

## Clinicopathological Profile and Immunohistochemical Expression of Primary Extranodal Non-Hodgkin Lymphoma in Tertiary Care Centre in Eastern India: 3 year Study

Dr. Bhawna Bhutoria Jain<sup>1</sup>, Dr Ayandip Nandi<sup>2\*</sup>, Uma Banerjee<sup>3</sup><sup>1</sup>Associate Professor, <sup>2</sup>Assistant professor, <sup>3</sup>Professor & HOD, Department of pathology, Medical College, Kolkata India

\*Corresponding author: Dr. Ayandip Nandi

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## Abstract

## Original Research Article

**Background:** Non-hodgkin Lymphoma (NHL) is a common killer throughout the world and a major percentage occur in extranodal tissue. Primary extranodal NHL has a varied presentation and is frequently associated with worse prognosis. Our study aims to evaluate clinicopathological profile of primary extranodal NHL presenting in our Institute over a period of 3years and study their immunohistochemical expression. **Material/Methods:** Total 223 cases of NHL during the period of January 2016 till December 2018 were evaluated retrospectively. Routine histology and complete panel of immunohistochemical analysis were done inside institute. Patient's records were evaluated for haematological, biochemical, radiological, clinical presentation and staging. **Results:** 42.1% of all NHLs were extranodal, mean age of population were 54.2years. Male: Female ratio being 1.8:1. Most common age group affected was 6<sup>th</sup> decade, commonest histological pattern being diffuse large B cell Lymphoma (DLBCL). Most common site affected was gastrointestinal tract, of which commonest site of presentation was stomach. B cell: T cell lymphoma ratio was 4.53:1. Bone marrow involvement was seen in 6.3% of cases. Most of the patients had Ann arbor stage II. **Conclusion:** Extranodal NHL has a rising trend in developing countries. Due to improved diagnostic techniques many of them are being diagnosed early but needs targeted/site specific algorithm for adequate management. Studies/specific trials are lacking regarding zonal distribution, management and survival analysis. A holistic approach may help in containment of extranodal NHL disease burden.

**Key-words:** Extranodal, Non-Hodgkin Lymphoma (NHL), Immunohistochemistry, Diffuse large B cell Lymphoma (DLBCL), Gastrointestinal Lymphoma.

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## INTRODUCTION

Non-Hodgkin's lymphoma (NHL) is a common hematological malignancy with varied clinicopathological, morphological & diagnostic features. The age-adjusted incidence rates for NHL in men and women in India are 2.9/100,000 and 1.5/100,000, respectively [1]. Compared to developed nations, the key differences in the presentation in India include: median age of 54 years (almost a decade less), higher male to female ratio, higher proportion of patients with B-symptoms (40-60 vs. 20-30%), poor performance status (ECOG score  $\geq 2$ ) at diagnosis (50 vs. 20-30%), higher frequency of diffuse large B-cell lymphomas (60-70 vs. <40%), lower frequency of follicular NHL (<20 vs. 30-40%) and T-cell type in 10-20 vs. <10% [1]. Extra-nodal lymphomas (ENL) account for around 25-30% among all NHL in various studies [2, 3] & are defined as NHLs that originates from tissues other than lymph.

Node and sometimes even from sites that do not normally contain lymphoid tissue. Compared to the United States, Asian countries, including those in the Far East, have higher rates of aggressive NHL, T-cell NHL, and extranodal (EN) diseases [1, 4-6]. They are subclassified into primary (p-ENL) and secondary ENL depending on their mode of presentation; secondary indicating that the lymphoma first presented in an extra nodal site [5] and subsequently involved the nodes.

There is wide variation in incidence, geographic distribution, organs affected, morphological types, stage at presentation and prognostic indices among extranodal lymphomas worldwide. Also, there is lack of prospective trials demonstrating organ-wise classification, staging and treatment protocol. However, common to all studies is the fact that ENL incidence is on the rise, possibly due to urban lifestyle, better healthcare facilities leading to increased detection, geographical and environmental factors like epidemic-

like increase of immunosuppressive and autoimmune disorders. In this institution-based observational cross-sectional study, we aim to subtype extranodal lymphomas presenting to our institute over past 3 years by morphological and immunohistochemical profile along with study the epidemiological, prognostic and clinicopathological parameters as applicable.

