

## DIAGNOSTIC AND PREDICTIVE VALUE OF SURVIVIN, P63 AND CD15 IN NON HODGKIN'S LYMPHOMAS

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### ABSTRACT

**Background:** Lymphoid proliferations are thought to be either benign reactive conditions, atypical lymphoid proliferations or malignant lymphomas. NHL's are a diverse group of malignant neoplasms that affect lymphoid tissue, their incidence has risen twofold over the past 10 years. IHC, is now routinely used to confirm the diagnosis of lymphoma.

**The aim:** Is to evaluate the expression of Survivin, P63 and CD15 in different types of reactive lymphoid hyperplasia and lymphoma and their ability to differentiate between some types of them. Also, correlate expression of the above markers as indicators of aggressiveness in NHL's.

**Methods:** A total of selected 80 cases of patients which were previously diagnosed as: 30 cases of Non Hodgkin's lymphoma, 20 cases of Hodgkin's lymphoma, and 30 cases of reactive lymphoid hyperplasia. The sections were stained with H&E and immunohistochemical markers of Survivin, p63 and CD15 separately.

**Results:** Expression of survivin & P63 immunostains in cases of aggressive lymphomas was (61.9%)&(71.4%) which are higher than their expression in cases of indolent lymphomas (38.1%)&(28.6%) respectively. Also, expression of survivin & P63 immunostains in cases of follicular lymphoma was (50%)&(33.3%) which are higher than their expression in cases of atypical lymphoid hyperplasia (40%)&(20%) respectively. P63 was frequently expressed in large cell lymphoma cases (64.2%). None of the cases of CHL demonstrated any P63 expression. CD15 is a specific marker of reed Sternberg cells of CHL and showed no expression in cases of lymphocytic predominance HL. CD15 was also occasionally expressed in cases of large cell lymphomas.

**Conclusion:** survivin and P63 markers can be used as immunohistochemical tools in distinguishing follicular lymphoma from lymphoid hyperplasia especially atypical cases. Also, survivin and P63 markers can be used as indicator of aggressiveness in NHL cases. CD15 is a specific marker of RS cells of CHL. Also, CD15 is occasionally expressed in cases of large cell lymphomas, so not used alone in differentiation between large cell lymphoma and CHL where morphological distinction is difficult. When adding a P63 immunostaining, the accuracy of distinction between these lesions was increased. so, P63 can be used as a potential tool in the differential diagnosis between these lesions.

**Key words,** IHC, CHL, RS cells, NLPHL, Survivin, P63, Immunohistochemistry.

### INTRODUCTION

Lymphoid proliferations are traditionally thought to be either benign conditions (reactive hyperplasia and lymphadenitis) or malignant lymphomas (1). Not all lymphoid lesions at present can be precisely placed into one of these categories. Therefore, there also exist a third group of lymphoid proliferations, the atypical lymphoid proliferations (AtLP). AtLP is a descriptive term that describes a diagnostic dilemma rather than a specific diagnosis. They show some features associated with lymphoma but lack the full criteria of malignancy. They have some likelihood for subsequent transformation into lymphomas, and therefore AtLP occupy a middle ground between benign and malignant lymphoid proliferations. Nevertheless, sometimes AtLP are not necessarily premalignant and may very well represent a fully benign situation mimicking malignancy. Up to date, there are no criteria for defining and classifying AtLP. Also,

worrisome features that predict whether a patient with AtLP will have a self-limited illness or one that will eventually result in lymphoma are not well known (2).

Lymphoma represent a major health problem throughout the world. It is already a common malignancy and is counting to increase rapidly. It is the fifth most common malignancy in the United States, its incidence is more common in developed countries and high incidence exist in parts of middle east and Africa (3). The exact incidence in Egypt is not precisely known due to lack of a National cancer registry. The only data available are relative frequency data of hospital-based registries (4).

The World Health Organization (WHO) classification of neoplasms of the hematopoietic and lymphoid tissues, published in 2001 and updated in 2008, represents a worldwide consensus on the diagnosis of these tumors, adopted for use by

pathologists, clinicians, and basic scientists (5). The WHO classification of 2008 identified new entities and variants, and incorporated new emerging concepts in the understanding of lymphoid neoplasms. However, when published in 2008 some questions were unresolved, such as the extent to which specific genetic or molecular alterations define certain tumors (6).

Immunophenotyping of lymphoid neoplasms is crucial in the morphologic evaluation of the tissue for proper classification of disease and is the method by which antibodies are used to detect cellular antigens in clinical samples. Some diseases have specific combinations of cell surface markers, which can aid in identifying the immunophenotype and diagnosing the disease and thus in choosing proper treatment. Two methods commonly used to identify the immunophenotype of lymphomas are flow cytometry and immunohistochemistry (7).

Survivin is a newly described unique member of the inhibitor of apoptosis protein (IAP) family. Its expression is undetectable in normal adult tissues but significantly up-regulated in transformed cell lines and several malignant tumors. It may protect cells against apoptosis and promote tumor growth and invasion (8). In the past few years survivin has emerged as a potential early predictor of malignancies. Survivin's over-expression appears to correspond with higher malignant grades and reduced survival rates in different cancers such as B-cell non-Hodgkin's lymphoma (9). Survivin was found to be over expressed in patients with lymphoma. As the expression of survivin is positively correlated with tumor progression and inversely correlated with the survival period of patients after chemotherapy, survivin has been proposed as an attractive target for new anti-cancer intervention (10).

p53 gene homologue (p63) was described recently and some authors related it to poor prognosis in malignant lymphoma, although p63 is expressed in germinal centre lymphocytes and seems to be related to the development of the lymphoma (11). The morphological and phenotypic features between anaplastic large cell lymphoma and classical Hodgkin's lymphoma are similar, making this differential diagnosis challenging, P63 protein expression is frequently expressed in a subset of anaplastic large cell lymphoma cases and may be used as a potential tool in the differential diagnosis between these two types of lymphoma (12).

CD15 is expressed in normal mature myeloid cells, granulocytes, macrophages but it has been predominantly used as an immunohistochemical marker to identify Reed-Sternberg cells (RS) in Classical Hodgkin Lymphoma (CHL), useful to distinguish it from reactive lymphadenitis, lymphocyte-predominance Hodgkin lymphoma, non-Hodgkin lymphomas and HD-like neoplasms (13,14).

## MATERIALS AND METHODS

### Subjects:

The material was collected from Pathology Department, faculty of Medicine, Zagazig University as well as from National Cancer Institute in Cairo during the period from September 2011 to august 2014. The material of the present retrospective study was based upon selected Archival paraffin embedded tissue blocks of patients previously diagnosed as non Hodgkin's Lymphoma (30 cases), Hodgkin's lymphoma (20 cases) and reactive lymphoid hyperplasia (30 cases).

### Samples:

Cases of Our work included : Thirty (30) cases of non-Hodgkin lymphoma which are 14 Cases of anaplastic diffuse large cell lymphoma, 6 cases of follicular lymphoma, 2 cases of Burkitt's lymphoma, 4 Cases mixed small & large cell lymphoma, and 4 cases small lymphocytic lymphoma. Twenty (20) cases of Hodgkin lymphoma which are 16 Cases of classic Hodgkin lymphoma (CHL), and 4 cases of nodular lymphocytic predominance HL (NLPHL). Thirty (30) cases of reactive lymphoid hyperplasia including 10 cases of atypical lymphoid hyperplasia.

### Histopathologic examination:

Tissue specimens were fixed in 10% buffered formalin and embedded in paraffin. Consecutive 4 µm sections were prepared and stained with hematoxylin & eosin (H&E) for histopathological examination. H&E stained sections clearly demonstrate the changes of the lymph node architecture, the pattern of lymphoid proliferations either benign or malignant, diffuse or nodular. It easily clarifies the morphology of neoplastic lymphoid cells and was also used to select representative areas of the tumor for subsequent immuno- histochemical studies.

### Immunohistochemical Analysis:

Immunohistochemical staining was carried out using streptavidin-biotin immunoperoxidase technique (Dako-cytomation, Glostrup, Denmark).

