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Detection of Cyclin D1 Protein and Ki67 among Sudanese B lymphoma Patients

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Abstract:

This is a descriptive retrospective case study aimed to detect cyclin d1, Ki67 index among lymphoma patients by using immunohistochemical method.

Fifty formalin fixed paraffin embedded blocks (FFPB) specimens were selected 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 4 years to 80 years with mean age 51 years. Most of the patients were aggregating at age older than 40 years representing 39 (78%) and the remaining 11 (22%) were younger than 40 years. Thirty three (66%) of the patients were males and 17 (34%) patients were females. Histopathological diagnosed of samples revealed that 20 (40%) samples were diffuse large B cell lymphoma, 13(26%) samples were small lymphocytic lymphoma, 8 (16%) samples were Mantle lymphoma, 5(10%) samples were follicular lymphoma and 4(8%) samples were Burkitt lymphoma patients.

Cyclin d1 expression showed positive in 2 (4%) samples and negative in18 (36%) samples among diffuse large B cell lymphoma, among small lymphocytic lymphoma showed 3 (6%) positive samples and 10 (20%) negative samples were, all Mantle lymphoma samples

showed positive result 8 (16%), 2 (4%) positive samples and 3 (6%) negative samples among follicular lymphoma and no expression among Burkitt lymphoma with statistical significance association (P value = 0.00).
Ki67 index showed that diffuse large B cell lymphoma mean (50 ±11.5), small lymphocytic lymphoma mean (20± 16), Mantle lymphoma mean (55 ±3), Burkitt lymphoma mean (97± 11), and follicular lymphoma mean (30± 22) with statistical significance association (P value = 0.00). The study concluded that cyclin d1 and Ki67 index are associated with B lymphomas type.

Key words: B lymphoma, cyclin d1, Ki67

INTRODUCTION:

The lymphomas is heterogeneous group of hematological malignances that originate in lymphoid tissues, lymphoma may arise with in single multiple lymph nodes or extra nodal sites commonly involving the lymphoid tissue of GIT and CNS, the two major type of lymphomas Hodgkin's and non Hodgkin's lymphoma[(1)].

Each year, more than 500000 individuals worldwide are diagnosed with non- Hodgkin's lymphoma (NHL), making it the most common hematologic malignancy [(2)].

NHLs are broadly classed as B cell or T cell lymphomas, depending on the lymphocyte linage that give rise to the malignancy. B-cell lymphomas represent approximately 90% of NHLs whereas T-cell lymphomas represent approximately10% [(2)].

In Sudan, little work has been done concerning NHLs. In Soba teaching hospital (Sudan) during the period 1979-1989 they found that there were 1205 patients with malignancy, 51 patients of them with NHLs (comprising 5.4% of all malignant tumors). The male-female ratio was 4.1:1, the age of patients

ranged between few months to 90 years old, and the age group (40-70) years show higher frequency of NHLs [3].

The risk factors that have been identified are sex, age, and race, occupational exposures, immune system deficiency, helicobacter pylori [4] and relation to infections (e.g. EBV) [5].
The diagnosis of lymphoma is made is by histological examination of neoplastic lymphocytes and low –power architecture of excised lymph node give important clues to the diagnosis blood test imaging, molecular techniques, cytogenetic [6]. flowcytometry, and immunohistochemical analysis [7]. The sub typing of B-cell lymphomas is often confirmed by using immunohistochemistry to characterize the patrrern of expression of several biomarkers in patient tissue sample, it classified to diffuse large B cell, follicular lymphoma, small lymphocytic lymphoma, Mantle cell lymphoma, marginal zone b cell lymphoma, mucosa –associated lymphoid tissue, spleenic marginal zone, and Burkitt lymphoma [8].

Immunohistochemistry is technique using microscopy to characterize the cell in tissue sample it involves the use of labeled antibodies to detect and localize the expression of biomarker [9].Immunohistochemistry is helpful in tumor diagnosis of difficult neoplasm and reclassification of these tumor, the methodology is relatively simple, and under stringent condition fairly reliable [10].

Cyclins function as regulators of CDK kinesis, different cyclins exhibit distinct expression and degradation patterns which contribute to the temporal coordination of each mitotic event, this cyclin forms a complex with and functions as a regulatory subunit of CDK4 or CDK6, whose activity is required for cell cycle G1/S transition [11].

The nuclear protein cyclin d1 is of considerable interest in lymphoma genesis because of its well-defined molecular role as a permissive factor for the progression of the G1phase of the cell cycle [12] [13]. Cyclin d1 is expressed at very low levels in normal b cells [14].