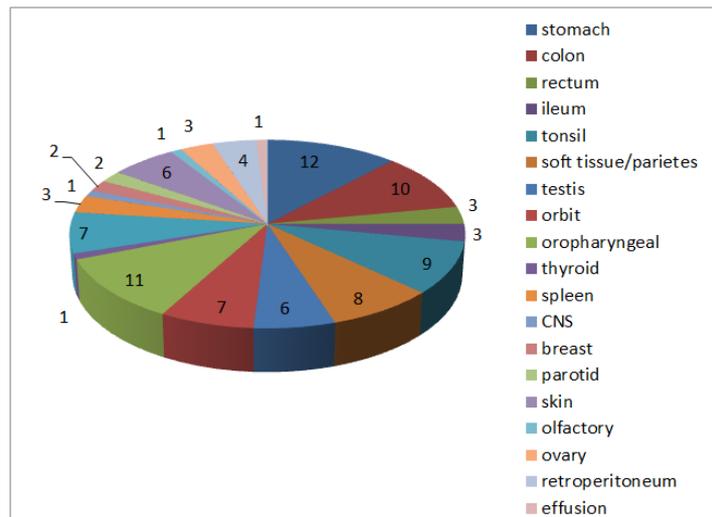
### MATERIAL & METHODS

From January 2016 till December 2018 over a span of 3 years all excision biopsy/trucut biopsy specimens showing Non-Hodgkin lymphomas were evaluated for this study. Patients' records were evaluated for age, sex, ethnicity, presenting symptom, routine blood count, Liver function test, serum Lactate dehydrogenase level, serology status, complete radiology work-up including chest X-ray, Chest CT scan/abdominal CT/PET-CT to rule out nodal/liver/spleen involvement. Modified Ann-Arbor staging was evaluated where complete detail was available. Routine histology was carried out in formalin-fixed paraffin embedded sections stained with hematoxyline & eosin. Immunohistochemical analysis was performed using panel of monoclonal antibodies by Polymer detection technique [8]. Antigen retrieval was done by TRIS-EDTA buffer using alkaline pH (pH8) by pascal pressure cooker. Antibody panels applied comprised of CD45, CD3, CD20, Tdt, CD19, CD79a, CD5, CD23, CD15, CD30, CD10, Bcl-2, Bcl-6, CyclinD1, PAX-5, CD138, Ki-67, EMA, CD4, CD8. Clonality assays were not performed due to lack of facility but supportive evidence was gathered by immunohistochemical marker expression distribution patterns. Cytogenetic & molecular test reports were

retrieved where available. Tonsil & waldeyer's ring without cervical lymph node involvement was considered as extranodal in our study following the example of Otter R *et al.* [9], D'amore AF *et al.* [10] Chan JK *et al.* [11]. Disseminated disease (secondary lymph nodes/marrow involvement) but with clear past record of primary disease at an extranodal site was considered as extranodal lymphoma as proposed by Krol *et al.* [12]. Hodgkin lymphoma and plasmacytoma were excluded from study. Primary mediastinal lymphomas predominantly arising in posterior mediastinum were considered of nodal origin and were also excluded from study.

