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CLINICOPATHOLOGICAL CORRELATION OF HODGKIN LYMPHOMA AN IMMUNOPROFILE AND REVIEW OF LITERATURE

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ABSTRACT

Introduction: Hodgkin lymphoma (HL) is a lymphoid malignancy that amounts for less than 1% of all de novo neoplasms occurring every year globally and nearly 30% of all lymphomas. **Objectives**: To evaluate the expression of several immunohistochemical markers on formalin fixed tissue, paraffin embedded sections of Hodgkin lymphoma subtypes and correlate them with age, sex and site. **Methods**: A total of 14 cases of Hodgkin lymphoma collected during two years 2012-2013. H&E, IHC were done. The markers used were CD30,CD15, CD20,CD3, LCA, EMA using En Vision system. **Results:** All cases were Classical Hodgkin lymphoma (cHL), 42.9% were males, 57.1% were females. nodular sclerosis 14.3% were males, 42.9% were females with a total of 57.1%, with M:F ratio 1:3, mixed cellularity 21.4% were males and 14.3% were females, with a total of 35.7%, with the M:F ratio 1.5:1, unclassified cHL was a male patient 7.1%. The most common age group affected was ≤ 29 (64.3%) followed by 30-59, (21.4%). Most of the cases were nodal 92.9% with cervical anatomic localization predominant 50% and only 7.1% extranodal in the breast which is a rare location. In respect to phenotyping, CD30 was positive in all the cases 100%, CD15 was positive in 50% of the cases, CD20 was positive in 14.3% of the cases, CD3, LCA, EMA were negative in all. **Conclusions:** IHC is a very important investigation for HL and other lymphomas and for immunotherapy. The most common subtype of HL was nodular sclerosis with female predominant, followed by mixed cellularity. The most common age group affected was ≤ 29 (64.3%). Most of the cases were nodal with cervical predominant, except one extranodal in the breast. CD30 expressed in all cases, while CD15 expressed in 50%, CD20 expressed in 14.3%.

KEYWORDS: Hodgkin lymphoma, immunhistochemistry, Yemen.

INTRODUCTION

Hodgkin lymphoma is a lymphoid malignancy that amounts for less than 1% of all de novo neoplasms occurring every year globally,[1,2] and nearly 30% of all lymphomas. The total prevalence has not seemingly changed, in comparing with non- Hodgkin lymphomas where there has been a stable increase in incidence.[3]

It has long been documented that HL was not a single disease. Currently HL has been classified on the bases of immunophenotype of the Reed Sternberg (RS) cell into classical HL (cHL) and nodular lymphocyte predominance HL (NLPHL). The immunoprofiles of the RS cells and the background lymphocytes have currently been shown to influence the performance and reaction to treatment of HL.[4]

The stamp of Hodgkin lymphoma (HL) is the presence of large, mononucleated Hodgkin and multinucleated Reed/Sternberg cells. These cells symbolize the tumor cells, but commonly embrance less than 1% of the cellular infiltrate in the lymphoma tissue.[5] Due to the scarcity of Hodgkin and Reed/Sternberg (HRS) cells and their uncommon phenotype, the derivation of these cells from germinal center (GC) B cells in both the lymphocyte predominant (LP) and the classical subtypes of HL could elucidated only recently.[6] Only in very unusual cases, HRS cells of classical HL denote transformed T cells.[7,8] Most of the T- cell antigen positive classical HL cases do not show T-cell receptor rearrangement but Ig gene rearrangement in the HRS cells as a substitute so that the manifestation of T- cell antigens is often aberrant.[8]

Hodgkin's Reed Sternberg (HRS) cells are characteristically positive for CD15 and CD30 and often loss appearance of pan B-cell markers CD19, CD20, CD22, CD45 and CD79a.[9] The HRS-cells, also express the Pax-5/B-cell-specific activator protein.[10] Additional characteristic finding is the lack of the transcription factor Oct 2 and / or co-activator BOB-1. The latter is critical for the stimulation of immunoglobulin transcription.[11]

The lymphocyte predominant (LP) cells of NLPHL are LCA (CD45) positive, express the B-cell associated antigens CD19, CD20, CD22, CD79a, but are negative for CD30 and CD15, in contrast to the pattern from true HRS cells.[10,12] A subset of LP cells approximately 40% express epithelial membrane antigen (EMA), while the true HRS cells are negative.[10] The CD3 is a marker for T cells and natural killer cells.[13] It is definite for T-cell derivation.