3–5 µm thick sections cut from formalin-fixed, paraffin-embedded blocks, mounted on positive charged slides, were deparaffinized in xylene and rehydrated in graded alcohol. The mounted sections were immersed and boiled in ready to use Dako target retrieval solution (PH 6.0) in a microwave for 20 min, and then washed in phosphate buffer saline (PBS) (pH 7.3). Thereafter, blocking of endogenous peroxidase activity with 6% H<sub>2</sub>O<sub>2</sub> in methanol was carried out. The slides were then incubated over night using a Survivin, Rabbit polyclonal antibody, Prediluted Ab which is ready to use. P63, mouse monoclonal antibody, clone BC4A4, dilution 1:100. CD15, mouse monoclonal antibody, (clone MMA ; same as LeuM1), dilution 1:200.

Incubation with a secondary antibody and product visualization were performed (Dako Cytomation, Glostrup, Denmark) with diaminobenzidine substrate as the chromogen. The slides were finally counterstained with Mayer's haematoxylin, and washed with distilled water and PBS.

Positive controls were stained at the same staining setting with the studied cases. Prostatic carcinoma was used as positive control for survivin, Basal epithelial cells of normal prostate was taken as positive control for P63, and Reed Sternberg cells was used as positive control for CD15. Their negative controls were obtained by omission of the primary antibody.

#### **Immunohistochemical Evaluation:**

##### **Evaluation of Survivin immunostaining:**

The immunoreactivity of Survivin was in nucleus and cytoplasm. The dark brown staining indicates positive expression of Survivin. We evaluated the extent and intensity of Survivin expression. The percentage of cells positive for Survivin was determined and graded as follows: 0 = 0%-5%, 1 = 6%-25%, 2 = 26%-50%, 3 = 51%-75%, and 4 = 76%- 100%. The intensity of Survivin staining was graded as follows: 0 = None (Negative -ve), 1 = Weak (Mild positive +), 2 = Moderate (Moderate positive ++), 3 = Intense (Strong positive +++)(15).

##### **Evaluation of P63 immunostaining:**

Only nuclear staining above 5% was interpreted as positive for p63 expression. Immunostaining results for p63 were semi-quantitatively evaluated according to the percentage of positive tumor cells in each case, cases were divided into 4 groups according to the following scores (16,12): None (negative -ve) ≤5%, Weak (mild positive +) >5%

to 10%, Moderate (moderate positive ++) >10% to 50%, and Intense (strong positive +++) >50%.

##### **Evaluation of CD15 immunostaining:**

A case was considered positive if 10% or more of the Hodgkin-Reed-Sternberg cells stained positive. Reed-Sternberg cells display a characteristic pattern of CD15 positivity, with membranous staining combined with staining of the golgi apparatus (granular paranuclear) (13,17). CD15 is present on >95% of granulocytes including neutrophils and eosinophils, which are used as internal control.

##### **Statistical analysis**

Data were checked, entered and analyzed using SPSS version 19 and EPI-INFO 6 for data processing and statistics. For categorical variables ANOVA test or chi-square was used. P-value less than 0.05 was considered significant.

## **RESULTS**

### **Results of Survivin immunostaining**

\* Expression of survivin immunostain in cases of aggressive lymphomas as large cell and Burkitt's lymphoma (61.9%) is higher than its expression in cases of indolent lymphomas as follicular, small lymphocytic lymphoma (38.1%). so, may be used as indicator of aggressiveness in lymphoma (Fig.1&2).

\* Expression of survivin immunostain and its immunoreactive scores in cases of follicular lymphoma (50%) is higher than its expression in cases of atypical lymphoid hyperplasia(40%). So, survivin may be used as immunohistochemical tool to differentiate between these two lesions (Fig.3&4).

### **Results of P63 immunostaining**

\* Expression of P63 immunostain in aggressive lymphomas as large cell and Burkitt's lymphoma (71.4%) is higher than its expression in indolent lymphomas as follicular, small lymphocytic lymphoma (28.6%). so, may be used as indicator of aggressiveness in lymphoma (Fig.5&6).

\* Expression of P63 immunostain in cases of follicular lymphoma (33.3%) is higher than its expression in cases of atypical lymphoid hyperplasia(20%). So, P63 may be used as immunohistochemical tool to differentiate between these two lesions(Fig.7) .

\* P63 is frequently expressed in anaplastic diffuse large cell lymphoma cases (64.2%) (Fig.6). None of the cases of classic HL demonstrated any P63 expression (Fig.8). So, may be used as a potential tool in the differential diagnosis between these two lesions.

### **Results of CD15 immunostaining**

CD15 is a specific marker of reed Sternberg cells of CHL (Fig.9) and showed no expression in cases of lymphocytic predominance HL (Fig.10), so used to

differentiate between these two lesions. CD15 also occasionally expressed in cases of diffuse anaplastic large cell lymphomas (Fig.11).

**Table (1):** Expression of Survivin, P63, and CD15 in NHL's and its relation with clinicopathologic features of these cases.

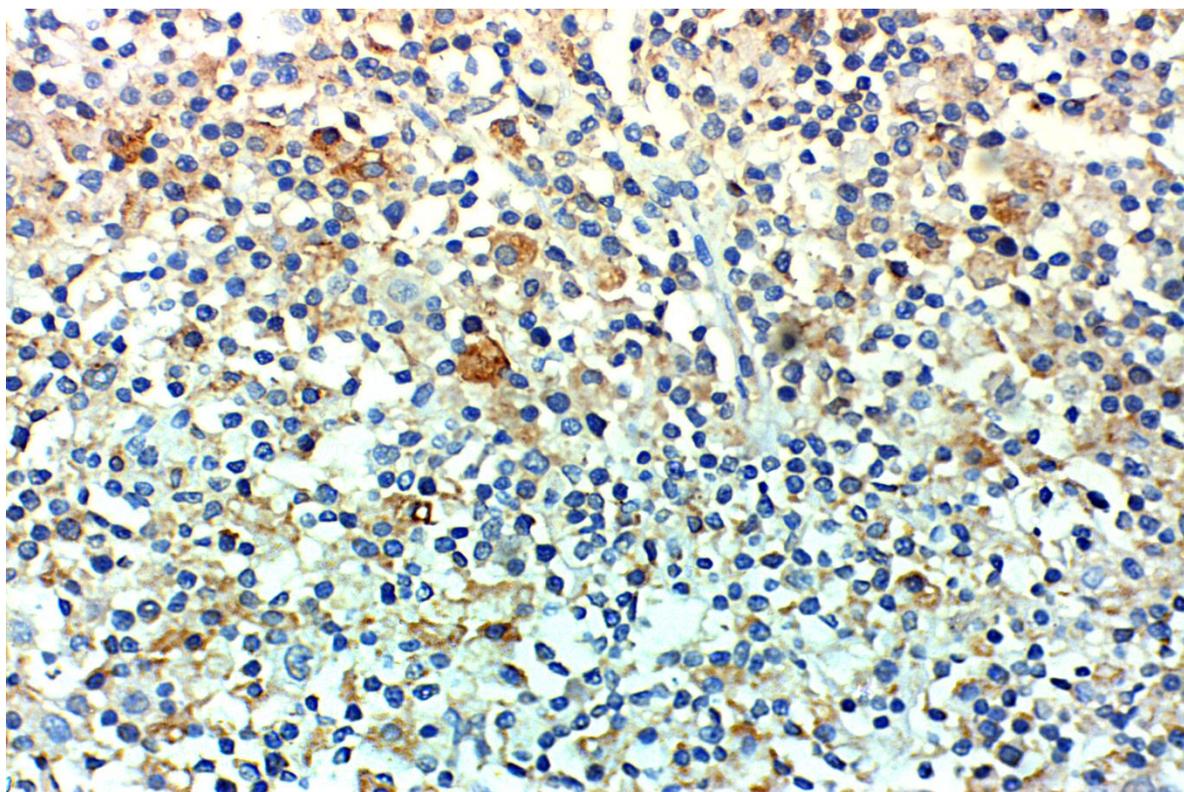
Group	Survivin		P63		CD15		P value of survivin	P value of P63	P value of CD15	
	+	-	+	-	+	-				
30										
<b>Gender</b>										
M	22	15(71.4)	7(77.8)	8(57.1)	14(87.5)	2(66.7)	20(74.1)	0.719	0.061	0.783
F	8	6(28.6)	2(22.2)	6(42.9)	2(12.5)	1(33.3)	7(25.9)			
<b>AgeY's</b>										
<45	13	10(47.6)	3(33.3)	8(57.1)	5(31.3)	2(66.7)	11(40.7)	0.469	0.153	0.389
≥45	17	11(52.4)	6(66.7)	6(42.9)	11(68.7)	1(33.3)	16(59.3)			
<b>Location</b>										
Cervial	16	12(57.1)	4(44.4)	7(50.0)	9(56.3)	1(33.3)	15(55.6)	0.522	0.732	0.464
Others	14	9(42.9)	5(55.6)	7(50.0)	7(43.7)	2(66.7)	12(44.4)			
<b>Malignan cy</b>										
Indolent	14	8(38.1)	6(66.7)	4(28.6)	10(62.5)	0(0.0)	14(51.9)	0.15	0.063	0.087
Aggressiv e	16	13(61.9)	3(33.3)	10(71.4)	6(37.5)	3(100.0)	13(48.1)			

**Table(2):** Expression of Survivin, P63, and CD15 in HL's and its relation with clinicopathologic features of these cases.

