

## Research article

**A clinicopathological and immunophenotypic study of nodal Hodgkin's lymphomas**Christol Blanch Moras<sup>1</sup>, Pritilata Rout<sup>2</sup><sup>1</sup>Department of Pathology, Father Muller Medical College, Mangalore, Karnataka, India<sup>2</sup>Department of Pathology, St. John's Medical College, Bangalore, Karnataka, India

(Received: June 2022      Revised: September 2022      Accepted: October 2022)

Corresponding author: **Christol Moras**. Email: christol.b.moras@fathermuller.in**ABSTRACT**

**Introduction and Aim:** Hodgkin's lymphoma is a B cell lymphoma, the diagnosis of which is characterized by the presence of neoplastic Hodgkin's/Reed Sternberg cells (HRS) and the unique cellular microenvironment. Clinical examinations, morphology and immunophenotypic study aids in the accurate diagnosis of Hodgkin's lymphoma (HL). The literature available on the clinicopathological and immunohistochemistry (IHC) profile of Hodgkin's lymphoma in Indian population is inadequate. This study was carried out to describe the clinicopathological and immunohistochemical profile of patients in an Indian tertiary care hospital.

**Methodology:** An observational, descriptive study was carried out and included all diagnosed cases of nodal HL for a period of 4 and half years. The demographic details and common clinical presentation were retrieved from the records. The Haematoxylin and eosin (H and E) slides and IHC stained slides for CD15, CD30, CD20, LCA and CD3 were retrieved and studied. Each case was assigned to one of the following histological subtypes. mixed cellularity (MC), nodular sclerosis (NS), lymphocyte rich (LR), lymphocyte depleted (LD) and nodular lymphocyte predominant Hodgkin's lymphoma (NLPHL). Those cases where histological subtyping could not be done due to equivocal features were grouped as unclassifiable.

**Results:** A total number of 65 cases of Hodgkin's lymphoma were included. A unimodal distribution and male preponderance was noted. The subtype of Mixed cellularity predominated the series followed by nodular sclerosis, lymphocyte rich and lymphocyte depleted. There was only one case of Nodular Lymphocyte Predominant Hodgkin's lymphoma. Immunoreactivity of CD 30 was seen in all cases of classical Hodgkin's Lymphoma.

**Conclusion:** Our study reaffirms the findings from previous research ,highlighting the difference in clinicopathological profile of HL from its western counterpart. The usage of immunohistochemistry should be done for all cases of HL since it aids in the diagnosis and also in identification of potential therapeutic targets.

**Keywords:** Hodgkin's lymphoma; immunohistochemistry; morphology.

**INTRODUCTION**

**H**odgkin's lymphoma is a B cell derived lymphoma, the diagnosis of which is denoted by distinctive neoplastic Hodgkin's/ Reed Sternberg cells (HRS) and the unique cellular microenvironment. Clinical examinations, morphology and immunophenotypic study aids in the accurate diagnosis of Hodgkin's lymphoma (HL). Hodgkin's lymphoma is broadly classified into classical Hodgkin's Lymphoma (CHL) and Nodular Lymphocyte Predominant Hodgkin's Lymphoma. CHL is further subclassified into mixed cellularity (MC), nodular sclerosis (NS), lymphocyte rich (LR), lymphocyte depleted (LD).

Therapeutic advances have helped in the identification of newer targets including neoplastic and microenvironment components thereby having an impact on prognosis of the disease (1,2,3). The literature available on the clinicopathological and immunohistochemistry (IHC) profile of Hodgkin's Lymphoma in Indian population is inadequate. This

study is an attempt to describe the clinicopathological and immunohistochemical profile of patients in an Indian tertiary care hospital.

**MATERIALS AND METHODS**

This was an observational and descriptive study done in a tertiary care hospital. Primary objective was to study the clinical profile, morphological spectrum and IHC profile of HL. The study was approved by the institutional board review and ethical committee.