### RESULTS & ANALYSIS

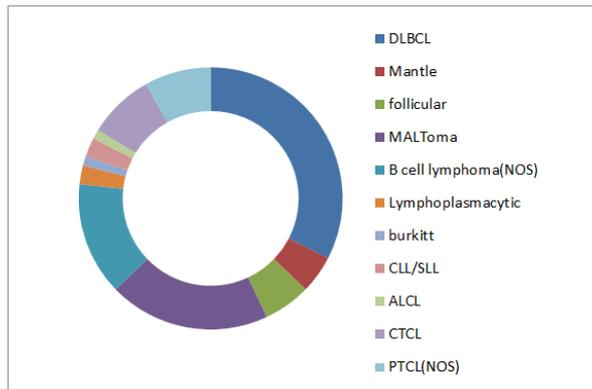
Total number of NHL diagnosed during 3 year period was 223; out of which 94 cases were extranodal lymphomas (42.1%).Such high percentage of ENL is possibly due to selective referral of complicated cases to tertiary centre; may also be due to inclusion of oropharyngeal tonsil & cutaneous ENLs. Demographically lowest age affected was 8 year old male and highest age was 72 years. Mean age being 54.2years. Male was more frequently affected than female (M: F=1.8:1), most frequently age group affected was 5<sup>th</sup>-6<sup>th</sup> decade (40.2%). Site affected were in decreasing order: Gastrointestinal tract/GIT (27%) followed by oropharyngeal (19%) and cutaneous (7.4%). Rare sites involved were: orbit, testis, ovary, breast, lung, thyroid, chest wall, abdominal parietes, spleen, CNS, parotid, olfactory and pleural effusion. Among GIT gastric lymphomas were most prevalent followed by colonic.



**Fig-1: Pie diagram showing relative percentage of site specific extranodal lymphomas**

Histologically diffuse large B cell lymphoma (DLBCL) were most prevalent (40.9%) followed by extranodal marginal zone lymphoma /MALToma(18%). Immunohistochemically B cell lymphomas were more

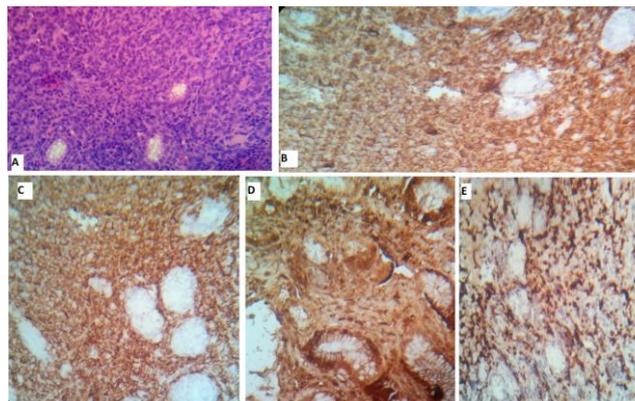
prevalent than T cell (4.53:1). Cutaneous T cell lymphomas (CTCL) were most predominant among all T cell NHL followed by peripheral T cell lymphoma (NOS) and anaplastic large cell lymphoma (ALCL).



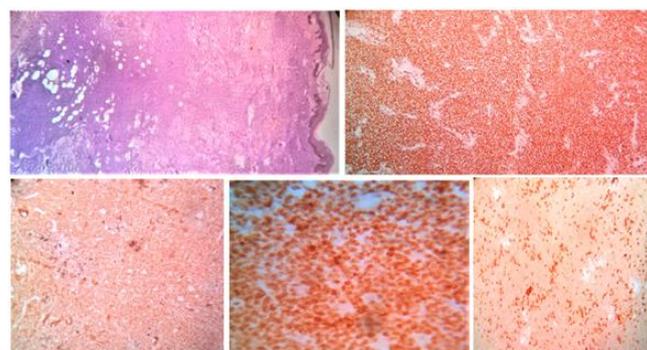
**Fig-2: Histological variants of extranodal lymphoma**

57.89% showed Ki-67 index more than 40%, i.e. very high proliferation activity, 26.3% showed ki-67 10-40% range, only 15.7% showed low proliferation index. Bone marrow involvement was seen in 6 cases (6.3%) most of them being DLBCL followed by Mantle and Small lymphocytic lymphoma(SLL).In pediatric age group, most frequently affected site was testis followed by olfactory, lymphoblastic lymphoma being the commonest variant. Type B symptoms were present

in only nine patients. Two patients were known HIV-positive under Anti-retroviral therapy, both had DLBCL of colon. Ann Arbor staging was available for 29 cases, most of those were of stage II (48%).Total number of CD20 positivity was 68.4% among all p-ENLs. Among all DLBCL, 11.9% showed BCL-6 positivity in more than 20% of tumour cells indicating their germinal center differentiation.