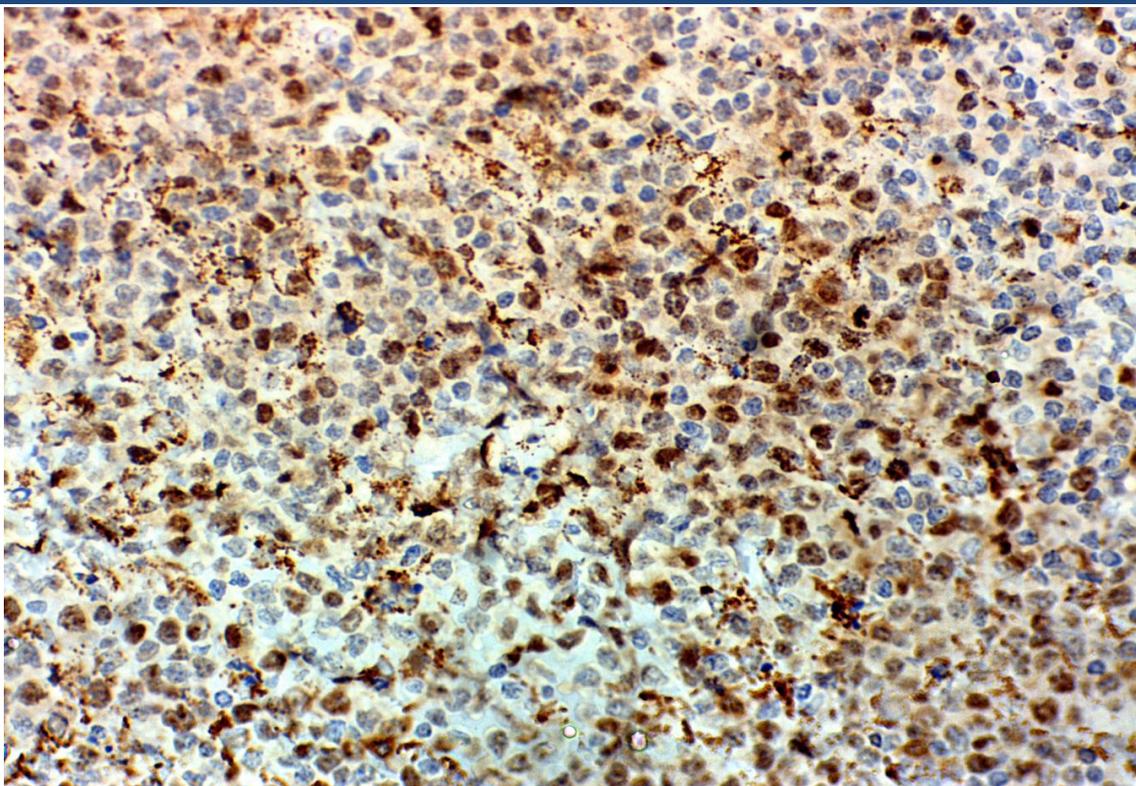
Group	Survivin		P63		CD15		P value of survivin	P value of P63	P value of CD15	
	+	-	+	-	+	-				
20										
<b>Gender</b>										
M	13	7(63.6)	6(66.7)	1(100.0)	12(63.2)	9(64.3)	4(66.7)	0.887	0.452	0.918
F	7	4(36.4)	3(33.3)	0(0.0)	7(36.8)	5(35.7)	2(33.3)			
<b>AgeY's</b>										
<45	14	7(63.6)	7(77.8)	1(100.0)	13(68.4)	9(64.3)	5(83.3)	0.492	0.502	0.394
≥45	6	4(36.4)	2(22.2)	0(0.0)	6(31.6)	5(35.7)	1(16.7)			
<b>Location</b>										
Cervial	12	9(81.8)	3(33.3)	1(100.0)	11(57.9)	8(57.1)	4(66.7)	0.027*	0.402	0.69
Others	8	2(18.2)	6(66.7)	0(0.0)	8(42.1)	6(42.9)	2(33.3)			
<b>Maligna ncy</b>										
cHL	16	9(81.8)	7(77.8)	0(0.0)	16(84.2)	14(100.0)	2(33.3)	0.822	0.04*	<0.001
NLPHL	4	2(18.2)	2(22.2)	1(100.0)	3(15.8)	0(0.0)	4(66.7)			**

**Table (3):** Expression of Survivin, P63, and CD15 in reactive hyperplasia's and its relation with clinicopathologic features of these cases.

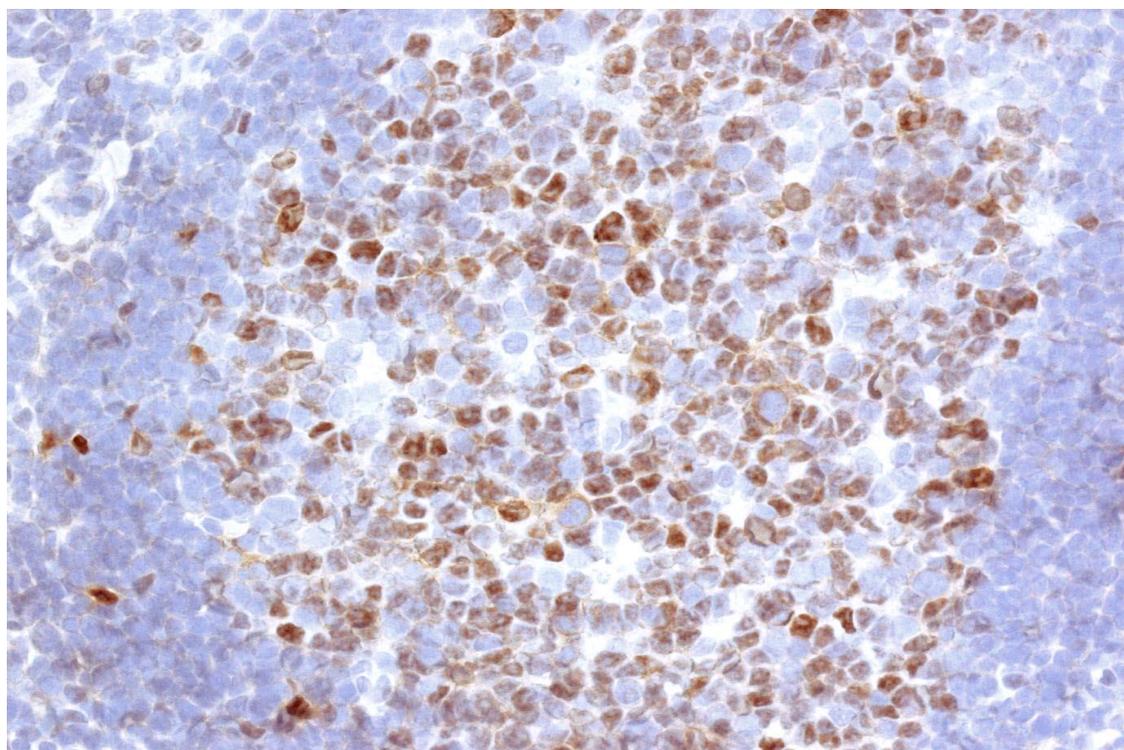
Group	30	Survivin		P63		CD15		P value of survivin	P value of P63	P value of CD15
		+	-	+	-	+	-			
<b>Gender</b>										
M	17	5(62.5)	12(54.5)	2(66.7)	15(55.6)	0(0.0)	17(56.7)	0.697	0.713	
F	13	3(37.5)	10(45.5)	1(33.3)	12(44.4)	0(0.0)	13(43.3)			
<b>AgeY's</b>										
<45	21	5(62.5)	16(72.7)	3(100.0)	18(66.7)	0(0.0)	21(70.)	0.589	0.232	
≥45	9	3(37.5)	6(27.3)	0(0.0)	9(33.3)	0(0.0)	9(30.0)			
<b>Location</b>										
Cervial	14	8(100.0)	6(27.3)	3(100.0)	11(40.7)	0(0.0)	14(46.7)	<0.001**	0.051	
Others	16	0(0.0)	16(72.7)	0(0.0)	16(59.3)	0(0.0)	16(53.3)			
<b>Lesion</b>										
Reactive	20	4(50.0)	16(72.7)	1(33.3)	19(70.4)	0(0.0)	20(66.7)	0.243	0.197	
Atypical	10	4(50.0)	6(27.3)	2(66.7)	8(29.6)	0(0.0)	10(33.3)			



