Original Article

Immunoprofile of Hodgkin's lymphoma in India

Patkar N, Mehta J[1], Kulkarni B, Pande R, Advani S[2], Borges A

Departments of Histopathology and [2]Medical Oncology, Asian Institute of Oncology, SL Raheja Hospital, Mumbai, India. [1]Centre of Excellence, Department of Histopathology, Wellspring Laboratories, Mumbai, India

Correspondence to: Dr Nikhil Patkar, E-mail: nvpatkar@gmail.com

Abstract

AIMS AND BACKGROUND: The immunoprofile of the Reed Sternberg cell with respect to immunoreactivity for CD20 and lack of CD15 has been described as a poor prognostic factor. Large scale studies analyzing the immunoprofile of Hodgkin's lymphoma (HL) from India are lacking. The aim of this study was to obtain baseline information on relative frequencies and immunoprofiles of the two major types of HL and comparing reports from developed and developing countries. **MATERIALS AND METHODS:** 451 cases of HL were classified as per the WHO into classical (n= 397) HL (cHL) and nodular lymphocyte predominant HL (NLPHL) (n=54). Cases of cHL were divided into 5 immunophenotypic groups; Group A (CD15+,CD30+,CD20-), Group B (CD15-,CD30+,CD20-), Group C (CD15+,CD30+,CD20+), Group D (CD15-,CD30+,CD20+)and Group E (CD15-,CD30-,CD20+). In cases of NLPHL, the immunophenotype of lymphocytes in the background, whether T(CD3) or B(CD20) rich was observed. **RESULTS:** Most cases of cHL belonged to Group A (44.58%) followed by Group B (40.05%), C(5.54%), D(9.57%) and E(0.25%). Half, (50.89%) the cases of cHL were immunonegative for CD15, whereas CD20 was expressed by 15.61% of the cases. Three (5.55%) cases of NLPHL showed a CD3 (T) cell rich background. Significant differences were also observed with respect to the age distribution of cHL as compared to the west. **CONCLUSION:** Our study demonstrates that India has a high number of CD15 negative and a relatively higher number of CD20 positive cHL cases as compared to the western population. Favorable treatment response and good cure rates that one sees in western cHL may not apply to India.

Keywords: Hodgkin's lymphoma, immunophenotype

Introduction

Hodgkin's lymphoma (HL), first described by Thomas Hodgkin in 1832 is one of the most enigmatic diseases known. In recent years, immunophenotyping has contributed significantly to our understanding the nature and biology of the diagnostic Reed Sternberg (RS) cell.

It had long been recognized that HL was not a single disease. Today HL has been classified on the basis of immunophenotype of the RS cell into classical HL (cHL) and nodular lymphocyte predominance HL (NLPHL). Moreover, the immunoprofiles of the RS cells and the background lymphocytes have recently been shown to impact the behavior and response to treatment of HL.

HL is not an uncommon lymphoid tumor in India.[1,2] Data from some of the referral cancer hospitals in the Indian subcontinent have suggested that demographic profile of HL and its response to treatment is different to that in reports from the west. Large-scale studies which have analyzed the immunoprofile of HL in India are few and between.

In this context we reviewed 451 consecutive cases of HL referred to our hospital with the aims of obtaining baseline information on relative frequencies and immunoprofiles of the two major types and comparing reports from developed and developing countries.

