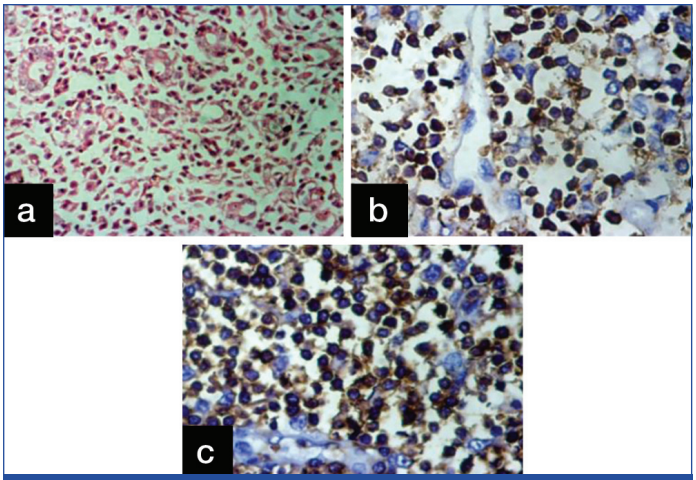
[Table/Fig-10]: a) Primary Peripheral T-cell lymphoma NOS of Liver (H&E 100X) on IHC showed positivity for CD3 and CD5; b-d) Primary Diffuse Large B-cell lymphoma of Brain showing diffuse sheets of medium to large lymphoid cells (H&E200X). On IHC showed positivity for CD19, CD20, CD10, CD79a, BCL2 and BCL6.



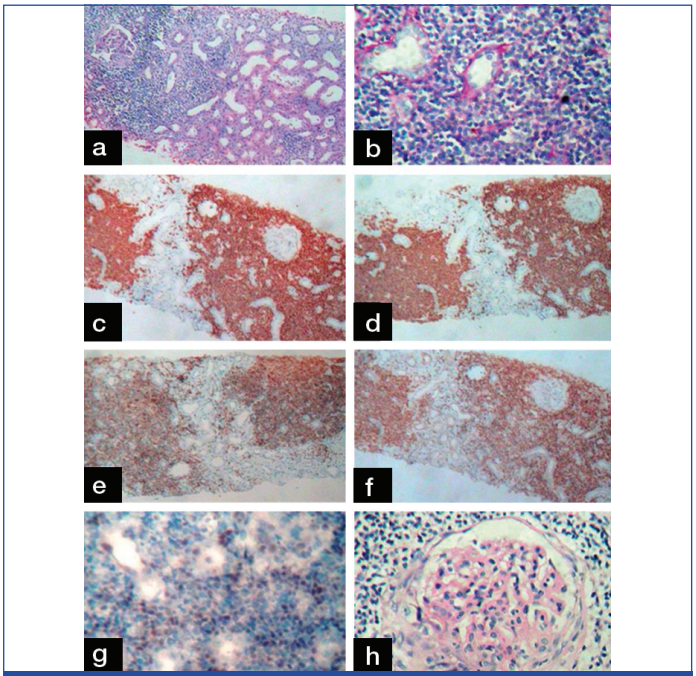
[Table/Fig-11]: Primary Diffuse Large B-cell lymphoma of colon; a) Showing diffuse sheets of medium to large lymphoid cells infiltrating lamina propria and mucosal glands (H&E 40X); b) Neoplastic cells showing anisonucleosis with many cells showing prominent nucleoli (H&E 400X); c) Cells show intense membranous positivity for CD10 (IHC 400X); d) Cells show moderate membranous positivity for CD19 (IHC 400X); e) Cells showing intense membranous positivity for CD20 (IHC 400X); f) Cells show nuclear and cytoplasmic positivity for BCL2 (IHC 400X).



[Table/Fig-12]: MALT Lymphoma ileum; a) Showing diffuse sheets of small to medium sized neoplastic lymphoid cells infiltrating lamina propria and mucosal glands (H&E 100X); b) Neoplastic cells showing pleomorphic nuclei, condensed chromatin, inconspicuous nucleoli (H&E 400X); c,d) Cells show positivity for CD19 and CD20 (IHC 400X).



[Table/Fig-13]: Primary Peripheral T-cell lymphoma NOS of stomach; a) Showing diffuse infiltrates of small to medium sized neoplastic lymphoid cells infiltrating lamina propria and mucosal glands (H&E 100X); b,c) Cells show positivity for CD3 and CD5 (IHC 400X).