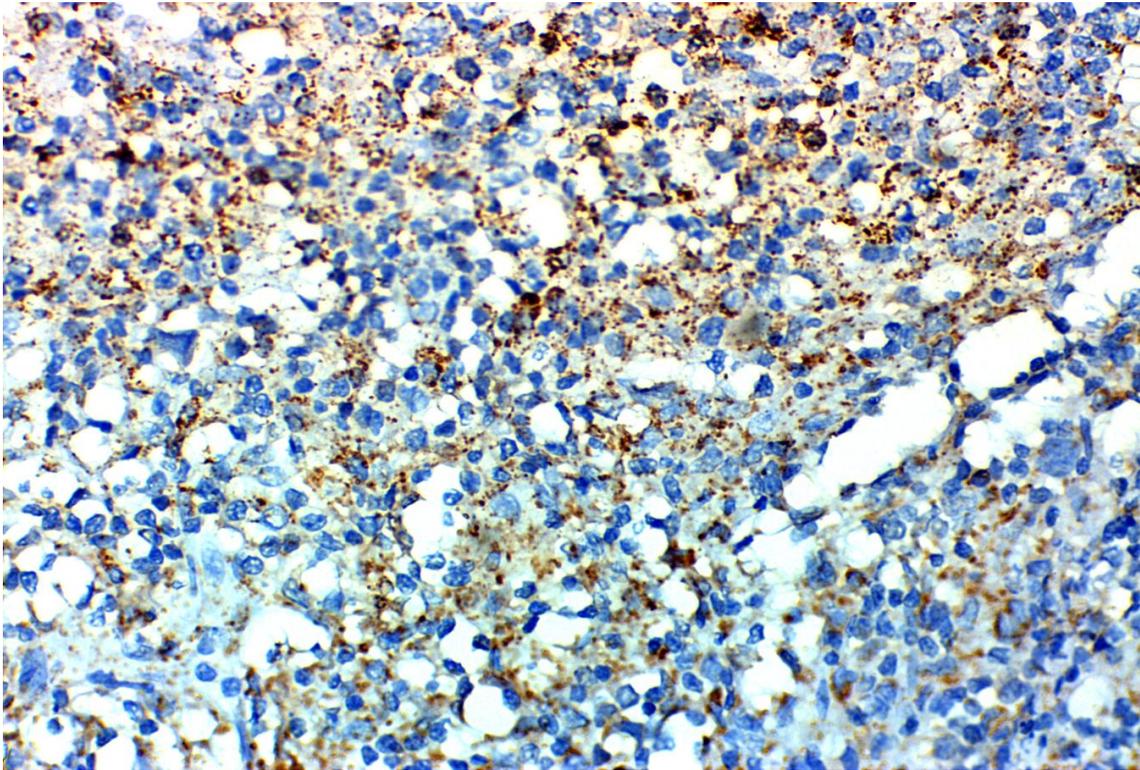
**Fig. (1):** Mixed small and large cell lymphoma stained with Survivin immunostain showing moderate (++) cytoplasmic staining of lymphoid cells. (Counter stain, Mayer's H, X 400)



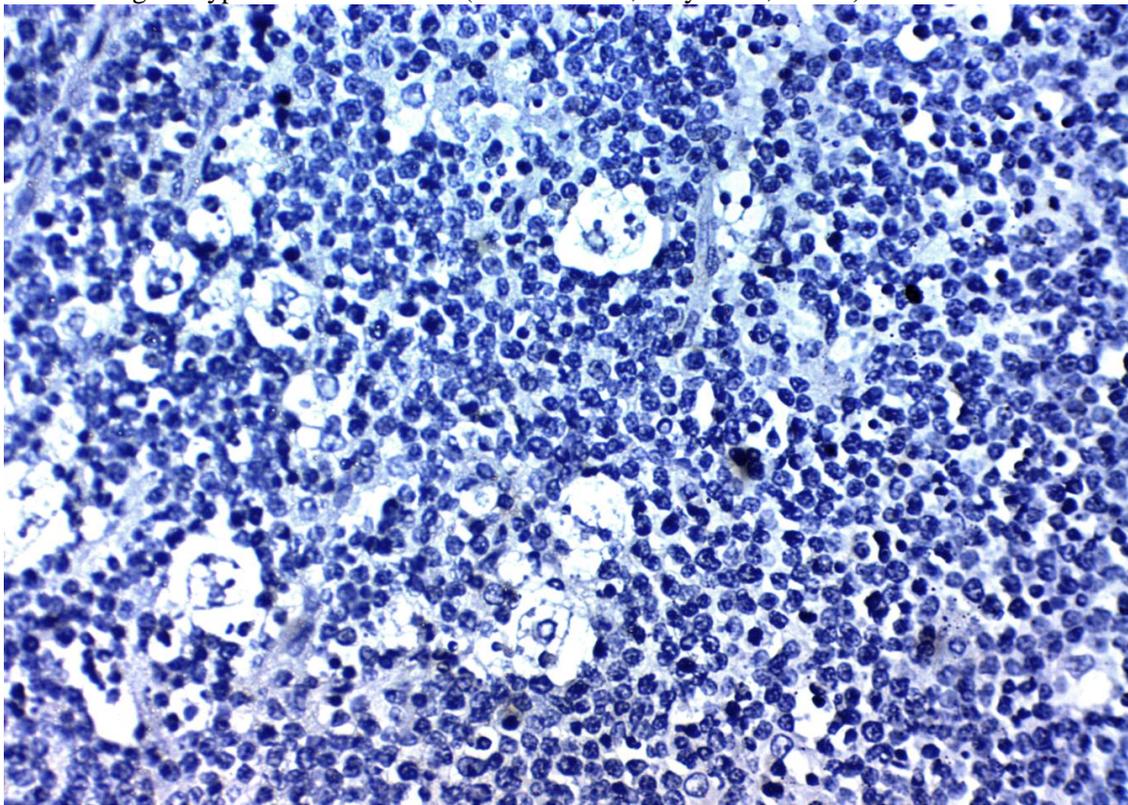
**Fig. (2):** Anaplastic diffuse large cell lymphoma stained with Survivin immunostain showing severe (+++) nuclear and cytoplasmic staining of lymphoid cells.(Counter stain, Mayer's H, X 400)



