عمـــادة البحـــث العلمـــي
DEANSHIP OF SCIENTIFIC RESEARCH

Sudan Journal of Science and Technology
Journal homepage: http://jst.sustech.edu/

Expression of MUM1, CD30, CD15 and CD20 by neoplastic cells of Lymphomas

Abu Elgasim Abass Awad Elkareem [*1] and Nada Mohammed Ahmed Abdalla [1]
Histopathology and Cytology, College of Medical Laboratory Science, Sudan University of Science and Technology, Khartoum – Sudan Email: gassomy2@gmail.com

ARTICLE INFO

Article history
Received:05.07.2015
Accepted: 15.07.2015
Available online:01.10.2015

Keywords:
Lymphoma, MUM1, CD30, CD15, CD20.

ABSTRACT

In a descriptive retrospective study aiming to detect MUM1, CD30, CD15 and CD20 expression among lymphoma patients, immunohistochemistry was used. Thirty formalin fixed paraffin embedded blocks specimens (FFPB) were prepared for this study. Sections were cut by rotary microtome then stained by immunohistochemical method (new indirect method). The ages of the involved patients ranged between one year to75 years with mean age 28 (±23.3) years. Most of the patients were aggregating at age older than 41 years representing 18 (60%) and the remaining 12 (40%) were younger than 41 years. Twenty (66.7%) of the patients were males and 10 (33.3%) were females. Histopathological diagnosis of the samples revealed diffuse large B-cell non Hodgkin's lymphoma's (NHL) in 18 (60%) patients, five (16.7) patients were mantle NHL and the remaining 7 (23.3%) patients were classical Hodgkin's lymphoma's (HL). MUM1 showed positive expression in 15 (50%) patients, and negative in 15 (50%) patients. The relation between histopathological diagnosis and MUM1 expression showed 10 (66.7%) positive patients and 8 (53.3%) negative patients among diffuse large B-cell NHL. Five (33.3%) positive patients and 2 (13.3%) negative patients were classical HL, five (33.3%) has no reaction with MUM1 protein represented as mantle cell NHL with statistical significance (P value < 0.05). Among the study subjects CD30 and CD15 were positive in 7 (23.3%) patients, and negative in 23 (76.7%) patients. CD20 was positive in 23 (76.7%) patients, and negative in 7 (23.3%) patients. CD20 status showed positive

expression in 23(76.6%) patients and negative in 7 (23.3%), with statistical significance (P value < 0.05). It is conclude that MUM1 receptor, CD30, CD15 and CD20 are associated with lymphomas type.

INTRODUCTION

Lymphomas are a heterogeneous group of hematologic malignancies that originate in lymphoid tissues. A lymphoma may arise within a single or multiple lymph nodes or in extranodal sites. They are commonly involving the lymphoid tissue of the gastrointestinal tract and central nervous system. The two major types of lymphomas are Hodgkin's and non Hodgkin's lymphomas (NHL) (Koda-Kimmle, 2009). It is estimated that these hematologic malignancies account for >60,000 new cases annually, with seven time as many patients affected by non Hodgkin's lymphoma compared with Hodgkin's disease (Koda-Kimmle, 2009). The risk factors that have been identified are sex, age, race, occupational exposures, immune system deficiency, Helicobacter pylori (Xue *et al*., 2001), and relation to infections (e. g Epstein Bar Viruse) (Parkin, 2011).