**Inclusion criteria**

All cases of nodal Hodgkin's lymphoma diagnosed during a 4 and a half years period at an Indian tertiary care hospital were included.

**Exclusion criteria**

Those cases where IHC was not done were excluded from the study.

The demographic details like age, sex and common clinical presentation were retrieved from the records. The haematoxylin and eosin (H and E) and IHC slides

were retrieved and analysed. The H and E slides were studied for presence of RS cells and its variants such as classical, popcorn, mononuclear, lacunar variants. The background infiltrate like eosinophils, plasma cells, histiocytes, lymphocytes were noted.

The IHC stained slides for CD15 (clone BRA4F1), CD30(clone Ber-H2), CD20(clone L-26), LCA (Clone PD7/26/16 and 2B11) and CD3(clone -PS1) were retrieved and studied. Polymer technology with DAB detection system was used. Pressure cooking for antigen retrieval was done. All the tests were interpreted in conjunction with positive and negative controls. The pattern, intensity of staining and percentage of positivity of neoplastic cells (RS cells) were noted. In addition, the nature of background of non-neoplastic lymphocytes (whether T or B) was also noted.

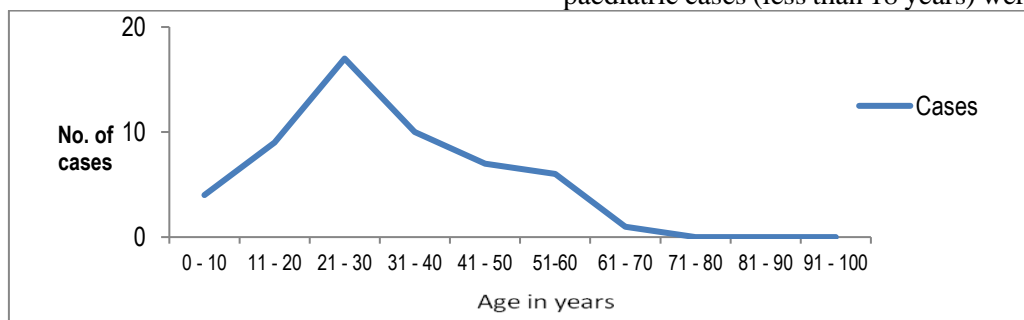
Based on these features, each case was assigned to one of the following histological subtypes. mixed cellularity (MC), nodular sclerosis (NS), lymphocyte rich (LR), lymphocyte depleted (LD) and nodular

lymphocyte predominant Hodgkin's lymphoma (NLPHL). Those cases where histological subtyping could not be done due to equivocal features were grouped as Unclassifiable Any other feature like fibrosis, necrosis and granuloma were also observed against its histological subtype.

Microsoft excel 2010 was used to calculate the data and total numbers, mean value and percentage of different types were calculated by descriptive statistics. The aim was to study the clinical profile, morphological spectrum and IHC profile of HL.

**RESULTS**

In the present study, a total number of 65 cases of Hodgkin's lymphoma diagnosed during 4 and a half years period at a tertiary care hospital were included. Out of the 65 cases, both in the adult age group and in the paediatric age group the males predominated. The male to female ratio was 2.25:1. The age of the study group ranged from 4 years to 65 years. The mean age was 31years. Among them, the total numbers of paediatric cases (less than 18 years) were 13 in number.



**Fig. 1:** Age distribution of cases

As seen in Fig. 1, the maximum number of cases occurred in the third decade. The age ranged from 4 to 65 years. Superficial lymph node enlargement was the commonest presentation seen in 89.2% of the cases. Also noted was fever, weight loss and night sweats as summarized in Table 1. Splenomegaly was seen in 38 cases and hepatomegaly was seen in 33 cases. Both splenomegaly and hepatomegaly was seen in 24 cases.