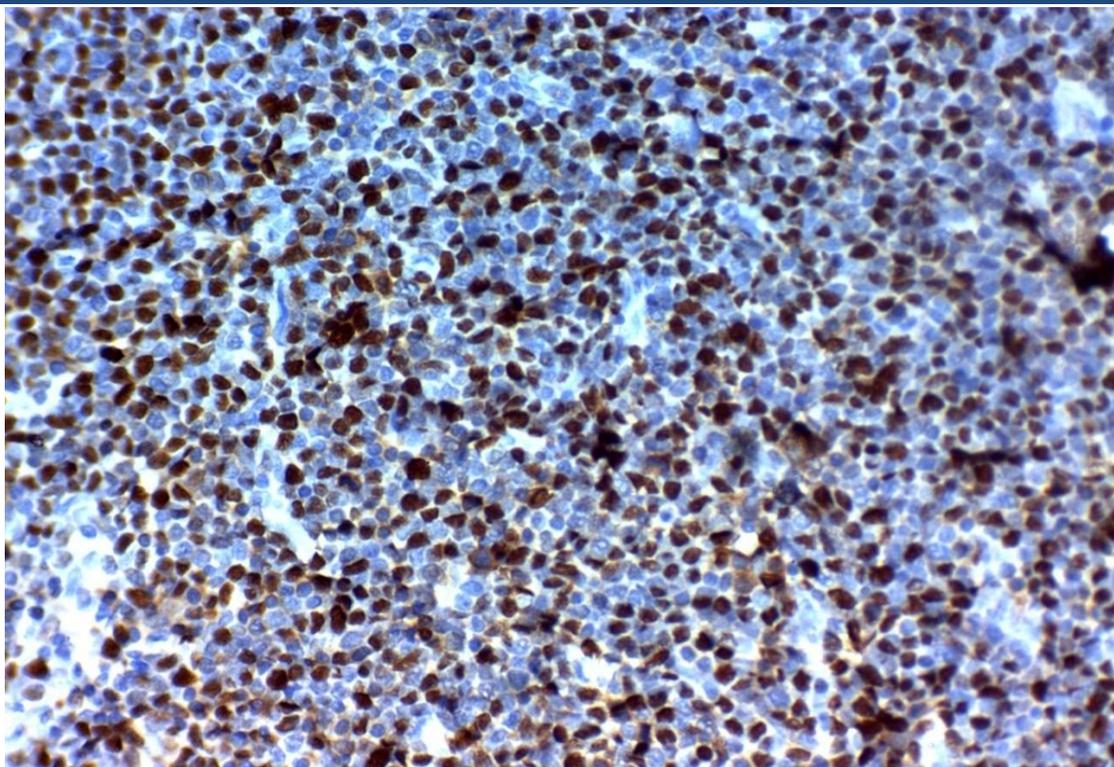
**Fig. (3):** Follicular lymphoma stained with Survivin immunostain showing moderate (++) nuclear and cytoplasmic staining of neoplastic follicular cells.(Counter stain, Mayer's H, X 400)



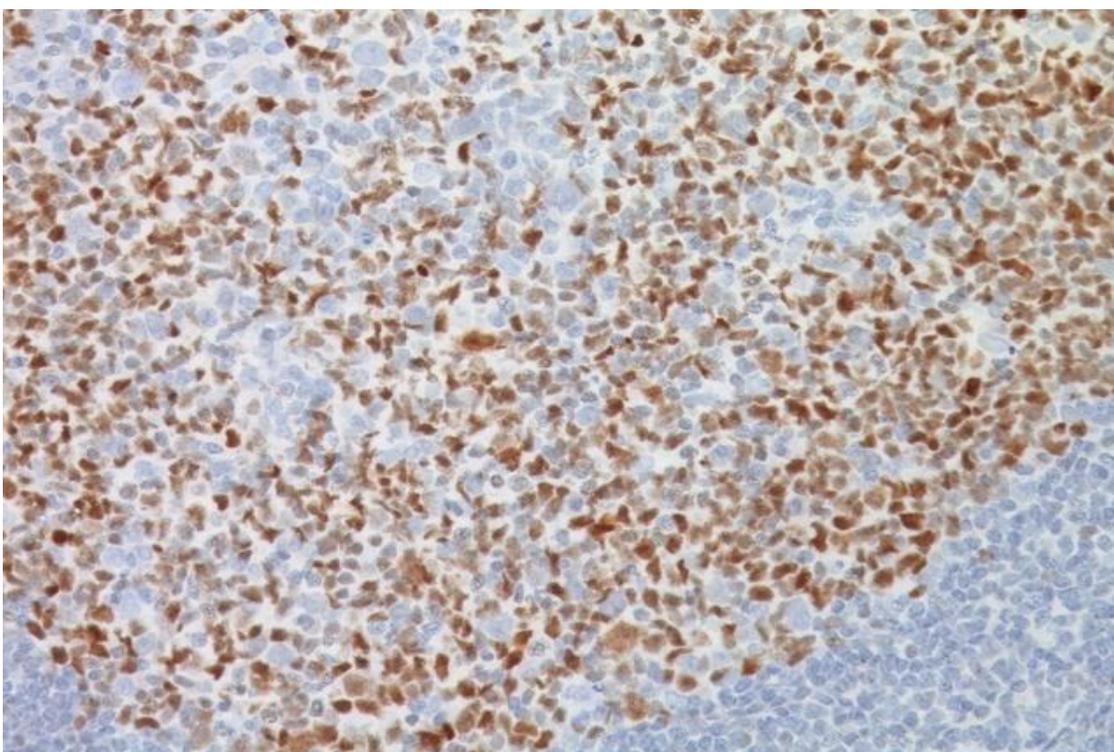
**Fig. (4):** Atypical reactive hyperplasia stained with Survivin immunostain showing moderate (++) cytoplasmic staining of atypical follicular cells.(Counter stain, Mayer's H, X 400)



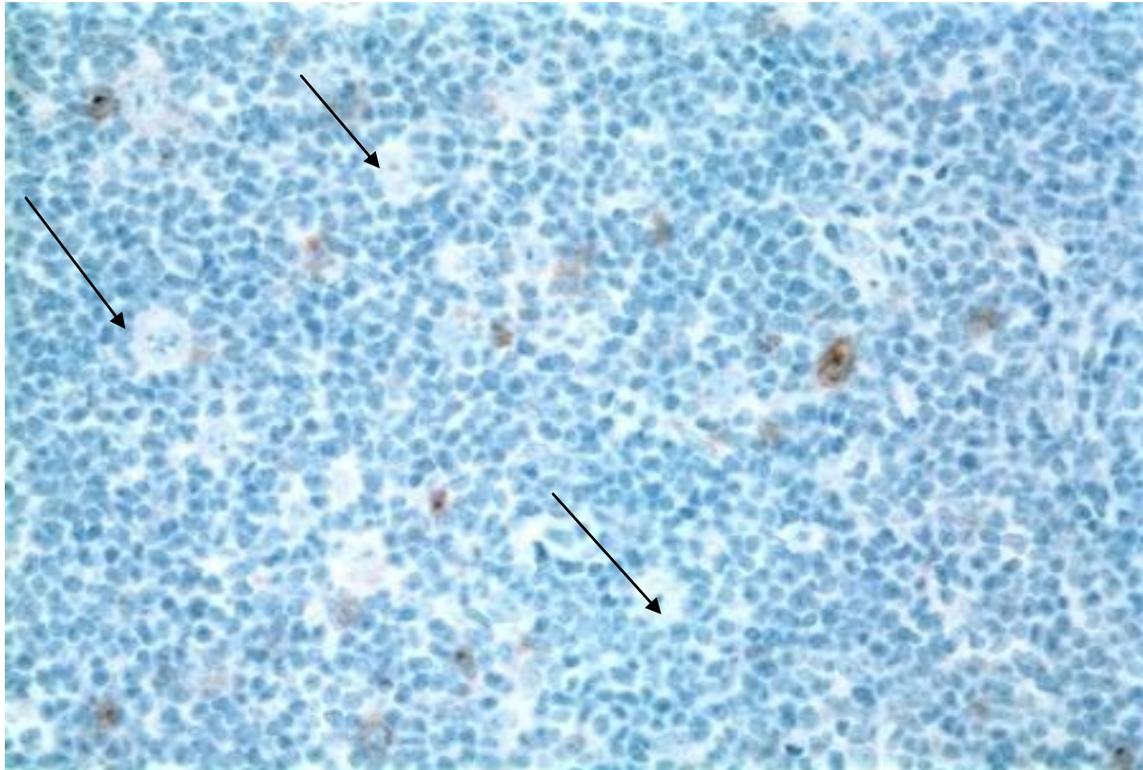
**Fig. (5):** Burkitt's lymphoma stained with P63 immunostain showing negative staining.(Counter stain, Mayer's H, X 400)