The aim of this study is to assess the diagnostic role of immunohistochemical markers for immunophenotyping of Hodgkin lymphoma on formalin fixed, paraffin embedded sections and to subclassify our cases according to WHO classification and correlate them with age and sex, site of biopsy, and to evaluate the expression of several markers and to compare our results with other geographic regions.

PATIENTS AND METHODS

Tissue materials from 14 cases of Hodgkin lymphoma, 6 males and 8 Females were investigated during 2012-2013 from one laboratory in Sana'a governorate. Only 3 children were ≤14 while others were >14 years. Data of age, sex, site of biopsy were collected from the records of histopathology and immunohistochemistry of the patients. All the slides were reviewed to know the phenotype of HRS cell. The Hodgkin lymphoma cases were grouped according to the WHO classifications.[14] and categorized into 3 groups ≤ 29, 30-59, ≥ 60. The data collected in SPSS data collection sheet version 19 and statistical analysis was carried out using chi square test. P-value ≤ 0.05 considered significant. Tables were prepared to summarize the results.

All the biopsies were fixed in 10% neutral buffered formalin and processed for paraffin embedding, sectioning and staining with H&E. Immunohistochemistry (IHC) was done on 3 µm thick sections of representative tumor areas. Of all cases, histological slides were deparaffinized in xylene, different alcohol series, endogenous peroxidase blockage for 20 min with 3% H_2O_2 methanol, followed by antigen retrieval in pressure cooker (citrate buffer PH 6.0). The sections were placed in phosphate buffer saline (PBS) (PH.7.4). Overnight incubation with primary antibodies at 4◦C, rinsing in PBS 3x5min. Secondary antibody labelled polymers with horseradish peroxidase. Color was developed with Diaminobenzidin (DAB) + Buffer substrate for liquid DAB. Positive and negative control slides were used. The markers used were CD30, CD15, CD3, CD20, LCA, EMA. For CD30 and CD15, both membranous and paranuclear golgi apparatus staining were regarded positive, where as membranous staining is considered positive for the other markers including CD20, LCA,EMA and cytoplasmic staining is considered positive for CD3.

RESULTS

A total of 14 cases were analyzed, all cases were cHL, 6 males (42.9%), 8 females (57.1%), nodular sclerosis 14.3% were males, 42.9% were females with a total of 57.1%, with M:F ratio 1:3, mixed cellularity 21.4% were males and 14.3% were females, with a total 35.7%, with the M:F ratio 1.5:1, unclassified cHL was a male patient 7.1%.

In our study no cases were found in the subtypes nodular lymphocyte predominant Hodgkin lymphoma(NLPHL), cHL lymphocyte rich or lymphocyte depletion. (Table 1) The most common age group affected was ≤ 29 (64.3%), followed by 30-59, (21.4%), (Table.1).

Most of the cases were nodal 92.9% with cervical anatomic localization predominant 50% and only one case extranodal 7.1% in the breast which is a rare location. (Tables 1).

Table 1. Distribution of the subtypes of Hodgkin lymphoma according to age, sex, site.

Age	**NScHL %**	**MCcHL %**	**Unclassified %**	**Total %**	**P-value**
≤ 29	6 (42.9)	3(21.4)	-	9(64.3)	0.820
30-59	1(7.1)	1 (7.1)	1(7.1)	3(21.4)	0.478
≥ 60	1(7.1)	1(7.1)	-	2(14.3)	0.603
Total	**8(57.1)**	**5(35.7)**	**1(7.1)**	**14(100)**	
Sex					
Male	2(14.3)	3 (21.4)	1(7.1)	6(42.9)	0.659
Female	6 (42.9)	2 (14.3)	-	8(57.1)	
Total	**8 (57.1)**	**5(35.7)**	**1(7.1)**	**14(100)**	
Site					
Nodal					
Cervical	3(21.4)	3 (21.4)	1(7.1)	7(50)	0.884
Supraclavicular	-	1(7.1)	-	1(7.1)	0.204
Submandibular	1(7.1)	-	-	1(7.1)	0.572
Inguinal	1(7.1)	-	-	1(7.1)	0.572
Axillary	-	1(7.1)	-	1(7.1)	0.204
Unknown	2 (14.3)	-	-	2(14.3)	0.406
Extranodal					
Breast	1 (7.1)	-	-	1 (7.1)	0.572
Total	**8 (57.1)**	**5(35.7)**	**1(7.1)**	**14(100)**	
Mann-whitney U test					