**Table 1:** Patients' characteristics

Parameters	Characteristic	Number (%)
Gender	Male	45 (69.2)
	Female	20 (30.8)
Symptoms at presentation	Fever	49 (75.3)
	Weight loss	24 (36.9)
	Night sweats	18 (27.6)
	Lymphadenopathy	58 (89.2)
Lymph nodes involved	Cervical	35 (53.8)
	Axillary	19 (29.2)
	Inguinal	15 (23.1)
	Mediastinal	12 (18.4)
	Abdominal	10 (15.3)
Organomegaly	Hepatomegaly	33 (50.7)
	Splenomegaly	38 (58.4)

The subtype of mixed cellularity predominated the series followed by nodular sclerosis, lymphocyte rich

and lymphocyte depleted as summarised in Table 2. There was only one case of NLPHL. Unclassifiable category comprised 3.07% of cases.

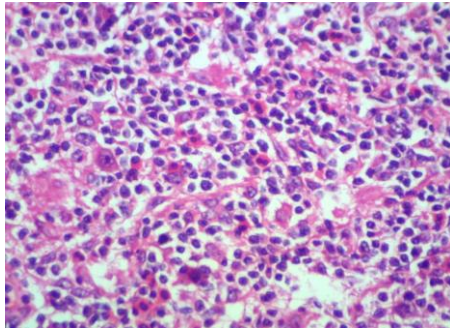
**Table 2:** Morphological data

Histopathological subtypes	Number (n=65) (%)
Mixed cellularity	33 (50.8)
Nodular sclerosis	24 (36.9)
Lymphocyte rich	4 (6.15)
Lymphocyte depleted	1 (1.53)
Nodular Lymphocyte predominant	1 (1.53)
Unclassifiable	2 (3.07)

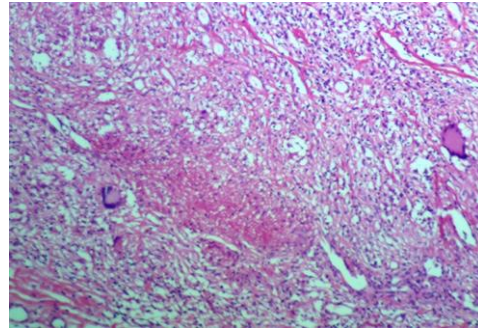
Extra nodal extension to bone marrow, spleen and liver were seen in 5 CHL cases (8.06%). Granuloma formation was seen in 14 cases of CHL (23.3%) The only case of NLPHL had infiltration to the bone marrow. Immunoreactivity of neoplastic RS cells for CD 30 was a common feature of CHL and was seen in all 62 cases. CD 15 expression was seen in 58 cases of CHL .CD 20 expression was not seen by any of the RS cells in Classical HL. LCA or CD3 expression was not seen in any of the RS cells. The background population was T cell rich in majority cases of CHL.7 cases

demonstrated variable B and T cell background. There was only 1 case of NLPHL, the popcorn cells of which

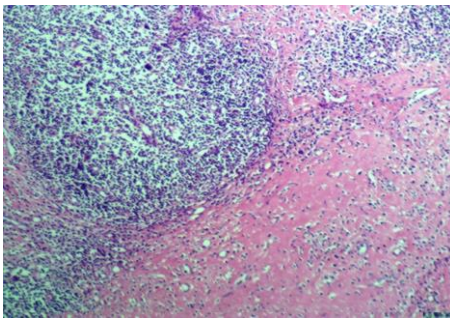
expressed LCA and CD 20. This case also showed a CD3 cell rich background.