Materials and Methods

We studied 2242 consecutive cases of lymphoma

diagnosed over a period of two and a half years at two referral laboratories. Out of these cases, 451 were of the Hodgkin's subtype. The cases in this study accounted for a fair representation of a "regular" population from Western India. Clinical details like age, sex and site of disease were noted in each case. On studying the morphology a panel of antibodies (which in addition to other antibodies, included antibodies for HL mentioned below) was ordered depending upon the differential diagnosis. As a part of processing, tissue sections were deparaffinized in xylene and rehydrated in a graded series of ethanols. Endogenous peroxidase was blocked by 3% hydrogen peroxide in methanol for 10 minutes at room temperature. Heat-induced epitope retrieval was done by heating these sections in 10-mMol/L sodium citrate buffer (pH 6.0) using the microwave technique for 10 minutes. After cooling at room temperature for 20 minutes the slides were treated with TRIS buffered saline. The sections were then incubated with the primary antibodies, CD15 (concentration 1:50), CD30 (1:50), CD20 (1:100), CD 3(1:100) and LCA (Leucocyte common antigen) (1:100). EMA (Epithelial membrane antigen) (1:50) was used in some of the cases of NLPHL. All the antibodies were sourced from Dako (Glostrup, Denmark) The link streptavidin avidin biotin system (Diagnostic Biosystems), based on streptavidin-biotin-peroxidase complex, was applied for the detection of the immunoreaction. Color was developed with 3,3'-diaminobenzidine and hydrogen peroxide (Diagnostic Biosystems), and all slides were subsequently counterstained with hematoxylin. Granulocytes were used as internal controls for analysis of CD15. Although not a specific internal control, CD30 expression in plasma cells was used as a surrogate marker see that the antibody had worked. The same were interpreted as positive if granular brown staining was seen in the golgi zone and/or the cell membrane of the RS cells. As suggested by Tzankov *et al*, a case was considered CD20-positive if there was specific membranous staining in >10% of the HRS cells.[3] HRS cells expressing CD20 were negative for LCA. As far as the histological subtype of HL was concerned the cases were divided into cHL and NLPHL. Where possible, the cases of cHL were histologically subtyped into nodular sclerosis, mixed cellularity, lymphocyte depleted and lymphocyte rich.

The cases of cHL were divided into 5 groups; Group A(CD15+, CD30+, CD20-), Group B(CD15-, CD30+, CD20-), Group C(CD15+, CD30+, CD20+), Group D(CD15-, CD30+, CD20+), Group E(CD15-, CD30-, CD20+). In all cases, the RS cells were immunonegative for LCA and CD3. In cases of NLPHL the background population was noted.

Results

Out of the 451 cases of HL, classical HL formed a majority of 397 (88.02%) cases, NLPHL forming the rest (11.97%).

The age of cHL ranged from 2 to 82 years, with an average of 35 years, whereas, the age of NLPHL ranged from 5 to 78 years, with an average of 35 years. The age distribution curves of cHL and NLPHL were as seen in Figure 1.

A majority of the cases of cHL occurred in males (71%); cases of nodular sclerosis too, were male predominant (66%). NLPHL, too, was male predominant (78%).

Classical HL occurred in superficial lymph nodes as well as deep nodal sites like retroperitoneal nodes. Cases of NLPHL, on the other hand occurred almost exclusively in superficial nodes, notably cervical, axillary and inguinal lymph nodes. Only three cases of cHL were extranodal, two occurred in the lung and one in the colon whereas; a single case of NLPHL was extranodal, occurring in the breast.

Histological typing of cHL was possible in 283 out of 397 cases. Histological subtyping could not be done on all the cases of cHL due to small amount of tissue present (e.g. core biopsy) or equivocal histological features. These were termed as unclassifiable cHL. Cases of cHL were distributed as follows: mixed cellularity - 142, nodular sclerosis - 116, lymphocyte rich - 22 and lymphocyte depleted - 3 cases. Immunoreactivity for CD30 was almost a universal feature of cHL, seen in 99.74% of the cases. Half (49.11%) of the cases expressed CD15, whereas CD20 was expressed by 15.61% of the cases. None of the RS cells expressed LCA or CD3. In almost all cases of cHL, the background cell population in cHL was T cell rich.

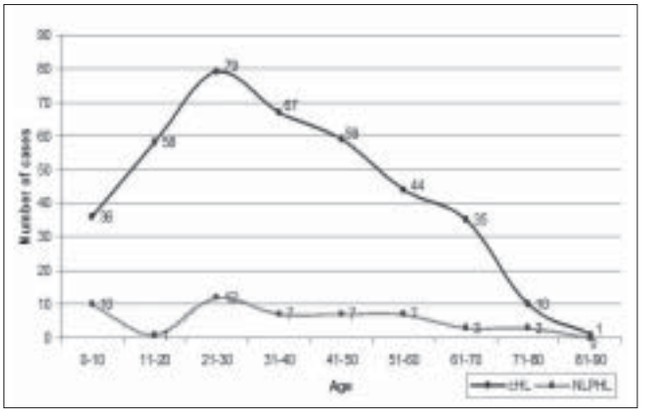

Figure 1: Age distribution curves of cHL and NLPHL

Two cases of lymphocyte rich cHL demonstrated a B-lymphocyte rich background. Distribution of cases of cHL according to 5 prognostically relevant groups was as seen in Table 1. A majority of cases belonged to Group A expressing both CD15 and CD30. This group was closely followed by group B cases which were immunonegative for CD15 antigen and were expressing only CD30. Twenty two cases belonged to group C expressing CD15, CD30 and CD20, whereas 38 cases in group D were immunoreactive for CD30 and CD20. We encountered only a single case of cHL expressing CD20 only.