[Table/Fig-14]: Primary Mantle cell lymphoma of kidney; a) Core biopsy showing glomeruli, tubules and diffusely infiltrating neoplastic lymphoid cells (H&E 100X); b) Showing monomorphic small to medium sized neoplastic lymphoid cells with inconspicuous nucleoli (H&E 400X); c) Neoplastic cells show membranous and cytoplasmic positivity for LCA (IHC 100X); d) Cells show membranous and cytoplasmic positivity for CD20 (IHC 100X); e) Cells show membranous and cytoplasmic positivity for CD5 (IHC 100X); f) Cells show cytoplasmic positivity for BCL2 (IHC 100X); g) Cells showing nuclear positivity for Cyclin D1 (IHC 100X); h) Crescentic glomerulonephritis, glomeruli showing cellular crescents (H&E 400X).

incidence of lymphomas are high, the incidence was particularly high in France (42%) and Kuwait (43%) [19]. The incidence of extra nodal NHLs varies greatly between countries, approximately 25-40% of NHL patients present with pENLs in developing countries. In developed countries the incidence was high in USA (24%), Canada (27%), Israel (36%), Lebanon (44%), Denmark (37%) and Holland (41%) [20]. In the present study, the incidence of extra nodal non Hodgkin's lymphomas was 77 (24.29%), nearly similar incidence was reported in some studies (26.9%, 22%, 22.6% 24%, 23% [15,17,18,21,22] some studies reported high incidence (54.7%, 46.6% 87.8%) [16,20,23]. In the present study, the most common site of occurrence for primary extra nodal NHLs was GIT 35 (45.45%) followed by head and neck 16 (20.77%) followed by mediastinum, kidney, testis, skin and others. The GIT was the common site in many studies (30.4%, 62.5%, 43%, 87.8%) [20-23]. CNS was the most common site reported by few authors (20.3%, 29.5%) [16,17] some reported head and neck as the most common site [7,18,24-26]. One possible explanation for head and neck being the most common site could be due to usage of agricultural pesticides, environmental pollutants, poor dental hygiene and tobacco chewing [27,28].

In the GIT, the most common site was stomach similar to reports in the literature [17,18,21,23,29]. Mishra P et al., reported ileum as the most common site followed by stomach [18]. [Table/Fig-15] shows comparison of epidemiological, histological features, sites of occurrence of pENLs between the present study and published literature [7,15-18,20-25,29]. Among the 77 cases of primary extra nodal non Hodgkin's lymphomas reported in the present study, 62 cases (80.51%) accounted for B-cell immunophenotype. Among them, DLBCL was the most common subtype accounting for 51 (66.23%) and was the most common subtype in GIT, head and neck, kidney and testis which was similar in many studies in the literature (62.9%, 69%, 54% 56.7%, 67.7%, 71%, 58.6%, 31%) [Table/Fig-15] [16-18,20-24].

The second most common subtype among the B-cell immunophenotypes was MALT lymphomas accounting for 9 (11.68%) cases and it was the third most common subtype following DLBCL and PTL-NOS among all cases and it was the second most common subtype noted in GIT in the present study [Table/Fig-11-13] also reported in many studies (1.42% 12%, 13.41%, 19.79%, 7.2%, 10.5%) [Table/Fig-15] [16,18,20-23]. Out of the 9 MALT lymphomas, 2 (22.22%) cases were shown to have association with *Helicobacter pylori*. Study done by Arora N et al., reported *H.pylori* in 44% of MALT lymphomas cases and other reported literature accounts for 92 [29].

In the present study, follicular lymphomas were distinctively absent from extra nodal sites. Geographic variation in molecular expression profiling in follicular lymphomas as well as between nodal and extra nodal sites may be possible explanations for complete absence of this entity in the present study and more over high grade transformation of pre-existent low-grade MALT or follicular lymphoma into DLBCL at extra nodal sites might be also another explanation for this observation [7,30].

T-cell phenotype was observed in 15/77 (19.48%) patients and they were the second most common among primary extra nodal NHLs. Among them, 14 cases were peripheral T-cell lymphomas NOS [Table/Fig-5,10,13] and the remaining one case was Anaplastic large cell lymphoma [Table/Fig-5,9]. In study done by Yang QP et al., peripheral T-cell lymphoma NOS was the most common type in different parts of China similar to the present study and Extra nodal Natural Killer T-cell Lymphoma (ENKTCL) was common in Hong Kong [7]. Padhi S et al., has reported eight T-cell lymphomas only in skin [17].