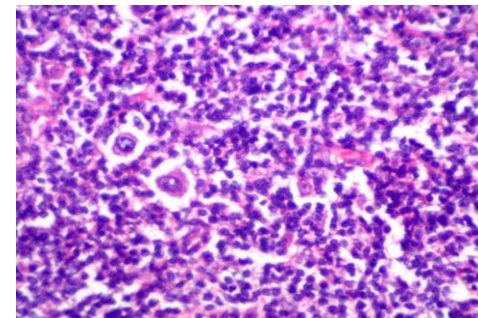
**Fig. 2:** MC-HL. RS cells are seen admixed with a polymorphous lymphoid infiltrate rich in eosinophils (40x)



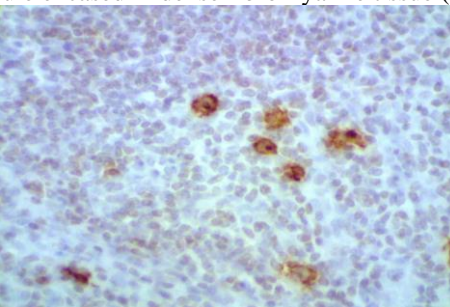
**Fig. 3:** Granuloma with epithelioid cells and multinucleated giant cells. Also seen is necrosis (10x)



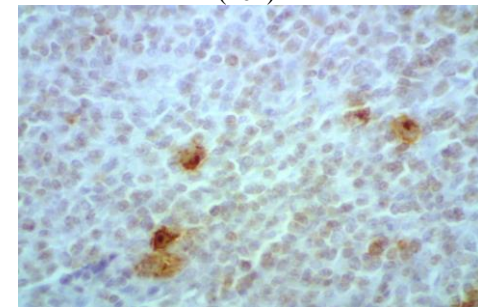
**Fig. 4:** Nodular sclerosis HL. The lymphoid nodule encased in dense fibro-hyaline tissue (10x)



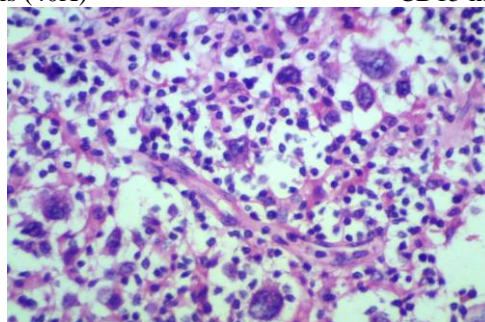
**Fig. 5:** Lacunar cells of nodular sclerosis (40x)



**Fig. 6:** Membrane immunoreactivity for CD 30 in RS cells (40X)



**Fig.7:** Membrane immunoreactivity for CD15 in RS cells (40X)



**Fig. 8:** lymph node showing popcorn cells of NLPHL (40x)

## DISCUSSION

Hodgkin's Lymphoma is a unique lymphoma distinguished by the classical Reed Sternberg cells in a non-neoplastic inflammatory background comprising of lymphocytes, eosinophils, neutrophils, histiocytes, epithelioid cells, plasma cells and fibroblasts in varying proportions depending on the morphological subtype.

Our study showed a unimodal distribution with maximum number of cases occurring in the third decade. This is in concurrence with other studies done

by Patekar *et al.*, Siddique *et al.*, and is in discordance with the study done by Geeta *et al.*, which detected bimodal age distribution of the disease with first peak in 11-20 years and second peak >50 years (4-6).

Majority of the cases occurred in males, a feature which is consistent in various studies. Male predominance was observed in all histological subtypes. This could be attributed to various factors like socioeconomic factors, immunocompetency of the population and individual susceptibility to the causative factor which influences the biological behaviour of the disease (7,8).

Superficial lymph node enlargement was the common presentation in our study. This finding is like other studies reported from India. Superficial involvement was attributed to the fact that the disease spread follows the physiological direction of lymph flow (9). Mixed cellularity was common in all age groups of our patients. This is in concurrence with other similar Indian studies (10,11) and is in contrast from western studies where nodular sclerosis is common (12). Our study did not reveal any variations among percentage of cases of each histologic subtype in different age groups. This contrasts with a study done by Siddiqui, *et al.*, which revealed that mixed cellularity was present in >80% of cases in age group more than fifty and that nodular sclerosis reached its peak in age group of 21 to 30years (5).

