Immunohistochemical study of CD15 expression in CD30+ classical Hodgkin's lymphoma

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الخلاصه

سرطان الغدد اللمفاوية هو نوع هودجكين HL: تعريفه على انه ورم خلايا الـ B-cell والذي يتضمن نوعين متميزيين من الامراض , كلاسيكي وعقدي.

تهدف الدراسة لتقييم التعبير المناعي النسيجي الكيمياوي للـ CD15 في CD30 للنوع الكلاسيكي من HL . هذه الدراسه الاستعاديه تتضمن تنقيح لـ 52 عينة عقد لمفاويه من مرضى حديثي الاصابه والتشخيص لمرض الـ HL للفترة من كانون الثاني 2005 الى اب 2008 .

تم جمعها من مستشفى اليرموك التعليمي ومن مدينة الطب في بغداد ومختبر الاورام الخاص. تمت مراجعة الشرائح الزجاجية واعادة فحصها وكذلك تم تأكيد تشخيص HL باستخدام صبغة CD30 حيث اعطت نتائج ايجابيه في الدراسه الحاليه.

كل الخزعات تم تثبيتها باستخدام الفورمالين وغمرت بشمع البارافين استخدمت مقاطع نسيجيه بقطر 4 مايكرون وصبغت باستخدام الهيماتوكسلين والايوسين وكذلك اضداد CD15 الاحادية النسيله طبقا الي التعليمات المرفقه بالعده الجاهزه .هذه الدراسه اشارت الى ايجابية CD15 الكلاسيكي من HL وجاءت بنسب 6 ,(100%) 10 ,(75%) 24 (100%) and 0 (0%) لكل من MC, NS, LD ,LR على التوالي.

استنتج ان العينات تتطلب من تحليل نسيجي كيمياوي للـ cHL بالاضافه الى مستضدات الـ CD30.

Abstract

Background: Hodgkin lymphoma (HL) is a histologically defined B-cell neoplasm that includes two distinct types of disease, classical and nodular lymphocyte-predominant.

Aim of the study: To evaluate the immunohistochemical expression of CD15 in CD30+ classical Hodgkin’s lymphoma.

Patients and Methods: This retrospective study included revision of 52 lymph nodes specimens from patients newly diagnosed with HL from January 2005 to September 2010.

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These samples were collected from Al-Yarmouk Teaching Hospital, Baghdad Medical City and Al-Atheer private laboratory. Only well-preserved samples that stood a retrospective confirmatory morphological revision and revealed IHC CD30 stain {from DAKO} positive reactivity were included in this study.

All biopsies were formalin-fixed and paraffin embedded. A 4 micron sections obtained and stained with hematoxylin and eosin and CD15 monoclonal antibody {from DAKO} according to the kit-included instructions.

Results: This study revealed that the CD15 positive classical HL are 24 (75%), 10 (100%), 6 (100%) and 0 (0%) for MC, NS, LD and LR respectively.

Conclusions: All cases of cHD should undergo immunophenotypic analysis for CD15 in addition to CD30 antigen.

Introduction

Hodgkin's Lymphoma (HL), formerly known as Hodgkin's disease, is a type of lymphoma first described by Thomas Hodgkin in 1832 [(1)]. It is a histologically defined B-cell neoplasm. The cell of origin is a germinal centre B cell and the disease is defined by the presence of the characteristic neoplastic cells, Reed–Sternberg cells and Hodgkin's cells or their variants, in a setting of inflammatory cells with or without fibrosis [(2)].

HL encompasses two distinct types of disease that differ in etiology, epidemiology, clinical features, pathology and prognosis. They are designated classical HL, which constitutes about 95% of the cases [(3)], and nodular lymphocyte-predominant HL (NLPHL), which constitutes only about 5% of the cases [(4)].

Classical HL is further subdivided into lymphocyte-rich (LR), mixed cellularity (MC), nodular sclerosis (NS) and lymphocyte-depleted (LD) subtypes on the basis of the ratio between neoplastic cells and reactive cells, the specific cytological features of the neoplastic cells and the presence or absence of fibrous bands in the affected lymph nodes [(5)].

HL commences in a single lymphocyte usually in a lymph node and spreads initially by lymphatics to contiguous lymph nodes [2].

HL is nowadays one of the few highly curable adulthood cancers; hence diagnostic accuracy is of utmost significance [6].

The excellent result of treatment of HL with combined chemotherapy is partially offset by treatment induced acute and late toxic, often fatal side effects like hematological and cardiopulmonary toxicities and most of all secondary myelodysplastic syndrome/leukemias and solid tumors [7].