Swami R et al., reported T-cell lymphomas as the second most common similar to the present study [15]. Mishra P et al., reported 13% of PTL NOS, 3% of ENKTCL and 2% of ALCLs [18]. The PTL NOS occuring at different sites like bone, soft tissue, waldeyer's ring, small intestine and ENKTCLs at different sites were reported by Fujita A et al., [20]. Farooq S et al., reported only two cases of PTL NOS out of 96 cases of pENLs [Table/Fig-15] [21].

S. No.	Author's name and year of publication	Place of study	Total study period in years	ENLs n	NHLs n (%)	Parameters compared				
						Age range (Mean age) years	M:F	Anatomic sites		Histological subtypes MC
								Common	Uncommon	
1	Yang QP et al., [7] 2011,	China	9	5549	2968 (53.5)	1-90 (50)	1.8:1	WR (23.7) GIT (22.3) NPNS (20.4) Skin (11.3)	CNS, Orbit, Bone, Thyroid, Salivary gland, Testes, Breast	DLBCL ENKTCL MALT
2	Swami R et al., [15] 2020	North India	1	63	17 (26.9%)	5-92	2.4:1	GIT (23) Retroperitoneum (23, Mediastinum (15)	Neck, breast, skin CNS, lung	DLBCL
3	Mehta J et al., [16] 2016	North India	2	128	70 (54.7%)	4-89	3:1	GIT (20) CNS (18.6) Head and Neck (17)	Bone marrow Urinary bladder	DLBCL PBL SLL
4	Padhi S et al., [17] 2012	South India	5	308	68 (22)	6-75 (43)	2:1	CNS (29.5) GIT (25) NPNS (11.8)	Bone, Testes Spleen, vulva, Lacrimal gland, Kidney	DLBCL, MALT B-NHL (U), IPSID, PEL, MF
5	Mishra P et al., [18] 2015	South India	3	300	68 (22.6)	2-75	1.3:1.	Head and neck (37) GIT (29) Soft tissue (9) Orbit (9)	Palate, Breast Bone, Thyroid Adrenal, Ovary	DLBCL PTCL Maltoma
6	Fujita A et al., [20] 2009	Japan	7	847	395 (46.6)	15-92 .	-	GIT (30.4) WR (17.8) Orbit (7.0)	Bone, Thyroid, Skin, Prostate, Testis, CNS, Breast, Uterus	DLBCL MALT
7	Farooq S et al., [21] 2014	North India	5	400)	96 (24)	12-76 (44.5)	2:1	GIT (62.5) WR (29.16)	Nasopharynx, testis, CNS, orbit, breast, thyroid	DLBCL MALT
8	Al Shemmari SH et al., [22] 2008	Kuwait	5	422	97 (23%)	-	-	GIT (43) Head and neck (14.4)	Kidney, Ovary Testis, Brain Lung	DLBCL Follicular MALT
9	Temmim L et al., [23] 2004	Kuwait	16	-	935	0-60	2.1: 1)	Adult; Stomach: (19.7) Skin (7.8) Small intestine: (9.8) Paediatric Bowel (50.0) Pharynx: (12.3) Ovary: (9.6)	Cervix/Vagina Heart, Breast Urinary bladder	Adults-DLBCL, Mycosis fungoides Paediatric -BL, Tlymphoblasticlymphoma
10	Aravind S et al., [24] 2017 (Head and Neck lymphomas)	South India	3	-	16	21-100 (60.6)	1.28:1	Maxilla (25%), Nasal cavity paranasal sinus (19%), orbit (19%),	-	DLBCL, follicular lymphoma,. NK/Tcell nasal type extra nodal Lymphoma.
11	Singh D et al., [25], 2003	North India	3	241	106 (44)	-	-	Head and Neck (50.9) GIT (27.4)	Brain, Skin Bone, Testis, UB	DLBCL
12	Arora N et al., [29] 2011 (GIT lymphomas)	South India	10	-	361	3–88 (45)	3.93:1	Stomach (53.57) Small intestine (23.51) Ileo cecum(10.12)	Colon, Rectum	DLBCL MALT BL
13	Present Study	South India	7 years 6 months	317	77, (24.29%)	4-81 (48.42)	1.7:1.	GIT (45.45), Head and neck (20.77), Mediastinum (5.19), kidney (5.19),	Ovary, skin bone, brain, breast, paraspinal region, liver, lung, uterus	DLBCL PTL NOS MALT