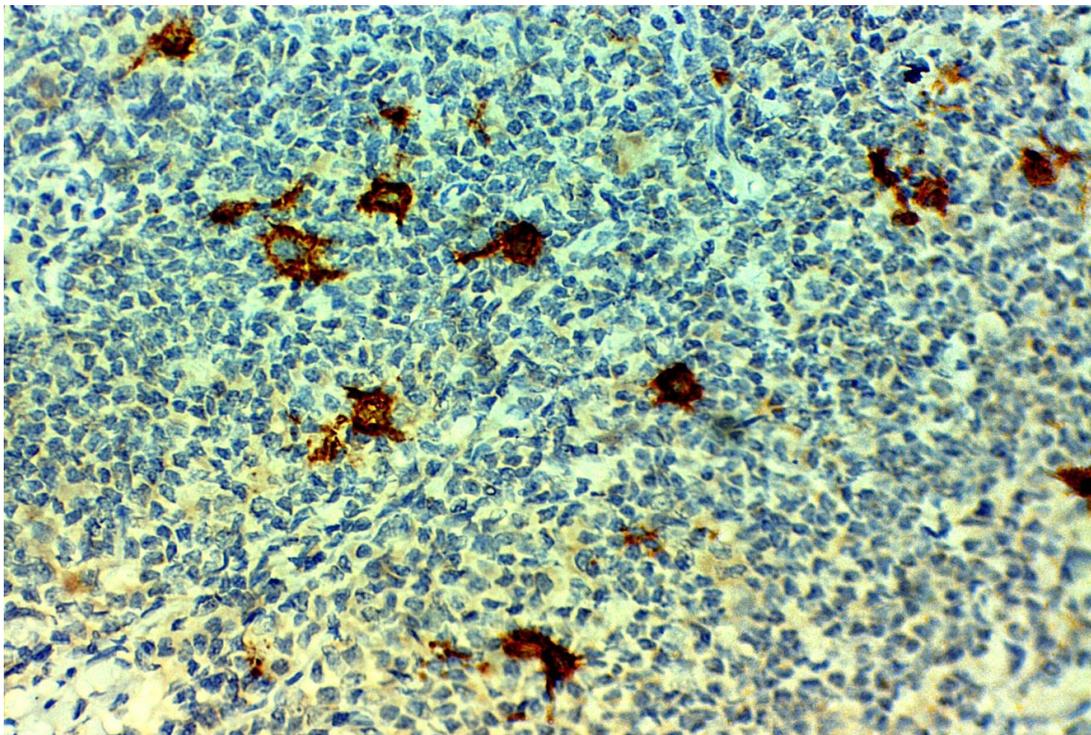
**Fig. (6):** Anaplastic diffuse large cell lymphoma stained with P63 immunostain showing severe (+++) nuclear staining of lymphoid cells.(Counter stain, Mayer's H, X 400)



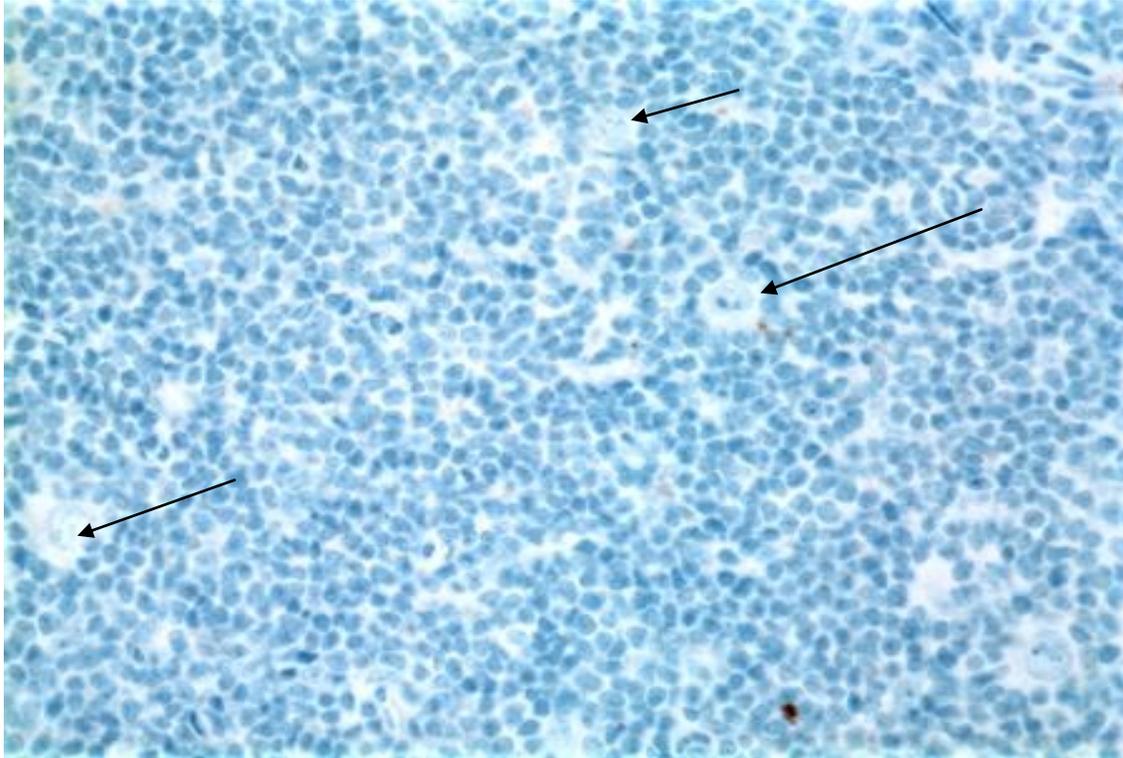
**Fig. (7):** Follicular lymphoma stained with P63 immunostain showing severe (+++) nuclear staining of neoplastic follicular cells. (Counter stain, Mayer's H, X 400)



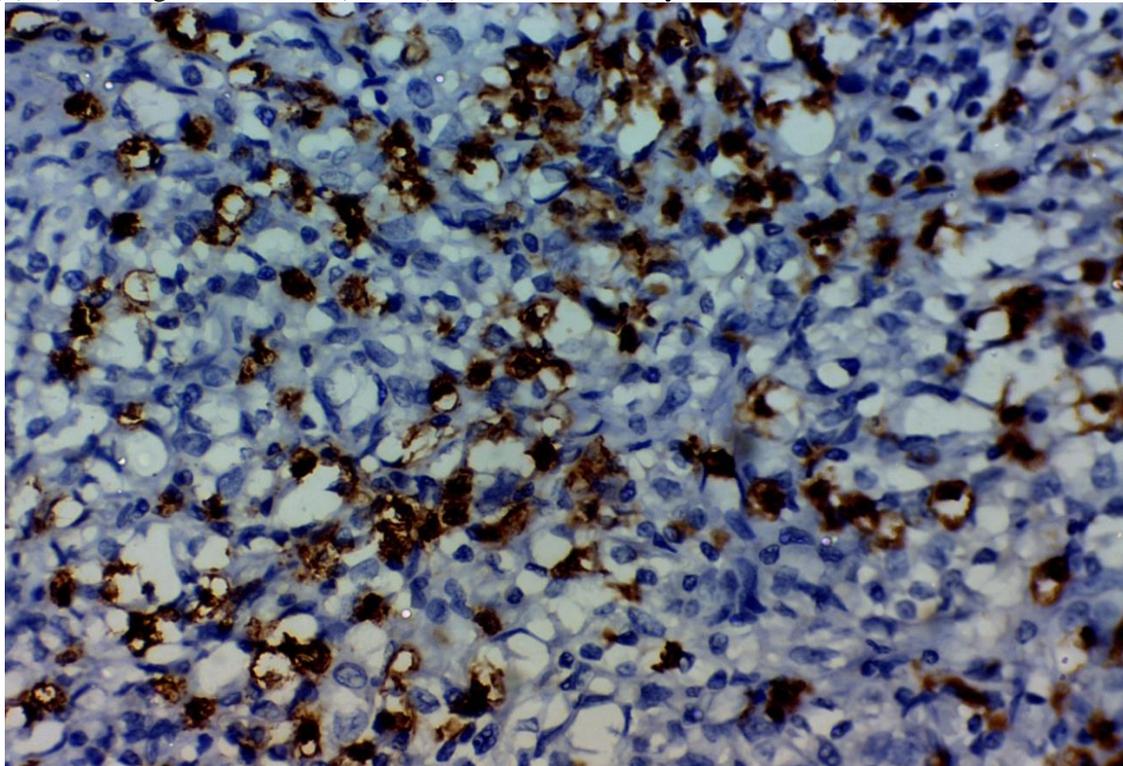
**Fig. (8):** CHL (mixed cellularity type) stained with P63 immunostain showing negative staining of R-S cells (arrows). A few reactive lymphoid cells reveal P63 nuclear expression.(Counter stain, Mayer's H, X 400)



**Fig. (9):** CHL (Mixed cellularity type) stained with CD15 immunostain showing (+ve) membranous and paranuclear staining of RS cells.(Counter stain, Mayer's H, X 400)



**Fig. (10):** Nodular lymphocytic predominance Hodgkin lymphoma (NLPHL) stained with CD15 immunostain showing (-ve) staining of L&H cells (arrows).(Counter stain, Mayer's H, X 400)



**Fig. (11):** Anaplastic diffuse large cell lymphoma stained with CD15 immunostain showing +ve membranous staining of Reed-Sternberg like cells.(Counter stain, Mayer's H, X400)

## DISCUSSION

Lymphoma represent a major health problem throughout the world. It is a common malignancy and is counting to increase rapidly. The exact incidence in Egypt is not known due to lack of a national cancer registry. The only data available are relative frequency data of hospital based registry (4).