In respect to phenotyping, CD30 was positive in all the cases. (100%). CD15 was positive in 50% of the cases. In MC subtype CD15 was +ve in 80% of cases and –ve in 20% of the cases. In NS subtype CD15 +ve in 37.5% of cases and –ve in 62.5% of cases. CD20 was Positive in two cases. CD3, LCA, EMA were negative in all cases. (Table 2).

Table 2. Immunohistochemical staining in H & RS cells.

No / Diagnosis	**CD30**	**CD15**	**CD20**	**CD3**	**LCA**	**EMA**
1 MC	+ve	+ve	+ve	-ve	-ve	-ve
2 NS	+ve	-ve	-ve	-ve	-ve	-ve
3 NS	+ve	+ve	-ve	-ve	-ve	-ve
4 NS	+ve	+ve	-ve	-ve	-ve	-ve
5 NS	+ve	-ve	-ve	-ve	-ve	-ve
6 MC	+ve	+ve	-ve	-ve	-ve	-ve
7 unclassified	+ve	-ve	+ve	-ve	-ve	-ve
8 NS	+ve Weak	+ve Strong	-ve	-ve	-ve	-ve
9 NS	+ve	-ve	-ve	-ve	-ve	-ve
10 NS	+ve	-ve	-ve	-ve	-ve	-ve
11 MC	+ve	+ve	-ve	-ve	-ve	-ve
12 MC	+ve	+ve	-ve	-ve	-ve	-ve
13 NS	+ve	-ve	-ve	-ve	-ve	-ve
14 MC	+ve	-ve	-ve	-ve	-ve	-ve

DISCUSSION

Hodgkin lymphoma is one of the most mysterious diseases known. In recent years, immunophenotyping has donated significantly to our understanding the nature and biology of the diagnostic Reed Sternberg cells(RS).[4]

Out of 55 cases of lymphoma, non-Hodgkin lymphoma was (74.5%) and Hodgkin lymphoma was (25.5%) with a ratio 2.9:1. Our results were in agreement to the study done in the middle east region where the incidence of HL was 27% in Saudi Arabia, 21.6% in Jordan and lower than Bahrain 33%, Oman 35% and United Arab Emarates 41%.[15]

All the Hodgkin lymphoma cases were classical HL, the most common subtype was nodular sclerosis 57.1%, Followed by mixed cellularity 35.7%.

Our results were similar to Mustafa et al.[15] in which nodular sclerosis was the most common 63.6% followed by mixed cellularity 20.3%. One study done by Fadhil et

al. found that 78.6% were NSHL. 19% were MCHL and 2.4% was NLPHL.[9]

In contrast with other studies from far east and western countries our results were intermediate. Remarkably, far east incidence of HL vary between 5% -10%.[16]

The patients ranged in age from 10-70, (mean age 29.85). the study of Mustafa S et al. the age ranged 5-81years (mean age 32).[9]

A study done in Scotland and New castle states that cHL still preserves its bimodal distribution. According to this study, the first peak is seen from 15-30 years of age followed by a severe, drop and a second peak in the sixth decade.[17]

In our current study, the most common age group affected was ≤ 29 (64.3%), followed by 30-59 (21.4%). In the study of Fadhil et al.[9] there was bimodal age group distribution in the first 3 decades 59.5% and 28.5% in the 5th & 6th decades with the peak incidence in the third decades 26.2%. The study of Fadhil et al.[9] is similar to our study in the first three decades, but our second peak was absent.