[Table/Fig-15]: Comparison of epidemiological, histological features, sites of occurrence of pENLs between the present study and published literature [7,15-18,20-25].
ENL: Extra nodal non hodgkin lymphoma; n: Number of patients studied; WR: Waldeyer's ring; GIT: Gastrointestinal tract including stomach, small and large intestine; NPNS: Nose, nasopharynx, and paranasal sinus; CNS: Central nervous system including brain and spinal cord; MC; Most common; DLBCL: Diffuse large B-cell lymphoma; FL: Follicular lymphoma; SLL: Small lymphocytic lymphoma; ENKTCL: Extra nodal natural killer/T-cell lymphoma of nasal type; MALT: Extra nodal marginal zone lymphoma of mucosa associated lymphoid tissue type; PTCL: Peripheral T-cell lymphoma; BL: Burkitt's lymphoma; ATLL: Adult T-cell lymphoma/leukaemia; B-NHL: B-cell non hodgkin lymphoma morphologically intermediate between burkitt lymphoma and DLBCL; IPSID: Immunoproliferative small intestinal disease; PEL: Primary effusion lymphoma involving pleural cavity; MF: Mycosis fungoides

According to WHO, primary renal lymphomas account for <1% of pENLs and the most common histological subtype being DLBCL. The present study reported 4 (5.19%) cases of primary extra nodal NHLs of which three were DLBCLs and one was mantle cell lymphoma [Table/Fig-14] similar to the given literature [16]. Primary mediastinal lymphomas account for 10% of lymphomas [31]. The most common primary mediastinal lymphomas are Hodgkin's Lymphoma (HL), Primary Mediastinal B-Cell Lymphoma (PMBCL) and T-Lymphoblastic Lymphoma (TLL) [32]. In the present study, 4 (5.19%) cases were mediastinal lymphomas of which 3 were DLBCLs, and one was PTL NOS. Mishra P et al., reported three (4%) mediastinal lymphomas of which two were DLBCLs and one was lymphoblastic lymphoma [18]. The present study reported 1 case of PTL NOS in the small intestine of post renal transplant patient. Arora N et al., reported nine cases of post-transplant lymphoproliferative disorders of them four cases were located in stomach and four in small intestine and one case was multifocal in GIT [29].

Primary bone lymphomas account for 3-5% of pENLs and majority are intermediate to high grade B-cell lymphomas [33]. The present study reported 1 (1.26%) case of DLBCL arising in bone similar to study done by Mishra P et al., (1.2%), Padhi S et al., (2 cases-3%) [17,18]. Higher number of primary bone lymphomas were reported by Al Shemmari SH et al., (6 cases-6%), Fujita A et al., (18 cases- 4.6%), Mehta J et al., (7 cases-7%) and Temmim L et al., [16,20,22,23].

In the present study, one case of plasmablastic lymphoma in the scalp with retroviral positivity was reported. Aravind S et al., reported a single case of plasmablastic lymphoma in maxilla with retroviral positivity [24]. Usually, plasmablastic lymphomas arise at the back drop of HIV infection. Study by Mehta J et al., reported nine cases of Plasmablastic lymphomas of them four arising in CNS, three in bone and one each in soft tissue and head and neck [Table/Fig-5,15] [16].

Different sites such as ovary, ankle, testis, breast, uterus, lung, skin, liver, orbit and paravertebral region were reported, majority of the

cases were DLBCLs except for PTL NOS occurring in liver, ovary and skin in the present study.

Limitation(s)

The present study was a morphological and epidemiological study, data regarding clinical presentation, disease stage, treatment received and outcomes were not included. Associated co-morbid conditions, occupation of patients, haematological, biochemical parameters were not included. Another drawback of the study was comparison with paediatric group was lacking due to very less number. Molecular diagnostic techniques such as cytogenetics/ fluorescence in-situ hybridisation were not performed in any of the cases due to lack of facilities.