Most of lymphoma are treatable and curable malignancies. However the most appropriate therapy require accurate diagnosis and careful staging evaluation. New insight into the biology of lymphoma in coming years might well improve our ability to evaluate patients and choose therapy (18).

As regards the histopathologic type, the present study revealed that non-Hodgkin lymphoma represented (37.5%), Hodgkin lymphoma represented (25%), and reactive hyperplasia represented (37.5%). This result coincides with the results of (18); (19) and (20) who recorded that the ratio of NHL : HL is 2:1.

In this study, diffuse anaplastic large cell lymphoma was the most frequent subtype of non-Hodgkin lymphoma which represented (46.7%) of all studied cases followed by follicular lymphoma (20%), small lymphocytic lymphoma (13.3%), mixed small and large cell lymphoma (13.3%) and lastly Burkitt's lymphoma (6.7%). These results were parallel to that described by (21) and (20) who reported that diffuse large cell lymphoma (DLBCL) is the most common subtype of B-cell lymphoma and account for (31%), follicular lymphoma account for (22%), small lymphocytic lymphoma account for (7%), and Burkitt's lymphoma account for (2%). Also (22) reported that there was a significant increase in diffuse large B cell lymphoma and decrease in small lymphocytic lymphoma. (4) reported that DLBCL is the most common type in NHL which represented 49% followed by Burkitt's lymphoma 6.9%, SLL 6%, FL 5.2%. This difference is may be due to low number of cases in this thesis and due to transformation of most cases of FL to DLBCL in late stage.

As regards to histopathologic subtypes of Hodgkin lymphoma, this work revealed that classic Hodgkin lymphoma cases represented (80%) and were more than cases of nodular lymphocyte predominance Hodgkin lymphoma (20%). This result coincide with the study carried by (23) who reported that NLPHL was rare variant accounting 5% of all cases of HL while classical Hodgkin lymphoma represented 95%. (4) in another study

stated that classical Hodgkin lymphoma represented 93%, NLPHL represented 1.1%, and Hodgkin's lymphoma unspecified represented 5.9% of their cases.

Survivin is useful in distinguishing follicular lymphoma from reactive hyperplasia but is not useful in distinguishing different types of lymphoma. In our work the expression of survivin in cases of Follicular lymphoma was (50%) and is higher than its expression in reactive hyperplasia (26.7%). So, may be used as immunohistochemical tool to differentiate between these two lesions. (24) reported that survivin is very valuable in differential diagnosis between follicular hyperplasia and neoplasia. Different results were mentioned by (25) and (26) who reported that survivin expression in reactive hyperplasia was (45%) and (10%) respectively.

Survivin expression may be used as indicator of aggressiveness in NHL cases. In our work survivin expression in aggressive lymphomas was (61.9%) which is higher than its expression in indolent lymphomas (38.1%). Also, (27) found expression of survivin in approximately 50% of high-grade NHL (centroblastic , immunoblastic), but not in low-grade lymphomas (lymphocytic). These findings suggest that apoptosis inhibition may be a general feature of neoplasia and identify survivin as a potential new target for apoptosis-based therapy in lymphoma.

Other study carried out by (25) proved that survivin was expressed in NHL as well as reactive lymphoid hyperplasia. However, its expression was significantly elevated in NHL compared to that in reactive lymphoid hyperplasia. The positive expression rate in inert, aggressive, highly aggressive lymphomas was 16.1%, 78.6%, and 93.3% respectively. so, overexpression of survivin was associated with tumor aggressiveness. Also, (28) and (26) showed that survivin expression was significantly higher in aggressive than in indolent NHL, while there was no statistically significant difference between indolent NHL and reactive hyperplasia. So, survivin expression is closely related to malignant grade and therefore may be considered an important prognostic factor of NHL.

P63 is useful in distinguishing follicular lymphoma from reactive hyperplasia. In our work the expression of P63 in cases of Follicular lymphoma was (33.3%) and is higher than its expression in reactive hyperplasia (10%). So, may be used as immunohistochemical tool to

differentiate between these two lesions. This result was parallel to that described by (12) who reported that P63 expression was (22%) in cases of follicular lymphoma. Also, (16) reported that P63 expression was (28%) in cases of follicular lymphoma. Different results were mentioned by (26) who reported that P63 expression in reactive hyperplasia was (40%). Also, (29) reported that P63 is very valuable in differential diagnosis between follicular hyperplasia and neoplasia as it is not expressed in cases of hyperplasia.

P63 expression may be used as indicator of aggressiveness in NHL cases. In our work P63 expression in aggressive lymphomas was (62.5%) which is higher than its expression in indolent lymphomas (28.6%). Also, (26) found expression of P63 in approximately (86.7%) of high-grade NHL, but was (76.9%) in low-grade lymphomas. So, there may be a close relationship between P63 protein and its expression in the regulation of lymphocyte proliferative kinetics.

It is well known, in some cases, the morphological and phenotypic features between diffuse anaplastic large cell lymphoma and classical Hodgkin's lymphoma are similar, making this differential diagnosis challenging, and establishing a histologic diagnosis for the gray zone between CHL and ALCL. In our work, (64.3%) of ALCL cases showed P63 nuclear positivity. In contrast, none of the cases of CHL demonstrated any P63 expression in Hodgkin cells. This result was parallel to that described by (12) who reported that P63 expression was (44%) in cases of ALCL and none of the cases of CHL demonstrated any P63 expression. Our finding of p63 expression in ALCL, in comparison to the consistently negative expression CHL, may be helpful in differential diagnosis. These results demonstrate that P63 protein expression is frequently expressed in a subset of ALCL cases and may be used as a potential tool in the differential diagnosis between ALCL and CHL.

In our work, CD15 positivity were detected in 3 cases out of 30 studied cases of NHL (10%), all 3 positive cases are diffuse anaplastic large cell lymphoma (21.4%), so CD15 was consistently absent but occasionally or infrequently expressed in these cases. Which, although rare, does occur. This result was parallel to that described by (30) who reported that CD15 expression was 3 cases out of 67 cases of large cell lymphoma. This observation is in agreement with (31) who showed that CD15 expression was 4 cases out of 41 cases of large cell

lymphoma. On the other hand, the study carried out by (32) reported that CD15 was not expressed in large B-cell lymphomas.

In our work, CD15 positivity were detected in 14 cases out of 20 studied cases of HL (70%). All positive cases are from 16 classic Hodgkin lymphoma cases (87.5%) and All 4 Nodular lymphocytic predominance HL cases were negative. Similar results were described by (23) ; (33) and (34) Who reported that CD15 is a specific marker of reed Sternberg cells of CHL and showed no expression in cases of lymphocytic predominance HL, so used to differentiate between these two lesions. Also, (35) reported that CD15 expression in HL was (65.4%). Different results were mentioned by (36) who reported that CD15 positivity was only (52%) of cases of CHL and was up to (4%) of cases of NLPHL.