The diagnosis of lymphomas is made by histological examination of the neoplastic lymphocytes. The low- power architecture of the excised lymph node gives important clues to the diagnosis (David and Robin, 2008). Furthermore blood test, imaging, molecular techniques, cytogenetic, flow cytometry, and immunohistochemical analysis are used (Hu *et al*., 1985). Immunohistochemistry is a technique using microscopy to characterize the cells in tissue sample. It involves the use of labeled antibodies to detect and localize or expression of biomarkers (Ramos-vara, 2005). Immunohistochemistry is helpful in tumor diagnosis of difficult neoplasm and reclassification for these tumors. The methodology is relatively simple, and under stringent condition fairly reliable (Hayat, 2002). MUM1 has received attention in the last years through its inclusion in immunohistochemical profiles for the identification of the diffuse large B-cell lymphoma (DLBCL) subtype with a non-germinal center-like phenotype (Hans *et al*., 2004). MUM1 has been proposed to play an important role in mediating B-cell activation and differentiation (Uranishi *et al*., 2005). MUM1/IRF4 is expressed in the nuclei and cytoplasm of plasma cells and a small percentage of germinal centers (GC) B-cells located in the "light zone." This antibody stains MUM1 protein, which is expressed in a subset of B-cells in the light zone of the germinal center, plasma cells, activated T-cells and a wide spectrum of related hematolymphoid neoplasms derived from these cells (Falini *et al*., 1998). Therefore this antibody is useful for the sub classification of lymphoid malignancies and an excellent marker for Hodgkin's and Reed-Sternberg cells of classic Hodgkin's disease in combination with anti-CD30 (Falini *et al*., 1998). MUM1 is expressed in late GC or post-GC B-cells and plasma cells but in contrast to what might be expected, MUM1 expression is not always associated with mutated IGHV, being observed in both mutated and non-mutated immunoglobulin heavy chain variable B-cell neoplasms (Craig *et al*., 2008).

There are few survey studies evaluating MUM1 in B-cell lymphomas, so the

incidence of MUM1 expression in lymphomas has not been well studied. Martinez *et al*., (2008) found that 3 in 28 cases (11%) of MUM1 expression in mantle cell lymphoma. On the other hand, Tumwine *et al*., (2008) reported an absence of MUM1 expression in 4 cases of mantle cell lymphoma studied. Cluster of differentiation (CD) markers are a group of special molecules on the surface of the cells in the body. There are more than 250 types of CD molecules. All cells in the body have one or more of them, but they are most useful for classifying white blood cells, lymphomas and leukemias are cancers of WBCs (Teng *et al*., (2005).

MATERIALS and METHODS

SLIDES PREPARATION

One section of 5µm thickness were obtained from formalin fixed paraffin embedded tissue (previously diagnosed by H&E stain) using a rotary microtome and taken in thermal coated slide and dried in hot plate oven at 80ºC for one hour (Bancroft and Marilyn, 2008).

IMMUNOHISTOCHEMICAL STAINING

Sections were brought to water and retrieved in water bath retrieval at 97ºC, then treated with hydrogen peroxide solution for fifteen minutes and washed in phosphate buffer saline (PBS) (PH 7.4) for five minutes, treated with anti- MUM1, CD30, CD15 or CD 20 primary antibodies (in separate slides) for thirty minutes, rinsed in PBS. Sections were treated with secondary polymer conjugate for thirty minutes and rinsed in phosphate buffer saline, treated with 3,3'-diaminobenzidine (DAB) for seven minutes, washed in phosphate buffer saline for five minutes, then counterstained in Mayer's haematoxylin for one minute and washed in water and blued in 0.05% ammoniated water for 16 second, then washed in tap water, dehydrated through ascending grades of ethanol (50%, 70%, 90%, 100%) two minutes for each then cleared in two change of xylene two minutes for each, and mounted in DPX mounting media (Bancroft and Marilyn, 2008).

RESULTS

Histopathological diagnosis revealed diffuse large B-cell NHL in 18 (60%) patients, five (16.7%) patients were mantle NHL and the remaining 7 (23.3%) patients were classical HL (Table 1). MUM1 receptor status showed positive expression in 15 (50%) patients, and negative in 15 (50%) patients (Table 2). CD30 and CD15 showed similar expression among patients. They were positive in 7 (23.3%) patients, and negative in 23 (76.7%) patients. CD20 receptor status was positive in 23 (76.7%) patients, and negative in 7 (23.3%) patients (Table 3). Histopathological diagnosis and MUM1 receptor expression showed positive in 10 (66.7%) patients and negative in 8 (53.3%) patients among diffuse large B-cell NHL. Five (33.3%) positive and 2 (13.3%) negative were classical HL. Five (33.3%) has no reaction with MUM1 protein were mantle cell NHL with significant association ($P < 0.05$) (Table 4). The histopathological diagnosis and CD30, CD15 and CD20 receptors status showed that 7 (23.3%) of demonstrated samples were positively expressed CD30 and CD15 marker, were CHL. 23 (76.7%) patients were CD20 positive were diffuse large B cell NHL with statistical significant association ($P < 0.05$) (Table 5).