A study from Saudi Arabia noticed dominance of HL cases in children and adolescents, but not the second peak in the elderly, the same as our study.[18]

The prevalence of HL in Asian women is lesser than that of men, but the general pattern is similar[9], in our study the female predominate than males, with the male to female ratio 3:4. Our study contrasts with all other studies done in Duhok in Northern Iraq M:F ratio 3:1, Turky 1.56:1, Jordan 1.5:1, Saudi Arabia 1.4:1, Iraq 1.6:1.[9] This is probably because of the small sample that we were having.

The immunohistochemical valuation of CD markers is an essential parameter in the evaluation and classification of HL. Although the CD marker status provides prognostic information, currently its major clinical value lies in the identification of these markers in HL subtypes, which has led to a rationale for many observations concerning the responses of progressive and recurrent HL subtypes to chemotherapy.[9]

In our current study CD30 was positive in all cases, this is similar to other studies done in Iraq by Fadhil et al. and other studies.[19] Most of the studies realized that CD30 is expressed in HRS cells in a greater proportion compared with CD15.[9]

In our current study CD15 was expressed in 50% of the cases. This result is similar with those stated from India,[4] Ukraine[20] who found CD 15 positivity in approximately 55.5% in India and 58.2% in Ukraine. When compared with a study from china[21] the current study revealed greater rates of CD15 expression, while it is lesser than reports from Egypt.[19] The reason may be ascribed to the properties of different antibodies, variation in the technique of incubation and antigen retrieval.[9]

The background cellular infiltrate of lymph node in HL, with T-lymphocytes predominating over B-cells, are seen in our study and other studies.[22] The cytokines produced T-cells may aid the progression and /or survival of HRS cells.[22] The manufacture and stimulation of numerous other cytokines may also clarify the influx of eosinophils (IL-5, eotaxin) and plasma cells (IL-6).[22] The expression of TGF β may account for fibrosis.[23]

Loss of immunoreactivity for CD15 is an opposing prognostic factor as these cases have a meaningfully poorer overall survival and freedom from treatment failure. Also CD15 negative patients had a greater incidence of reversions, free of other prognostic indicators.[24] Pileri et al.[24] have reported this, one should also ponder that a number of preanalytical variables such as B5 (increase expression of CD15) and neutral formalin (false negative CD15 in some cases) can also affect expression of CD15.[24]

Various studies have revealed positivity for CD20, ranging from 5-50%. The importance of CD20 expression by the RS cells is, as of now, a matter of disagreement. A current study by Portlock et al. decided that the presence of CD20 positive cells in cHL was a poor risk prognostic factors with initial therapy, for time to treatment failure and overall survival.[25] In our study CD20 were seen in two cases.(14.3%). In the study of Patkar et al.[4] CD20 was positive in 15.61% which is similar to our study. The worse prognosis was seen in cases which show positivity for CD20 and failed to express CD15, CD30.[4]

Classical HL cases rich in neoplastic cells may be similar to anaplastic large cell lymphoma (ALCL). Their documentation as classical HL is aided by confirming positivity for the B-cell specific activator protein BSAP (encoded by the PAX5 gene) on the neoplastic cells, because it is constantly negative in ALCL. Negativity for EMA and ALK kinase protein is also helpful.[26,27] The recognition of EBV encoded LMP-1 also in favor of classical HL. The most difficult differential diagnosis is with large B-cell lymphoma displaying anaplastic morphology. There may be a true biological overlap between such cases and cHL.

It is vital to consider immunophenotyping in all cases of HL as immunotherapy with rituximab is being used increasingly in NLPHL and lymphocyte predominant (rich) cHL. Similarly it has been used in deteriorated cases of cHL with favorable results. Immunotherapy with rituximab appears to have indicated a revolution of sorts. At present time the use of anti CD30 antibody (MDX 060) in the treatment of cHL is being investigated.[28,4]

CONCLUSIONS

We concluded that IHC is a very important investigation for HL and other lymphomas and for immunotherapy. The most common subtype of HL was nodular sclerosis with female predominant, followed by mixed cellularity. The most common age group affected was ≤ 29 (64.3%). Most of the cases were nodal with cervical predominant, except one extranodal in the breast. CD30 expressed in all cases, while CD15 expressed in 50%, CD20 expressed in 14.3%.

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