All the LandH cells of NLPHL expressed LCA and CD20. Weak and cytoplasmic CD30 expression was seen only in 4 cases. Three (5.55%) of these 54 cases showed a CD3 (T) cell rich background. A single case showed features that overlapped with T cell rich B cell lymphoma. Table 2 is a comparison between cHL and NLPHL cases.

Discussion

Hodgkin's disease, recently rechristened as Hodgkin's lymphoma has been subdivided into two main entities, classical Hodgkin's lymphoma (cHL) and nodular lymphocyte predominant Hodgkin's lymphoma (NLPHL).

NLPHL, in our study accounted for 11.97% cases of HL. This incidence is slightly higher as compared to western data according to which NLPHL forms 4-6% of cases.[4,5]

Table 1: Distribution of cases of classical Hodgkin's lymphoma

Group	CD15	CD30	CD20	Case(s)	Predominant histological subtype
A	+	+	-	177 (44.58%)	Mixed cellularity
B	-	+	-	159 (40.05%)	Nodular sclerosis
C	+	+	+	22 (5.54%)	Mixed cellularity
D	-	+	+	38 (9.57%)	Mixed cellularity
E	-	-	+	1 (0.25%)	Mixed cellularity

Table 2: Comparison between cHL and NLPHL

	cHL (n=397)	NLPHL (n=54)
Site of lymphadenopathy	Superficial and deep	Mostly superficial
Neoplastic cells	RS cells	LandH cells
Immunophenotype of neoplastic cells	CD15+/-, CD30+, LCA-, CD20+/-	CD15-, CD30-, LCA+, CD20+
Background population	Mostly T cell rich	Mostly B cell rich

Our study highlights the differences in the clinical and pathological profile of Indian cHL from its western counterpart. A recent study done in Scotland and New Castle states that cHL still maintains its bimodal distribution. According to this study, the first peak is seen from 15 to 30 years of age followed by a sharp fall and a second peak in the sixth decade.[6] We did not come across a bimodal age distribution. In our study, the age distribution of cHL shows a sharply rising incidence from the age of five years, which peaks in the third decade. This contrasts with that seen in the west.[6] As compared to the western population, a peak occurs a decade earlier and gradually declines, instead of a sharp fall as compared to the western data. This is attributed to the high (18.89%) percentage of cases that occur before 15 years in India. These findings in an Indian population have been documented two decades earlier in a publication from India by Dinshaw *et al*.[7] However; our findings are somewhat different from that noted by Dinshaw *et al*. In their study, a sharp fall is noted in the number of cases seen after the sixth decade. Our present study reports that a small second peak is noticeable in the sixth decade. This interesting observation could possibly reflect a changing trend in the epidemiology of cHL in India, rather than a referral bias. The age distribution curve of NLPHL in this study is strikingly identical to the Scotland and New Castle study. This finding further strengthens the belief that NLPHL and cHL are two distinct disease entities with different etiologies and clinical behavior. To the best of our knowledge, these findings have not been demonstrated in the Indian subcontinent, till date.

We divided cases of cHL into 5 groups as described by von Wasielewski *et al*.[8] The immunophenotype of cases in Group A represents the archetypical immunophenotype of cHL. In our study, although these cases are in a majority (44.58%), the incidence is much less that observed by von Wasielewski *et al* (83%). Group B cases differed from Group A in lacking immunoreactivity for CD15. This group accounted for 40% of the cases of cHL. Loss of immunoreactivity for CD15 is an adverse prognostic factor as these cases have a significantly worse overall survival and freedom from treatment failure. Also, CD15 negative patients had a higher incidence of relapses, independent of other prognostic indicators. On the other hand, it is interesting to note that the expression of CD15 antigen in lung, colon, hepatocellular and thyroid carcinoma is associated with an adverse prognostic outcome.[5,8,9] Having stated this one should also consider that a

number of preanalytical variables such as B5 fixation (increased expression of CD15) and neutral formalin (false negative CD15 in some cases) can also influence expression of CD15. These reasons could also explain the high degree of CD15 negativity seen in our study.