**Fig-3: Gastric lymphoma.3A: H&E stained section showing lymphoepithelial lesion and monomorphic lymphoid infiltrate.3B:CD45 showing diffuse positivity.3C:CD20 showing diffuse positivity.3D:BCL6 showing sparse staining of 20%, 3E: Ki-67 showing high proliferation index (60%). (x400) A diagnosis of DLBCL was made.**



**Fig-4: Cutaneous lymphoma. A: H&E section shows large lymphoid cell in typical 'Bottom-heavy' infiltrate. B: CD20 showing diffuse positivity. C: BCL2 showing diffuse positivity. D: BCL6 showing diffuse positivity. E: ki-67 showing proliferative index of 30%.CD3, CD5, Alk-1 was negative. A diagnosis of cutaneous DLBCL was made (All pictures have magnification of x400)**

## DISCUSSION

Judith A Ferry discussed “Lymphomas arising in extranodal sites are intriguing. The types of lymphomas encountered vary widely from one extranodal site to another. For many types of extranodal lymphomas, there are distinctive clinicopathologic features, sometimes including association with an underlying immunodeficiency syndrome, autoimmune disease, infection, or other immunologic disorder, or a predilection to affect patients of certain ethnic origins [13]”. The frequency of the primary extranodal NHL is high where the total lymphoma incidence is high. A study by Singh *et al.* [14] from North India reported an incidence of 44% (106/241 cases over 3 year period). Similarly, a study from South India by Padhi *et al.* [15] have a reported an incidence of 22%. Studies from Pakistan [16], Korea [17] and China[18] have also reported incidences ranging from 45% to 62%. Pai A *et al.* [19] also reported an incidence of 35.96% in South India which is in stark contrast with European countries where incidence shown to be within 25-30% range in various studies [3, 4]. It has also been observed that during the last two decades the incidence of lymphomas has increased, and that EN-NHL increased more rapidly than the nodal type [20, 21].

In present study Mean age of patients were 54.2 years which are comparable with Padhi *et al.* [15] who have found patients diagnosed to have p ENL had a mean age of 48.2 years with lesser duration of symptoms and lesser B symptoms. We have found a sex ratio of 1.8:1 which is also comparable with Padhi *et al.*[15] from South India who have found 2:1 ratio of male preponderance.

DLBCL is the most common histological variant in p-ENLs worldwide. We have found DLBCL to be commonest histological variant which is comparable with Arora N *et al.* [22], Yun J *et al.* [23] & Mondal SK *et al.* [24] followed by MALT lymphomas (18%).

The incidence of the GIT lymphomas has been increasing throughout the world, and it has now been reported to be the most common site of involvement among the extranodal lymphomas [24, 25]. The head and neck region including the Waldeyer's ring have reported to be common sites of origin of pENL in few studies from India [14, 16] Pakistan [15] and China [17]. The common sites of origin of pENL in our study were GIT followed by oropharyngeal region, and this is comparable to the other studies from Asian continent [14, 16, 24].

The pattern of involvement of the primary gastrointestinal lymphomas is similar to both European and asian studies, stomach being the commonest site followed by colon and small intestine [12-18]. Despite the advances in diagnosis, better understanding of

molecular biology and several possible targeted therapies in horizon, prognosis for p-ENLs remain poor till date. Therapeutic outcome has been worse for patients with pENL involving rare sites as shown by Yun *et al.*[23] Studies have also shown that the age, stage of the lymphoma, performance status, and serum LDH level have been independent prognostic variables, whereas the site of involvement (nodal versus extranodal site) did not bear any prognostic significance as shown by Lal *et al.*[25] This cross sectional & observational study could not correlate the outcomes as the long-term follow-up data pertaining to the survival analysis are lacking. However, we do believe at this point of time that more studies of similar kind with focus on outcomes and also highlighting the genetic profile, must be carried out to understand the complex biology of the primary extranodal pENLs.

## CONCLUSION

Extranodal NHL has a rising trend in developing countries. Due to improved diagnostic techniques many of them are being diagnosed early but needs targeted/site specific algorithm for adequate management. Large population based registry/therapy-specific trials are lacking regarding zonal distribution, management and survival analysis. A holistic approach may help in containment of extranodal NHL disease burden.

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