It is therefore of pivotal importance to maintain the high standard of cure rates but at the same time to reduce substantially the toxic burden of tumor reductive strategies by optimizing therapy through better definition of the risk groups [8], i.e., reduction of therapy and its side effects in good risk patients and the identification of those at high risk who require more aggressive treatment and possibly more novel approaches [9].

Antibodies against CD15 and CD30 are often used to support morphological diagnosis of HL. The classical HL is CD15+ and CD30+ in general. However, the results for CD15 are less clear-cut in many studies, showing up to 40% of classical HL that lack positivity for this maker [6]. Lack of CD15 expression in classical HL is an independent negative prognostic factor for relapses and survival. Therefore, immunohistochemistry (IHC) is able to identify classical HL cases with unfavorable clinical outcome [10].

CD30 is very useful for the diagnosis of classical HL as it is almost always positive, yet from the prognosis point of view it would not give much information [11].

CD30 is a cell membrane protein of the tumor necrosis factor receptor family; it is expressed by activated T and B cells. It is a positive regulator of apoptosis, and also has been shown to limit the proliferative potential of autoreactive CD8 effector T cells and protect the body against autoimmunity. CD30 is associated with anaplastic large cell lymphoma, in embryonal carcinoma and on classical Hodgkin Lymphoma cells. However, as the clinical presentation and histopathological picture is distinct for each, then

staining with CD30 can be considered pathognomonic for HL in the proper settings [6].

CD15 (3-fucosyl-N-acetyl-lactosamine), also called Lewis x and SSEA-1 (stage-specific embryonic antigen-1), is a cluster of differentiation antigen representing an immunologically significant molecule. It is a carbohydrate adhesion molecule that can be expressed on glycoproteins, glycolipids and proteoglycans. In neutrophils it mediates phagocytosis and chemotaxis. It is expressed in patients with classical HL, some B-cell chronic lymphocytic leukemias, acute lymphoblastic leukemias, and most acute nonlymphocytic leukemias [12].

CD15 is characteristic, but not specific, for H&RS cells because it can be detected, although rarely, in B and T cell lymphomas and in non-lymphoid tumors [6].

Aim of the study

To evaluate the immunohistochemical expression of CD15 in CD30+ classical Hodgkin's lymphoma.

Patients, materials and methods

This is a descriptive retrospective study included revision of 52 lymph nodes specimens from patients newly diagnosed with HL from January 2005 to September 2010. These samples were collected from Al-Yarmouk Teaching Hospital, Baghdad Medical City and Al-Atheer private laboratory.Only well-preserved, properly labeled samples, with clinical data regarding age, sex, site of lymph node biopsy and histological subtypes that stood a retrospective confirmatory morphological revision and revealed IHC CD30 stain {from DAKO} positive reactivity were included in this study. All cases of nodular lymphocyte predominant HL were excluded.All biopsies were formalin-fixed and paraffin embedded. A 4 micron sections obtained and stained with hematoxylin and eosin and CD15 monoclonal antibody {from DAKO} according to the kit-included instructions. CD30 and CD15 were considered reactive if the Reed–Sternberg cells,

Hodgkin's cells or their variants showed intense cherry red granular cytoplasmic and/or paranuclear or membranous staining.

Expression of CD30 and CD15 is based on a cutoff of more than 10% positivity of HRS cells.

Results

In this study the Male:Female ratio is 1.6:1. Age range from 4-80 years with an age mean of about 29 year.

Forty (77%) out of 52 of the CD30 positive cHL cases are CD15 positive.

The descriptive statistics of HD patients are listed in table 1.

Table 1: Descriptive statistics of HL patients included in this study:

Parameter				HD patients (n=15)	
				No.	%
Sex	MC, LD and LR	Males		30	63.8
		Females		17	36.2
	NS	Males		2	40
		Females		3	60
Age groups (years)		1-10 (n=5)	Males	3	60
			Females	2	40
		11-20 (n=17)	Males	10	58.8
			Females	7	41.2
		21-30 (n=10)	Males	6	60
			Females	4	40
		31-40 (n=7)	Males	5	71.4
			Females	2	28.6
		41-50 (n=6)	Males	5	83.3
			Females	1	16.7
		51-60 (n=3)	Males	1	33.3
			Females	2	67.7
		61-70 (n=2)	Males	2	67.7
			Females	1	33.3
		71-80 (n=1)	Males	0	0
			Females	1	100
Site of lymph node biopsy used for initial diagnosis		Cervical		35	67.3
		Axillary		6	11.5
		Supraclavicular		5	9.6
		Inguinal		2	3.8
		Submandibular		2	3.8
		Mesenteric		1	1.9
		Mediastinum		1	1.9
Reactivity to CD15 according to WHO classification of classical HL	Classical HL (CD30 +)	MC (n=30)	-	24	75
				8	25
		NS (n=10)	-	10	100
			-	0	0
		LD (n=6)		6	100
			-	0	0
		LR (n=4)	-	0	0
				4	100

Discussion

Hodgkin's lymphoma (HL) is a rare malignancy; nevertheless, its prognosis is very good with the majority of patients are cured with the current therapy [6].