Table (1): Distribution of the study subjects by histopathological diagnosis.

Diagnosis	Frequency	Percentage (%)
Diffuse large B-cell NHL	18	60.0
Mantle cell NHL	5	16.7
Classical HL	7	23.3
Total	30	100%

Table (2): Distribution of MUM1 receptors status among the study subjects

MUM1	Frequency	Percentage (%)
Positive	15	50
Negative	15	50
Total	30	100%

Table (3): Expression of CD30, CD15 and CD20 receptors among the study subjects.

Variable	Positive	Negative	Total
CD30	7 (23.3%)	23 (76.7%)	30 (100%)
CD15	7 (23.3%)	23 (76.7%)	30 (100%)
CD20	23 (76.7%)	7 (23.3%)	30 (100%)

Table (4): Relation between the expression of MUM1 protein and histopathological diagnosis.

Diagnosis	MUM1		Total
	Positive	Negative	
Diffuse large B-cell NHL	10 (33.3%)	8 (26.7%)	18 (60.0%)
Mantle cell NHL	0 (0%)	5 (16.7%)	5 (16.7%)
Classical HL	5 (16.7%)	2 (15.0%)	7 (23.3%)
Total	15 (50%)	15 (50%)	30 (100%)

P value= 0.039

Table (5): Distribution of CD30, CD15 and CD20 receptors status by histopathological diagnosis.

Variable	Category	CD30	CD15	CD20
Diffuse large B-cell NHL	Positive	0	0	18
	Negative	18	18	0
Mantle cell NHL	Positive	0	0	5
	Negative	5	5	0
Classical HL	Positive	7	7	0
	Negative	0	0	7
P value		0.00	0.00	0.00

DISCUSSION

In this study most patients were aggregating at age ranges older than 41years. This result showed that there was an increase susceptibility of lymphomas involvement with increase of age. This finding was supported with Gelfand *et al*., (2006) who reported that the older people (older than age 65) may have a slightly higher risk of developing lymphoma than younger people. Also Armitage *et al*., (2008) reported that non Hodgkin's lymphoma can develop in people of all ages, including children; it is most common in adults. The most common types of NHL usually appear in people in their 60 years and 70 years.

The present study demonstrates that there was an increase in number of males compared to females (2:1). This result supported by Alexander *et al*., (2007) who reported that women are known to have a lower incidence of non-Hodgkin lymphoma (NHL) than men (15.8 cases/100,000 person years versus 23.2/ 100,000 person per year from 1998 to 2002). It also agreed with Shenoy *et al*., (2011), who reported that the Hodgkin's lymphoma is nearly twice as common in males, it is higher in males than females.

MUM1 expression is restricted to cells in lymphocytic and melanocytic lineages. Furthermore, MUM1 expression was found to be higher in plasma cells among B lineage and in activated T cells among T lineage lymphocytes as determined by expression analysis of various hematopoietic cell lines. MUM1 receptor status was shown in half positivity of samples; it seemed that MUM1 are expressed in some type of lymphomas. This finding is compatible with Tsuboi *et al*., (2000) who reported the expression pattern of MUM1 in normal lymphoid tissues as well as in lymphoma and other lymphoid malignancies. This result also agreed with Gaidano (2000) who reported that MUM1 in lymphoid neoplasms, in accordance with its expression at late stages of B-cell differentiation, and after T-cell activation, MUM1 protein is strongly expressed in lymphoplasmacytic lymphoma, myeloma, DLBCL, primary effusion lymphoma, Hodgkin lymphoma and anaplastic large cell lymphoma.