All MCL show over expression of the cyclin d1 [15] . Cyclin d1 over expression in a highly specific and sensitive marker of MCLs, although it may be also deregulated in a minor subset of B-CLLs and in HCL[16].

In all tumor cells in the 112 cases expressed B cell-related antigen, 104 expressed cyclin d1[17].

Hasio retrospectively stained 206 consecutive DLBCLs for cyclin d1, and identified 3 (1.5%) positive cases, and reported cyclin d1-positive DLBCLs are rare [18].

(2.1%) of DLBCL expressed cyclin d1and compared with patients who had cyclin d1-negative DLBCL, men were more commonly affected with cyclin d1-positive DLBCL, and they were significantly younger [19]. Approximately 20% to 30% of SLL have cyclin d1 positive [20] Gladkikh *et al* found that Mantle cell lymphoma have level of cyclin d1 increase four orders than other b lymphomas while other b lymphomas still have level of cyclin d1 significant elevated above that of normal lymphocytes [21].

Ki-67, a nuclear non histone protein, is synthesized at the beginning of cell proliferation, and it is expressed in all phases of the cell cycle except during G0 phase [22]. Its strict association with cell proliferation and its co-expression with other well-known markers of proliferation indicate a pivotal role in cell division. Ki-67 expression has been widely used in clinical practice as an index, to evaluate the proliferative activity of lymphoma [23].

Manuel *et al* 1997 found that Ki67 expression was observed in all cases (P=0.0319)[24].

Follicular lymphoma had a much lower mean Ki-67 than DLBCL, and small lymphocytic lymphoma had the lowest values ,there are significant association between the Ki-67 (P=0.025)and patients [25].

1n Rassidakis *et al* study, reported that the mean proliferation fraction (Ki-67) immunoreactivity was 16.3% and 17.5% in FCL and MCL, respectively [26].

MATERIALS AND METHODS:

Slides preparation:

Two sections of 5µ m thickness were obtained from FFPB embedded tissue (previously diagnosed as lymphomas by H&E) using a rotary microtome and taken in thermal coated slide and dried in hot plate oven at 80ºC for one hour [27].

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.

One section treated with anti –cyclin d1primary antibodies and the other section with ki67 antibodies for thirty minutes, then rinsed in PBS. Sections then treated with secondary polymer conjugate for thirty minutes and rinsed in phosphate buffer saline, then treated with 3,3'diaminobenzidine (DAB) for seven minutes , then washed in phosphate buffer saline for five minutes, then counter stained in Mayer's haematoxylin for one minute and washed in water and blued in 0.05% ammoniated water for 16 seconds, 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 [27].

Result interpretation:

Results obtained from two sections were detected by researcher and confirmed by experienced histopathologist. Negative and positive controls were used for evaluation of the test sections.

Statistical analysis:

All information about the study population was entered a computer as well as obtained results. The data was analyzed

using SPSS computer program. Frequencies, means, chi-square tests and independent t test were calculated.

RESULTS:

Patients histopathological diagnosed as B lymphoma, 20 (40%) samples were diffuse large B cell lymphoma patients, 13 (26%) samples were small lymphocytic lymphoma patient, 8 (16%) samples were Mantle lymphoma patients, 5(10%) samples were follicular lymphoma patients, and 4 (8%) samples were Burkitt lymphoma patients Table (1).

The distribution of age group among study subjects showed 39 (78%) patients more than 40 years and11 (22%) patients less than 40 years Table (2).

Sex distribution showed that 33(66%) patient were male and 17(34%) patients were female Table (3).

Cyclin d1expression showed positive in 2 (4%) patients and negative in18 (36%) patients among diffuse large b cell lymphoma, among small lymphocytic lymphoma 3 (6%) positive patients and 10 (20%) negative patients, Mantle lymphoma patients showed 8 (16%) and no negative result, 2 (4%) patients positive, 3 (6%) negative patients among follicular lymphoma and no positive result was observed among Burkitt lymphoma with (P=0.00) Table (4).

The relation between ki67 index and histopathological diagnosis showed that, diffuse large B cell lymphoma mean (50 ±11.5), small lymphocytic lymphoma mean (20± 16), Mantle lymphoma mean (65 ±3), Burkitt lymphoma mean (97.5± 11.4), and follicular lymphoma mean (30.5± 22.5) (P=0.00) Table (5).

