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RESEARCH ARTICLE

SIGNIFICANCE OF CD3 AND CD20 IN DIAGNOSIS OF NON-HODGKIN LYMPHOMA IN SUDANESE PATIENTS

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ABSTRACT

Aim: The aim of this study was to evaluate the significance and importance of CD3 and CD20 in the diagnosis of lymphoma in formalin-fixed paraffin-embedded tissue sections in Sudanese patients.
Study design: Cross-sectional descriptive study.
Place and Duration of Study: Departments of histopathology at Radiation and Isotopes Center at Khartoum (RICK) and at the National Health Laboratoryduring the period between September 2015 and May 2016.
Material and Methods: Tissue sections obtained from 100 formalin-fixed paraffin-embedded tissue blocks of lymphoma lesions were immunohistochemically stained using monoclonal antibodies for CD3 and CD20.
Results: CD20 was positive in all B cell lymphomas and negative in all T cell lymphomas, while CD3 is positive in all T cell lymphomas and negative in all B cell lymphomas.
Conclusion: Immunohistochemistry is an important tool in diagnosis of lymphoma and differentiation between B cell lymphoma and T cell lymphoma.

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INTRODUCTION

Immunohistochemistry is one of the most important methods to be used for diagnosis and classification of lymphoma (Jaffe *et al*., 2008). As diagnostic biomarkers, CD20 and CD3 are among the commonest specific leukocyte markers that have been routinely used in the identification and assessment of lymphoid neoplasms (Pilozzi *et al*, 1998). The application of CD20 as B-cell marker and CD3 as T-cell marker is currently strongly recommended for assessment of lymphoproliferative diseases (Xiao *et al*., 2010). CD3 complex is closely associated at the lymphocyte cell surface with the T-cell antigen receptor. CD3 antigen is a highly specific marker for T-cells, and is present in majority of T-cell neoplasms, but is absent in B-cells (Anderson *et al*., 1991) CD20 is a transmembrane protein, with a molecular weight of 35 to 37 kDa, which is expressed early during B cell development and lost during terminal B cell differentiation into plasma cells (Magro *et al*, 2006). CD20 is classified as a pan-B cell marker, and its presence on benign and neoplastic lymphocytes is generally considered specific for B-lineage. However, recent studies have indicated that peripheral T cell lymphomas rarely express CD20 (Buckner *et al*., 2007). Only few cases of CD20-positive NK/T-cell lymphoma have been reported in the literature (Mohrmann and Arber, 2000; Balmer *et al*., 2009; Yokose *et al*., 2001; Oshima *et al*., 2009; Venizelos *et al*., 2008). Because CD20expression in T cell lymphoma is rare, obtaining a correctdiagnosis of this type of CD20 positive lymphoma can bedifficult. Accordingly, because misdiagnosis has a substantial impact on therapeutic strategy, careful morphologic evaluation and wide range of immunophenotypictools and molecular genetic studies must be employed toachieve an accurate diagnosis (Venizelos *et al*., 2008). Here, we report the expression of CD3 and CD20 in paraffin-embedded formalin-fixed tissue sections obtained from lymphoma lesions of Sudanese patients at Khartoum, the capital of Sudan, to evaluate their significance in lymphoma diagnosis in Sudan.

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MATERIALS AND METHODS

A total of 100 formalin-fixed paraffin- embedded tissue blocks from lymphoma lesions were included in this study. They were

obtained from the histopathology archives of the Radiation and Isotopes Center at Khartoum (RICK) and National Health Laboratory during the period between September 2015 and May 2016. Clinical data of patients was obtained from the hospital records. Using a rotary microtome, three tissue sections of 3µm- thick were cut from each block, one slidewas stained by Mayer's hematoxylin and eosin stain forconfirmation of the diagnosis obtained from the records, one salinized slide (Thermo) was stained using monoclonalantibody for CD3, and the third salinized slide was stained usingmonoclonal antibody for CD20. The immunohistochemical procedure was done as follows: each section on salinized slide was exposed to deparaffinization in xylene; slide was then rehydrated through graded series of alcohol and placed in distilled water. Sample was steamed for antigen retrieval for CD3 or CD20 using water bath. Endogenous peroxidase activity was blocked with 3% hydrogen peroxide and methanol for 10 min, and then the slide was incubated with 100-200 µl of primary antibody for 20 min at room temperature in a moisture chamber. After washing with PBS for 3 min, binding of antibody was detected by incubating for 20 minutes with dextran labeled polymer ((Thermo -ultra vision). Finally, the sectionwas washed in three changes of PBS, followed by adding 3, 3 diaminobenzidine tetra hydrochloride (DAB) as a chromogen to produce the characteristic brown stain for the visualization of the antibody/enzyme complex for up to 5 min. The slide was then counterstained with heamatoxylin and read under a light microscope.

RESULTS AND DISCUSSION

Most patients in this study (51%) were above 40 years of age (table No 1). Males were 56 and females were 44.That is consistent with several studies dealt with lymphoma (Chalterjee *et al*., 2004; Yagi *et al*., 1984; Tumwine *et al*., 2009). B cell lymphomas were 96% of cases and only 4% were T cell lymphomas (Ross and Oliver, 2010).

Table 1. Frequency of lymphoma cases within age groups

Type of lymphoma /Age groups	< 10 years	10 – 40 years	41 – 70 years	> 70 years	Total
B cell lymphoma	11	28	47	10	96
T cell lymphoma	0	0	4	0	4
Total	11	28	51	10	100

Majority of sites of lesions obtained (63%) were lymph nodes (Table 2) (Swerdlow *et al*., 2016).

Table 2. Frequency of lymphoma cases in different body sites

Type of tumor /Site of lesion	Lymph node	Abdominal mass	Colon mass	Gastric mass	Other sites	Total
B cell lymphoma	61	9	5	2	19	96
T cell lymphoma	2	0	0	0	2	4
Total	63	9	5	2	21	100

CD20 is positive in all B cell lymphomas (96%) and negative in all T cell lymphomas (4%), while CD3 is positive in all T cell lymphomas (4%) and negative in all B cell lymphomas (96%) (Table No 3) (Echeverri *et al*., 2002; Dave *et al*., 2006; Teeling *et al*., 2004).

Table 3. Marker expression (CD3 and CD20) in both types of lymphoma

Marker expression /Type of lymphoma	B cell lymphoma	T cell lymphoma	Total
Positive CD20	96%	0%	96%
Negative CD20	0%	4%	4%
Positive CD3	0%	4%	4%
Negative CD3	96%	0%	96%

P. value = 0.000

Correlation of age groups with marker expression (Table 4) or type of lymphoma (Table 5) was insignificant.

Table 4. Correlation of age groups with expression of CD3 and CD20

Expression/Age groups	< 10 years	10 – 40 years	41 – to 70 years	> 70 years	Total
Positive CD3	0	0	4	0	4
Negative CD3	11	28	47	10	96
Positive CD20	11	28	47	10	96
Negative CD20	0	0	4	0	4

P. value = 0.136

Table 5. Correlation of age groups with both types of lymphoma

Type of tumor/Age group	< 10 years	10 – 40 years	41 – to 70 years	> 70 years	Total
B cell lymphoma	11	28	47	10	96
T cell lymphoma	0	0	4	0	4
Total	11	28	51	10	100

P. value = 0.136

Conclusion

Immunohistochemistry by using CD3 and CD20 is an important tool in diagnosis of non-Hodgkin lymphoma and differentiation between B cell lymphoma and T cell lymphoma in our locality, as the case in different parts of the world.

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