CD30 receptor is expressed by activated, but not by resting, T and B cells. TNF receptor-associated factor 2 (TRAF2) and TNF receptor-associated factor 5 (TRAF5) can interact with this receptor, and mediate the signal transduction that leads to the activation of NF-kappa B. 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. CD15 are expressed in patients with Hodgkin's disease, some B-cell chronic lymphocytic leukemias, acute lymphoblastic leukemias, and most acute nonlymphocytic leukemias. CD20 is a marker for B-cells, a type of white blood cell (WBC) that protects the body from infections. There are two types of WBCs - B-cells and T cells. CD20 is present on the surface of B-cells but not T-cells. Our study showed strong relation between CD30 and CD15 and CD20 and subtypes of lymphoma. This study supported with Teng *et al*., (2005) who reported that CD30 is associated with anaplastic large cell lymphoma. Also Gorczyca *et al*., (2003) reported that CD30 and CD15 are also expressed on classical Hodgkin Lymphoma. This report also agreed with Swerdlow *et al*., (2003), who reported that in pathological conditions, CD30 is found at variable levels in different lymphomas of B-cell or T-cell derivation, and in several reactive conditions. However, strong and homogeneous CD30 expression in most neoplastic cells is restricted to fewer entities, mainly three groups of lymphoid neoplasms in classical Hodgkin's lymphoma, anaplastic

large cell lymphomas and primary cutaneous CD30+ T-cell lymphoproliferative disorders. Also this study supported with Olejniczak *et al*., (2006) who found that CD20 expression in DLBCL also showed marked variability and that some samples had dim CD20, similar to that of CLL.

REFERENCES

Alexander, D.D., Mink, PJ., Adami, H.O., Chang, E.T., Cole, P., Mandel, J.S., Trichopoulos, D., (2007). The non-Hodgkin lymphomas: a review of the epidemiologic literature. *International Journal of Cancer*: 120:1–39.

Armitage, J.O., Wyndham, H.W., Non-Hodgkin's lymphoma. In: Abeloff MD, Armitage, J.O., Niederhuber, J.E., Kastan, M.B., McKena, W.G., (2008). *Clinical Oncology*. 4th ed. Philadelphia, Pa: Elsevier Churchill Livingstone

Bancroft, J.D., Marilyn, G., (2008). *Theory and practice of histological techniques*, 6th ed, Churchill Livingstone, London, 125.

Craig, F., Soma, L., Melan, M., Kant, J., Swerdlow, S., (2008). MUM1/IRF4 expression in the circulating compartment of chronic lymphocytic leukemia. *Leuk Lymphoma*: 49:273–80.

David, A., Robin, R., (2008). *Muir's textbook of pathology* 4th ed, Edward Arnold London 2008.

Falini, B., Bigerna, B., Fizzotti, M., Pulford, K., Pileri, S.A., Delsol, G., (1998). ALK expression defines a distinct group of T/Null lymphomas (ALK lymphomas) with a wide morphological spectrum. *Am J Pathol*: 153:875.

Gaidano, G., and Carbone, A.. (2000). MUM1: a step ahead toward the understanding of lymphoma histogenesis. *Leukemia*: 14:563–566.

Gelfand, JM.. Shin, D.B., Neimann, A.L., Wang, X., Margolis, D.J., Troxel, A.B., (2006). The Risk of Lymphoma in Patients with Psoriasis. *The Journal for Investigative Dermatology*. 126: 2194-2201

Gorczyca, W., Tsang, P., Liu, Z.. Wu, CD., Dong, H.Y., Goldstein, M., Cohen, P., Gangi, M., Weisberger, J., (2003). CD30-positive T-cell lymphomas co-expressing CD15: an immunohistochemical analysis. *Int. J. Oncol*. (2): 319–24.