### CONCLUSION

survivin and P63 markers may be used as immunohistochemical tools in distinguishing follicular lymphoma from lymphoid hyperplasia especially atypical cases. Also, survivin and P63 markers may be used as indicator of aggressiveness in NHL cases due to their high expression in aggressive than in indolent lymphomas. CD15 is a specific marker of reed Sternberg cells of CHL and showed no expression in cases of lymphocytic predominance HL, so used to differentiate between these two lesions. Also, CD15 is occasionally expressed in cases of diffuse anaplastic large cell lymphomas, so not used alone in differentiation between diffuse anaplastic large cell lymphoma and Hodgkin's lymphoma. When adding a P63 immunostaining, the P63 is frequently expressed in diffuse anaplastic large cell lymphoma cases, none of the cases of classic HL demonstrated any P63 expression. So, may be used as a potential tool in the differential diagnosis between these two lesions.

### REFERENCES

- 1- Good DJ and Gascoyne RD. Atypical lymphoid hyperplasia mimicking lymphoma. *Hematol. Oncol. Clin. North Am.* 2009;23(4):729-745.
- 2- Hussein MR. Atypical lymphoid proliferations: the pathologist's view point. *Expert Rev Hematol.* 2013;6 (2):139-53.
- 3- Sanchez S, Holmes H, Katabi N, et al. Composite lymphocyte-rich Hodgkin lymphoma and peripheral T-cell lymphoma associated with Epstein-Barr virus: A case report and review of

- the literature. Archives of Pathology and Laboratory Medicine. 2006; 30 (1): 107-12.
- 4- Mokhtar N and Khaled H. Lymphoma first edition. National Cancer Institute, Cairo University. 2002.
  - 5- Swerdlow SH, Campo E, Harris NL, et al. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. Lyon, France: IARC Press; 2008.
  - 6- Campo E, Swerdlow SH, Harris NL, et al. The 2008 WHO classification of lymphoid neoplasms and beyond: evolving concepts and practical applications. Blood. 2011;117(19):5019-32.
  - 7- Jack AS and Burnett AK. Procedures for the primary diagnosis and follow-up of patients with lymphoma. In: Mauch PM, Armitage JO, Coiffier B, Dalla-Favera R, Harris NL, eds. *Non-Hodgkin's Lymphomas*. Philadelphia, PA: Lippincott Williams & Wilkins; 2004:81-95.
  - 8- Reis FR, Vasconcelos FC, Pereira DL, et al. Survivin and P-glycoprotein are associated and highly expressed in late phase chronic myeloid leukemia. Oncol Rep. 2011; 26:471-8.
  - 9- Mobahat M, Narendran A, And Riabowol. Survivin as a preferential target for cancer therapy. Int. J. Mol. sci. 2014;15:2494-2516.
  - 10- Coumar MS, Tsai F, Kanwar JR, Sarvagalla S, and Cheung CH. Treat cancers by targeting survivin: Just a dream or future reality?. Cancer Treatment Reviews. 2013; 39: 802–811.
  - 11- Hedvat CV, Teruya-Feldstein J, Puig P, et al. Expression of p63 in diffuse large B-cell lymphoma. Appl Immunohistochem Mol Morphol. 2005;13:237–42.
  - 12- Gualco G, Weiss LM, and Bacchi CE. Expression of P63 in anaplastic large cell lymphoma but not in classical Hodgkin's lymphoma. Hum Pathol. 2008; 39(10) 1505-10.
  - 13- Vassallo J, Lamant L, Brugieres L, Gaillard F, Campo E, et al. ALK-positive anaplastic large cell lymphoma mimicking nodular sclerosis Hodgkin's lymphoma: report of 10 cases. Am J Surg Pathol. 2006; 30:223–9.
  - 14- Inomata H, Takei M, Nakamura H, Fujiwara S, Shiraiwa H et al. Epstein-Barr-Virus-Infected CD15 (Lewis X)-Positive Hodgkin-Lymphoma-Like B Cells in Patients with Rheumatoid Arthritis. The Open Rheumatology Journal. 2009; 3, 41-47.
  - 15- Xing-Jiang Mao, Geng-Fu Wang, Zhi-Jun Chen et al. Expression of HMGB1 and its clinical significance in T-cell lymphoma, Asian pacific J cancer prev. 2012; 13:5569-71.
  - 16- Park CK and Oh YH. Expression of P63 in reactive hyperplasias and malignant lymphomas. J Korean Med sci. 2005; 20(5):752-8.
  - 17- Pellegrini W, Bresciani G, Zorzi AD, Marocolo D, Ungari M, Facchetti F. MMA Monoclonal Antibody Is A Superior Anti-CD15 Reagent For The Diagnosis Of Classical Hodgkin's Lymphoma. Haematologica. 2007; 92:708-9.
  - 18- Armitage JO. Staging No-Hodgkin Lymphoma. CA Cancer J Clin. 2005; 55: 368-77.
  - 19- El-Bolkainy MN, Nouh MA and El-Bolkainy TN. Topographic pathology of cancer. The National Cancer Institute, Cairo University Chapter 17-18. 2005.
  - 20- Alder E. Living with lymphoma, Chapter 2 symptom and diagnosis, Chapter 1-2 What is lymphoma. 2005.
  - 21- Javier DG and Ferres R. Large B cell diffuse lymphoma. [Htt://www.orpha.net/data/patho/GB/uk-BCL.pdf](http://www.orpha.net/data/patho/GB/uk-BCL.pdf). 2004.
  - 22- Mitterlechner T, Fiegl M, Muhlbock H, et al. Epidemiology of non-Hodgkin lymphomas in Tyrol/Austria from 1991 to 2000. J Clin Pathol. 2006; 59 (1): 48-55.
  - 23- Fraga M and Forteza J. Review Diagnosis of Hodgkin's disease: an update on histopathological and immunophenotypical features. Histol Histopathol (2007); 22: 923-35.
  - 24- Mazur G, Halon A, Wrobel T, et al. Survivin expression in lymph nodes, affected by lymphoma and reactive hyperplasia. Roczniki Akad Med Białymst. 2004; 49(1):76-8.
  - 25- Mao XJ, Wang GF, Chen ZJ, et al. Expression of HMGB1 and its clinical significance in T-cell lymphoma. Asian pacific J of cancer prevention. 2012;13:5569-71.
  - 26- Zou XL, Zhou Y, Liu XH, et al. Expression of survivin and P63 protein in B cell non-Hodgkin lymphoma and their effects on cell apoptosis and proliferation. Zhongguo Shi. 2007;15(1): 99-102.
  - 27- Ambrosini G, Adida C, Altieri DC. A novel anti-apoptosis gene, survivin, expressed in cancer and lymphoma. Nature Medicine. 1997; 3,917-21.
  - 28- Li J and Wu H. Expression of survivin in human non-Hodgkin lymphoma and its correlation with

- proliferation and angiogenesis. *Technology Med.sci.*2006; 26(5):504-7.
- 29- Liu Si-Guan. Expression and clinical significance of survivin and P63 in diffuse large B-cell lymphoma. *Journal of leukemia and lymphoma.*2010; 19(4):219-21.
- 30- Ewo GI, Kim LH and Peh SC. The pattern of CD15, CD30 and Bcl-2 expression in diffuse large B-cell lymphoma. *Med J Malaysia.*2006; 61:4.
- 31- Hartmann S, Eray M, Doring C, et al. Diffuse large B-cell lymphoma derived from nodular lymphocyte predominant Hodgkin lymphoma presents with variable histopathology. *BMC Cancer.*2014; 14:332.
- 32- Gobbi PG, Ferreri AJM, Ponzoni M and Levis A. Hodgkin lymphoma. *Critical Reviews in Oncology/Hematology.*2013;85: 216–37.
- 33- Fu X-H, Wang S-S, Huang Y, Xiao J, et al. Prognostic significance of CD20 expression in Hodgkin and Reed-sternberg cells of classical Hodgkin's lymphoma. *Chinese Journal of cancer.*2008; 27(11):450-56.
- 34- Eberle FC, Mani H, Jaffee E S. Histopathology of Hodgkin's lymphoma. *The cancer Journal.*2009; 15:2.
- 35- Tang H, Wei Q , Ge J, Jian W, et al. IMP3 as a supplemental diagnostic marker for Hodgkin lymphoma. *Human Pathology.* 2013; 44: 2167–72.
- 36- Nam-Cha S, Montes-Moreno S, Salcedo MT, et al. Lymphocyte-rich classical Hodgkin's lymphoma : distinctive tumor and microenvironment markers. *Modern paathol.*2009; 22: 1006-15.