CONCLUSION(S)

The pENLs are a diverse group of haemato-lymphoid malignancies, Non Hodgkin's lymphomas were the most pENLs the incidence of which is low in India compared to rest of the world, the prognosis of which depends on the extra nodal site. Gastrointestinal tract and Head and Neck lymphomas are the first and second common extra nodal sites noted. There was not much differences noted regarding the gender predilection, incidence and morphology compared to other studies. All the patients were immunocompetent. The diagnosis of pENLs can be challenging due to the morphological mimics and varied clinical presentation and that is the reason all of us should keep in mind the possibility of pENLs. In future, indepth studies with addition of genetic profile and therapeutic outcome should be conducted from time to time in order to understand the biology of these tumours.

REFERENCES

[1] Zucca E, Roggero E, Bertoni F, Conconi A, Cavalli F. Primary extranodal non-Hodgkin's lymphomas. Part 2: Head and neck, central nervous system and other less common sites. Ann Oncol. 1999;10(9):1023-33.
[2] Jemal A, Tiwari RC, Murray T. Cancer statistics. CA Cancer J Clin. 2004;54(1):08-29.
[3] Mahhdoomi R, Nayil K, Rayees A, Kirmani A, Ramzan A, Khalil MB, et al. Primary CNS lymphoma in immunocompetent: A review of literature and our experience from Kashmir. Turk Neurosurg. 2011;21(1):39-47.
[4] Yaqo RT, Hughson Md, Sulayvani FK, Al-Allwai NA. Malignant lymphoma in Northern Iraq: A retrospective analysis of 270 cases according to the World Health Organisation classification. Ind J Cancer. 2011;48(4):446-51.
[5] Rehman SA, Sidique M, Ahmad G, Naiz M. Extra nodal lymphoma. Prof Med J. 2005;12(3):223-29.
[6] Krol ADG, le Cessie S, Snijder S, Kluin-Nelemans JC, Kluin PM, Noordijk EM. Primary extranodal non-Hodgkin's lymphoma (NHL): The impact of alternative definitions tested in the comprehensive cancer centre West population-based NHL registry. Ann Oncol. 2003;14(1):131-39.
[7] Yang QP, Zhang WY, Yu JB, Zhao S, Xu H, Wang WY, et al. Subtype distribution of lymphomas in Southwest China: Analysis of 6,382 cases using WHO classification in a single institution. Diagnostic Pathology. 2011;6:77. Doi: 10.1186/1746-1596-6-77.
[8] Dawson IM, Cornes JS, Morson BC. Primary malignant lymphoid tumours of the intestinal tract. Report of 37 cases with a study of factors influencing prognosis. Br J Surg. 1961;49:80-89. Doi: 10.1002/bjs.18004921319.
[9] The Non-Hodgkin's Lymphoma Classification Project. A clinical evaluation of the International Lymphoma Study Group classification of non-Hodgkin's lymphoma. Blood. 1997;89(11):3909-18.
[10] Cavalli F. Extranodal lymphomas. In Magrath IT (ed): The Non-Hodgkin's Lymphomas (2nd edn). London: Arnold. 1997;1007-27.
[11] Chan JKC, Ng CS, Lo STH. Immunohistological characterization of malignant lymphomas of the waldeyer's ring other than the nasopharynx. Histopathology. 1987;11(9):885-99.
[12] Lillie RD. Histopathologic Technique and Practical Histochemistry (3rd ed). McGraw-Hill, New York, 1965.
[13] Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H, et al. (editors). World Health Organisation Classification of Tumours of Haematopoietic and Lymphoid Tissues, 4th Edn. IARC, Lyon, France, (2008) Pp.10.
[14] Anderson JR, Armitage JO, Weisenburger DD. Epidemiology of the non-Hodgkin's lymphomas: Distributions of the major subtypes differ by geographic locations. Non-Hodgkin's Lymphoma Classification Project. Ann Oncol. 1998;9(7):717-20.
[15] Swami R, Singh S, Singh P, Mani NS, Karandikar MN. Extranodal non-Hodgkin's lymphoma: A case series at a tertiary care hospital. IP Archives of Cytology and Histopathology Research. 2020;5(4):302-05.
[16] Mehta J, Thakrar MP, Meena M, Mittal A, Gupta K. Primary extranodal non-hodgkin lymphoma: A 2-year retrospective analysis from a tertiary care centre in Rajasthan. Int J Med Res Prof. 2016;2(4):76-80.