In our study, 62 cases (15.61%) of cHL were immunoreactive for CD20. Various studies have demonstrated positivity for CD20, ranging from 5 to 50%. The significance of CD20 expression by the RS cells is, as of now, a matter of controversy. A recent study by Portlock *et al.* concluded that the presence of CD20 positive cells in cHD was a poor risk prognostic factor with initial therapy, for time to treatment failure and overall survival. [10] Von Wasielewski *et al*, had earlier stated similar findings.[8] According to these studies, the worst prognosis was seen in cases which failed to express CD15 and CD30, expressing only CD20. We encountered only a single case of this type (Group E). Rassidakis *et al*, demonstrated that CD20 positivity in the RS cells was not associated with a different FFS as compared to CD20 negative cHL.[11] A similar opinion was voiced by Vassallo *et al*.[12] Meanwhile, a study done by Tzankov *et al*, showed that CD20 positive cHL cases have actually a better failure-free survival. They stated that CD20 expression was an independent positive prognostic factor as far as failure free survival was concerned. Along with other mechanisms, they postulated that an increase in CD20 (which resembled a Ca^{2+} ion channel) along with chemo and/or radiotherapy, might decrease apoptotic resistance or even activate programmed cell death. [3]

As far as NLPHD is concerned, immunoreactivity for CD20 is almost a universal feature of the popcorn cells. Along with immunoreactivity for LCA it is a feature of diagnostic importance. We encountered three cases of NLPHL with a T cell rich background; however the neoplastic cells in these cases expressed LCA and CD20. One of these cases could not be distinguished from TCRBCL. The distinction between NLPHL and TCRBCL although important for treatment, is always not easy. The analysis of the reactive background aids the diagnosis as by definition, small B cells are abundant in NLPHL, but rare in TCRBCL. When the neoplastic cells are diffusely scattered in a T-cell and histiocyte-rich background devoid of small B cells these cases could represent TCRBCL or secondary progression to TCRBCL.[13] It is important to analyze the immunophenotype of the background lymphocytes in cases of NLPHL which have lost their nodular architecture as these cases could represent a gray zone between NLPHL and T/HRBCL.[4,14,15]

Lastly, it is essential to consider imunophenotyping in all cases of HL as immunotherapy with rituximab is being used increasingly in NLPHL and lymphocyte predominant HL. Similarly it has been used in relapsed cases of cHL with promising results. Immunotherapy with rituximab seems to have heralded a revolution of sorts. Currently, the use of anti CD30 antibody (MDX-060) in the treatment of cHL is being investigated.[16-18]

Correa and O'Connor in their landmark article on HL established the interplay of diverse factors such as susceptibility to the etiologic agent, immunocompetence of the individual and socioeconomic factors governing a population as being determinants of biological behavior. They suggested that the difference in biological behavior of HL be best understood as a manifestation of host response peculiar to that environment. Accordingly, they sub classified HL into three types.[19] Talvalkar *et al*, stated that HL in India falls into the poor prognosis type 1 pattern.[20] This could possibly be attributed to the fact that, in India, primary EBV infection occurs early in life with the median age of primary infection being 1.4 years.[21] Early exposure to EBV has also been postulated to have an influence on the pathogenesis of HD. This could possibly be an important contributing factor (in addition to the immunoprofile) resulting in an unfavorable prognosis type as compared to the western countries. Thus, the favorable treatment response and good cure rates that one sees in western cHL may not apply to India. These findings have been confirmed by a recent study.[22]

To summarize, our study demonstrates that India has a high number of CD15 negative and a relatively higher number of CD20 positive cHL cases as compared to the western population. Although not universally available in this country, IHC is instrumental in diagnosing, classifying and prognosticating cases of HL and a panel of relevant antibodies must be used in every case. The increasing use of immunotherapy for HL further fortifies this view.

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