Table (1): Histopathological diagnosis of the study subjects:

Diagnosis	Frequency	Percent%
Diffuse large B cell lymphoma	20	40.0
Small lymphocytic lymphoma	13	26.0
Mantle lymphoma	8	16.0
Burkitt lymphoma	4	8.0
Follicular lymphoma	5	10.0
Total	50	100

Table (2): Distribution of age group among study subjects:

Age group (year)	Frequency	Percent%
<40	11	22.0
>40	39	78.0
Total	50	100.0

Table (3): Distribution of sex among the study subjects:

Sex	Frequency	Percent%
Male	33	66.0
Female	17	34.0
Total	50	100.0

Table (4): Relation between the expression of cyclin D1 protein and histopathological diagnosis.

Diagnosis	Cyclin D1 expression		Total
	Positive	Negative	
Diffuse large B cell lymphoma	2(4%)	18 (36%)	20(40%)
Burkitt lymphoma	0 (0%)	4 (8%)	4(8%)
Mantle lymphoma	8 (16%)	0 (0%)	8(16%)
Small lymphocytic lymphoma	3 (6%)	10 (20%)	13(26%)
Follicular lymphoma	2(4%)	3(6%)	5(10%)
Total	15 (33%)	35 (66%)	50 (100%)

P value=0.00

Table (5): Relation between ki67 index and histopathological diagnosis:

Diagnosis	Mean	P value
Diffuse large B cell lymphoma	50.5 ± 11.5	0.00
Small lymphocytic lymphoma	20.0 ± 16	
Mantle lymphoma	65.0 ± 2.9	
Burkitt Lymphoma	97.5 ± 11.4	
Follicular lymphoma	30.5 ± 22.5	

DISCUSSION:

Non Hodgkin's lymphomas is the tenth most common cancer worldwide, with nearly 386,000 new cases diagnosed in 2012 and majority of non Hodgkin's lymphomas are B lymphomas [(28)].

In this study patients' age ranged from 4 to 80 years, the result showed there was an increase susceptibility of lymphomas with increasing age. This finding was supported with Gelfand *et al* [(29)], who reported that the older people may have a slightly higher risk of developing lymphoma than

younger people. Also Armitage *et al* [30] 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 and 70 years. The present study demonstrated that there was an increase in number of males compared to females (2:1). This result supported by Alexander *et al*, [31] who reported that women are known to have a lower incidence of non- Hodgkin's 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*. [32], who reported that the Hodgkin's lymphoma is nearly twice as common in males.

In this study cyclin d1 expression is associated with B lymphoma (P=0.00) and expressed in all cases of Mantle lymphoma. This finding is compatible with de Boer et al[15], who found that all MCL showed over expression of the cyclin d1 and with Bosch et al [16], who reported that cyclin d1 over expression in a highly specific and sensitive marker of MCLs. In 20 DLBCL patient only 2 patient expresed cyclin d1, this result compatible with Hasio *et al* [18], who found that cyclin d1-positive DLBCLs are rare. And with Ok *et al* [19], who reported that (2.1%) of DLBCL expressed cyclin d1.

30% of small lymphocytic lymphoma patients expressed cyclin d1, this result compatible with Yang *et al* [20] who reported approximately 20% to 30% of SLL have cyclin d1 positive, also Burkitt lymphoma and follicular lymphoma expresed cyclin d1 matched with Gladkikh *et al* [21] , who found that Mantle cell lymphoma have level of cyclin d1 increase four orders than other B lymphomas while other B lymphomas still have level of cyclin d1 significant elevated above that of normal lymphocytes.

In this study also showed the expression of ki67 index among B lymphoma patient and we found that there is strong association between B lymphoma and ki67 (P=0.00), this findings is compatible with Manuel *et al* [24] who found that

Ki67 expression was observed in B lymphoma (P=0.0319). And also agreed with Broyde *et al* [25], who reported there is significant association between the Ki-67 and B lymphoma patient(P=0.025). Small lymphocytic lymphoma ki67 mean is (20± 16)which it is lower value than others , this finding is compatible with Broyde *et al*[25] who found small lymphocytic lymphoma has lowerest value.

Bukirtt lymphoma have a highest mean (97.5%) this result compatible with Chuang *et al* [33] who found that Burkitt lymphoma (BL) has a very high Ki-67 proliferation index .

CONCLUSION

The study concluded that there is strong association between cyclin d1, ki67 index and B lymphoma, cyclin d1 expressed in specific and sensitive manor in Mantle lymphoma.

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