[17] 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 Pacific J Cancer Prev. 2012;13(10):4889-95.
[18] Mishra P, Das S, Kar R, Jacob SE, Basu D. Primary extranodal non-Hodgkin lymphoma: A 3-year record-based descriptive study from a tertiary care centre in Southern India. Indian J Pathol Microbiol. 2015;58(3):296-300.
[19] Newton R, Ferlay J, Beral V, Devesa SS. The epidemiology of Non-Hodgkin's lymphoma: Comparison of nodal and extra-nodal sites. Int J Cancer. 1997;72(6):923-30.
[20] Fujita A, Tomita N, Fujita H, Motohashi K, Hyo R, Yamazaki E, et al. Features of primary extranodal lymphoma in Kanagawa: A human T-cell leukemia virus type 1 nonendemic area in Japan. Medical Oncology. 2009;26(1):49-54.
[21] Farooq S, Reshi R, Hassan Z, Nazir N, Beigh A, Abass F. Histopathological spectrum of primary extranodal non hodgkin's lymphomas in kashmir valley: A 5 years study. Int J Biol Med Res. 2014;5(4):4654-59.
[22] Al Shemmari SH, Ameen RM, Sajnani KP. Extranodal lymphoma: A comparative study. Hematology. 2008;13(3):163.
[23] Temmim L, Baker H, Amanguno H, Madda JP, Sinowatz F. Clinicopathological features of extranodal lymphomas: Kuwait experience. Oncology. 2004;67:382-89.
[24] Aravind S, Sangeetha KN, Sreejyothi HK, Vineetha R, Chandran KN. Primary extranodal lymphomas in the head and neck region: A retrospective study from a tertiary cancer centre in south India. International Journal of Recent Scientific Research. 2017;8(3):16245-48.
[25] Singh D, Kumar L, Goyal H, Raina V, Bijlani L, Wadhwa J, et al. Primary extranodal non-Hodgkin's lymphoma in Northern India. Proc Am Soc Clin Oncol. 2003;22:2457.
[26] Chen W, Tsai W, Chao T. The clinicopathological analysis of 303 cases with malignant lymphoma classified according to the World Health Organisation classification system in asingle institute of Taiwan. Ann Hematol. 2010;89(6):553-62.
[27] Vose J, Armitage J, Weisenburger D. International peripheral T-cell and natural killer/T-cell lymphoma study: Pathology findings and clinical outcomes. J Clin Oncol. 2008;26(25):4124-30.
[28] Ko OB, Lee DH, Kim SW, Lee JS, Kim S, Huh J, et al. Clinicopathologic characteristics of T-cell non-Hodgkin's lymphoma: A single institution experience. Korean J Intern Med. 2009;24(2):128-34.
[29] Arora N, Manipadam MT, Pulimood A, Ramakrishna BS, Chacko A, Kurian SS, et al. Gastrointestinal lymphomas: Pattern of distribution and histological subtypes: 10 years experience in a tertiary centre in South India. Indian J Pathol Microbiol. 2011;54(4):712-19.
[30] Biagi JJ, Seymour JF. Insight into the molecular pathogenesis of follicular lymphoma arising from analysis of geographic variation. Blood. 2002;99(12):4265-75.
[31] Duwe BV, Sterman DH, Musani AI. Tumours of the mediastinum. Chest. 2005;128(4):2893-909.
[32] Strollo DC, Rosado-de-Christenson ML, Jett JR. Primary mediastinal tumours: Part II. Tumours of the middle and posterior mediastinum. Chest. 1997;112(2):1344-57.
[33] Boddie AW Jr, Mullins JD, West G, Bouda D. Extranodal lymphoma: Surgical and other therapeutic alternatives. Curr Probl Cancer. 1982;6(9):01-64.

PARTICULARS OF CONTRIBUTORS:
1. Professor, Department of Pathology, Sri Venkateswara Institute of Medical Sciences, Tirupati, Andhra Pradesh, India.
2. Assistant Professor, Department of Pathology, Sri Venkateswara Institute of Medical Sciences, Tirupati, Andhra Pradesh, India.
3. Consultant Pathologist, Department of Laboratory Medicine, Amara Hospital, Tirupati, Andhra Pradesh, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Sivakumar Vulava,
#5, Old Assistant Professors Quarters, Block 1, Svims Campus,
Tirupati, Andhra Pradesh, India.
E-mail: sivavulava@gmail.com

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