In this study the incidence of HL was higher in males than in females, and this finding is in agreement with published statistics by Iraqi Ministry of health in 2001 [13], and in 1999 [14].

The incidence of HL was having a descending age peaks, starting its highest peak in the second decade of life. This is actually not very typical, and the explanation may be related to the small HL sample size.

The sites of biopsy used for the initial diagnosis of the disease were all lymph nodes, with the cervical lymph nodes being the most common. No extranodal biopsy sites were used in this study and this can be considered typical for HL.

In this study 77% of the CD30 positive cHL are also CD15 positive, i.e., 23% are CD15 negative. While abroad the percentage of cHL immunophenotype that has both CD30+ and CD15+ are 80-88% and only 12-20% has CD30+ but lack CD15 expression [6, 15]. This finding that the CD15 negative cHL is slightly more common in Iraq may impact negatively on prognosis as the clinical follow up revealed significant differences for freedom from treatment failure and overall survival between cases with typical immunophenotype and those with CD15 negativity.

Conclusions

All cases of cHD should undergo immunophenotypic analysis for CD15 in addition to CD30 antigen.

Recommendations

To perform a further confirmatory study to the findings of this research and to study cases of cHD for immunophenotypic expression of other markers that could affect prognosis as for CD20.

References

1. Bonadonna G: Historical review of Hodgkin disease. British Journal of Haematology, 2000; 110, 504-511.
2. Estella M, Barbara B, Andrew W: Lymphoid malignancies. 1st ed. 2007; Oxford clinical publishing.
3. Stein H, DelSol G, Pileri S, et al: Classical Hodgkin lymphoma. Pathology and Genetics of Tumours of Haematopoietic and Lymphoid Tissues, 2001; IARC Press.
4. Stein H, DelSol G, Pileri S, et al: Nodular lymphocyte predominant Hodgkin lymphoma. Pathology and Genetics of Tumours of Haematopoietic and Lymphoid Tissues, 2001; IARC.
5. John K: The new World Health Organization classification of lymphomas: the past, the present and the future. Hematological oncology, 2001; 19(4): 129-150.
6. Pileri S, Ascani S, Leoncini L, et al: Hodgkin's lymphoma: the pathologist's viewpoint, Review article. Journal of Clinical Pathology, 2002; 55: 162-176.
7. Diehl V, Klimm B, Re D: Hodgkin lymphoma: a curable disease: what comes next? European Journal of Haematology, 2005; 75 (Suppl. 66): 6-13.
8. Volker D., Harald S., Michael H., Raphael Z., Joseph M.C.: Hodgkin's lymphoma: Biology and treatment strategies for primary, refractory, and relapsed disease. Hematology, 2003.
9. Stein H., Marafioti T., Foss H.D., Helmut L., Michael H., Ioannis A., et al: Downregulation of BOB1, OBF2 and Oct2 in classical Hodgkin's disease but not in lymphocyte predominant Hodgkin's disease correlates with immunoglobulin transcription. Blood, 2001; 97: 496-501.
10. Alexandar T, Jens K, Falko F, et al: Prognostic Significance of CD20 Expression in Classical Hodgkin Lymphoma: A Clinicopathological Study of 119 Cases. Clinical cancer research, 2003; 9: 1381-1386.
11. Carol S, Gerard B, Jing Q: Adverse prognostic significance of CD20 positive Reed–Sternberg cells in classical Hodgkin's disease. British Journal of Haematology, 2004; 125: 701-708.

12. Nakayama F, Nishihara S, Iwasaki H, et al. CD15 expression in mature granulocytes is determined by alpha 1,3-fucosyltransferase IX, but in promyelocytes and monocytes by alpha 1,3-fucosyltransferase IV. Journal of Biological Chemistry, 2001; 276 (19): 16100-161006.
13. Ministry of health, Iraqi cancer board, Iraqi cancer registry center. Results of Iraqi cancer registry 1998-2000. 2001.
14. Ministry of health, Iraqi cancer board, Iraqi cancer registry center. Results of Iraqi cancer registry 1995-1997. 1999.
15. von Wasielewski R, Mengel M, Fischer R, et al: Classical Hodgkin's disease: clinical impact of the immunophenotype. American Journal of Pathology, 1997; 151: 1123-1130.