Extra nodal extension to bone marrow, spleen and liver was seen in 9.2% of all the cases. Hematogenous dissemination is the common cause for extra nodal involvement. In our study the frequency of marrow infiltration was found to be 6.3% in all cases. The frequency of marrow infiltration is lower as compared to other studies. In a study done by Sharma *et al.*, bone marrow involvement was found in 36.25% of cases (13). In a study done by Lone *et al.*, bone marrow infiltration was seen in 38% of cases (14). Bone marrow is being performed as an important investigation in the assessment of the spread of the disease. Involvement of bone marrow places the patient in advanced stage IV disease (5).

Granuloma formation was noted in 23.3 % of cases. The presence of non-caseous necrotizing epithelioid granuloma represents a local tissue response to tumour. Their presence in the tissue may mask the morphology of the Hodgkin's lymphoma and the diagnosis may be missed in core biopsy. However, the occurrence of granuloma in the neoplastic lymph node has a favourable prognosis (15,16).

Immunohistochemistry using a panel of antibodies is widely being used and helps in understanding the pathogenesis, establishing diagnosis, in prognostication and treatment. In our study CD30 expression was a general feature seen in all cases of CHL. This finding is comparable to study done by Patkar *et al.*, (4). CD30 immunoreactivity of the RS cells in CHL aids in establishing diagnosis (17). Availability of targeted therapy has also improved the response rate of HL patients. 6.4% of cases did not express CD 15 which is very low compared to study done by Patkar *et al.*, (4). In their study, CD15 negativity was seen in 40.05% of CHL cases. However, a study done by Benharroch, *et al.*, showed that 4% of their cases were not immunoreactive for CD15 (18). CD15 immunonegativity has adverse prognosis and an overall worse survival (4). It is surprising to note that

none of the CHL cases were positive for CD 20 in our study. Expression of CD 20 has unfavourable prognosis as stated by previous studies (19,20). Meanwhile a study done by Zuckerberg *et al.*, reported that CD 20 expression was seen in those tumours having monomorphic proliferation of mononuclear and binuclear RS cells with less of inflammatory infiltrate, whereas expression of CD 15 was seen in those cases with increased inflammatory infiltrate (18,21).

## CONCLUSION

Our study reaffirms the findings from previous research, highlighting the difference in clinicopathological profile of HL from its western counterpart. A unimodal distribution and male preponderance with superficial lymph node enlargement being the commonest presentation. Mixed cellularity was the predominant histological subtype and immunoreactivity of CD 30 was seen in all CHL cases. The usage of immunohistochemistry on paraffin section should be done for all cases of HL since it aids in the diagnosis and in recognition of potential therapeutic targets.

## CONFLICT OF INTEREST

There is no conflict of interest to declare.