Hans, C.P., Weisenburger, D.D., Greiner, T.C., Gascoyne, R.D., Delabie, J., Ott, G., Müller-Hermelink, HK., Campo, E., Braziel, R.M., Jaffe, E.S., Pan, Z., Farinha, P., Smith, L.M., Falini, B., Banham, A.H., Rosenwald, A., Staudt, L.M., Connors, J.M., Armitage, J.O., Chan, W.C,. (2004). Confirmation of the molecular classification of diffuse large B-cell lymphoma by immunohistochemistry using a tissue microarray. *Blood*: 103:275–282.

Hayat, M.A., (2002). *Microscopy, Immunohistochemistry, and antigen retrieval methods: for high and electron microscopy*. 1st ed. Springer: p1- 11.

Hu, E., Trela, M., Thompson, J., (1985). Detection of B-cell lymphoma in peripheral blood by DNA hybridisation. *Lancet*: 16:1092–5.

Koda-Kimmle, M.A., (2009). *Applied therapeutics: The clinical use of drugs*. Lippincott Williams & Wilkins Philadelphia, Pennsylvania

Martinez, A., Pittaluga, S., Rudelius, M., Davies-Hill, T., Sebasigari, D., Fountaine, T.J., Hewitt, S., Jaffe, E.S., Raffeld, M., (2008). Expression of the interferon regulatory factor 8/ICSBP-1 in human reactive lymphoid tissues and B-cell lymphomas: a novel germinal center marker. *Am J Surg Pathol*: 32(8):1190–200.

Olejniczak, S.H., Stewart, C.C., Donohue, K., Czuczman, M.S., (2006). A quantitative exploration of surface antigen expression in common B-cell malignancies using flow cytometry. *Immunol Invest*: 35:93-114.

Parkin, DM., (2011). Cancers attributable to infection in the UK in 2010. *Br J Cancer*: 105 (2):S49-S56

Ramos-vara, J.A., (2005). Technical aspects of immunohistochemistry. *Vet pathol*: 42: 405-426.

Shenoy, P., Maggioncalda. A., Malik, N.. and Flowers, CR., (2011). Incidence patterns and outcomes for Hodgkin's lymphoma patients in the United States, *Advances in Hematology* 2011:10.11

Swerdlow, A.J., (2003). Epidemiology of Hodgkin's disease and non-Hodgkin's lymphoma. *Eur J Nucl Med Mol Imaging*: 30 Suppl 1:S3-12.

Teng, L.H., Lu, D.H., Xu, Q.Z., Fu, Y.J., Yang, H., He, Z.L., (2005). Expression and diagnostic significance of OCT4, CD117 and CD30 in germ cell tumors (in Chinese). *Zhonghua Bing Li Xue Za Zhi* 34 (11): 711–5.

Tsuboi, K.. Iida. S.. Inagaki, H., Kato, M., Hayami, Y., Hanamura, I., Miura, K., Harada, S., Kikuchi, M., Komatsu, H., Banno, S., Wakita, A., Nakamura, S., Eimoto, T., Ueda, R., (2000). MUM1/IRF4 expression as a frequent event in mature lymphoid malignancies. *Leukemia*: 14(3):449–56.

Tumwine, LK., Campidelli, C., Righi, S., Neda, S., Byarugaba, W., Pileri, S.A., (2008). B-cell non-Hodgkin lymphomas in Uganda: an immunohistochemical appraisal on tissue microarray. *Hum Pathol*: 39:817–823.

Uranishi, M., Iida, S., Sanda, T., Tajima, E., Ito, M., Komatsu, H., Inagaki, H., Ueda, R., (2005). Multiple myeloma oncogene 1 (MUM1)/interferon regulatory factor 4 (IRF4) upregulates monokine induced by interferon-gamma (MIG) gene expression in B-cell malignancy. *Leukemia*: 19:1471–1478.

Xue, F.B., Xu, Y.Y., Wan, Y., Pan, B.R., Ren, J., Fan, D.M., (2001). Association of H. pylori infection with gastric carcinoma: a Meta analysis. *World J Gastroenterol*: 7(6):801-4.