## REFERENCES

1. Wang, H., Balakrishna, J.P., Pittaluga, S., Jaffe, E.S. Diagnosis of Hodgkin lymphoma in the modern era. *British Journal of Haematology*.2019;184: 45-59.
2. Pileri, S., Ascani, L., Leoncini, E., Sabattini, P., Zinzani, P.L., Piccaluga, P.P., *et al.*, Hodgkin's lymphoma: the pathologist's viewpoint. *Clin Pathol* .2002;55:162-176.
3. Swerdlow, S.H., Campo, E., Harris, N.L., Jaffe E.S., Pileri S.A., Stein H., *et al.*, World Health Organization classification of tumours of haematopoietic and lymphoid tissue. Lyon: IARC Press; 2017.
4. Patkar, N., Mehta, J., Kulkarni, B., Pande, R., Advani, S., Borges, A. Immunoprofile of Hodgkin's lymphoma in India. *Indian J Cancer* 2008; 45:59-63.
5. Siddiqui, N., Ayub, B., Badar, B., Zaidi, A. Hodgkin's Lymphoma in Pakistan: A clinico-epidemiological study of 658 cases at a cancer center in Lahore. *Asian Pacific J Cancer Prev*. 2006; 7(4): 651-655.
6. Geeta, P., Devadas, G. Clinicopathological Profile of Hodgkin's Lymphoma in Tertiary Care Hospital. *Journal of Dental and Medical Sciences*. 2017;16(3): 23-26.
7. Jarrett, R.F., Krajewski, A.S., Angus, B., Freeland, J., Taylor, P.R., Taylor, G.M. The Scotland and Newcastle epidemiological study of Hodgkin's disease: Impact of histopathological review and EBV status on incidence estimates. *J Clin Pathol* 2003; 56: 811-816.
8. Correa, P., O'Conor, G.T. Epidemiologic patterns of Hodgkin's disease. *Int J Cancer* 1971;8: 192-201.
9. Smithers, D.W., Lillcrap, S.C., Barnes, A. Patterns of lymph node involvement in relation to hypotheses about the modes of spread of Hodgkin's disease. *Cancer*. 1974;34(5):1779-1786.
10. Talvalkar, G.V., Sampat, M.B., Gangadharan, P. Hodgkin's lymphoma in western India: a review of 1082 cases. *Cancer*. 1982;15; 50(2):353-359.
11. Ramadas, K., Sankaranarayanan, N., Nair M.K., Padmanabhan, B. Adult Hodgkin's lymphoma in Kerala. *Cancer*. April 1994;15;73(8):2213-2217.

12. Shenoy, P., Maggioncalda, A., Malik, N., Flowers C. R. Incidence patterns and outcomes for Hodgkin's lymphoma patients in the United States. *Adv Hematol.* 2011;725219.
13. Sharma, S., Ahuja, A., Murari, M. Bone marrow biopsy in Hodgkin's disease. *Indian J Pathol Microbiol.* 2004 Jul;47(3):364-366.
14. Lone, A., Naeem, S. Frequency and pattern of bone marrow infiltration in Hodgkin's Lymphoma. 2011;27 :132-135.
15. Hou, W.H., Wei, P., Xie, J.L., Zheng, Y.Y., Zhou, X.G. Classical Hodgkin's lymphoma with necrotic granuloma-like morphological features. *Int J Clin Exp Med.* 2018;11: 9593-9602.
16. Sacks, E.L., Donaldson, S.S., Gordon, J., Dorfman, R.F. Epithelioid granulomas associated with Hodgkin's disease: clinical correlations in 55 previously untreated patients. *Cancer,* 1978;41: 562-567.
17. Ranuhardy, D., Suzanna, E., Sari, M.R., Hadisantoso, D.W., Andalucia, R., Abdillah, A. CD30, CD15, CD50, and PAX5 expressions as diagnostic markers for Hodgkin Lymphoma (HL) and systemic anaplastic large cell lymphoma (sALCL). *Acta Med Indones - Indones J Intern Med.* 2018; 50 (2):104-109.
18. Benharroch, D., Nalbandyan, K., Lazarev, I. CD20 Over-expression in Hodgkin-Reed-Sternberg cells of classical Hodgkin's Lymphoma: The neglected quest. *Journal of Cancer* 2015; 6(11): 1155-1159.
19. Portlock, C.S., Donnell, G.B., Qin, J., Straus, D., Yahalom, J., Zelenetz, A., *et al.*, Adverse prognostic significance of CD20 positive Reed-Sternberg cells in classical Hodgkin's disease. *Br J Hematol.* 2004;125: 701-708.
20. Wasielewski, V.R., Mengel, M., Fischer, R., Hansmann, M.L., Hubner, K., Franklin, J., *et al.*, Classical Hodgkin's disease: Clinical impact of the immunophenotype. *Am J Pathol.* 1997;151: 1123-1130.
21. Zuckerman, L.R., Collins, B.A., Ferry, J.A., Harris, N. L. Co-expression of CD15 and CD20 by Reed-Sternberg Cells. *American Journal of Pathology.* 1991;139(3): 475-483.