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Expression of Ki67and p53 Proteins in Hodgkin 's Lymphomas and Non Hodgkin's Lymphomapatients using immunohistochemistry

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Abstract :

Background: Hodgkin's lymphoma (HL) and Non-Hodgkin's lymphoma(NHL) are characterized by a profound disturbance of the cell cycle and apoptosis regulation., Ki-67 and P53 expression has been shown to be associated with adverse clinical outcome in a variety of malignant hematological disorders, including HL and NHL.
Aim: This study aimed to clarify Ki-67 expression level and its correlation with p53 expression in HL and NHL.
Material and methods: total of 28 newly diagnosed HL and NHL patients were investigated for Ki-67 expression and its correlation to p53 expression by immunohistochemical technique.
Results: Ki-67 expression was observed in 46.7 % of HL patients while 7.7% of NHL patients gave positive Ki-67 expression. A significant Association between thetype of lymphoma and Ki67 expression ($p<0.05$) noticed.

The histopathologcal type appeared to be 57.1% mixed cellularity and 37.5% of them showed positive Ki67 expression , while 42.9 % were intermediate grade and only 16.7 % showed positive Ki67 expression . There were no significant correlation between the histopathologcal type and Ki67 expression. only 4 (14.3 %) of the cases were p53 negative ,while the rest 24 cases were p53 positive ,and the majority (66.7%) were within score 2 . Eight cases (28.6 %) were positive for Ki-67 and p53 , Ki-67 was negative in 71.4% cases , and four of them were negative for p53 and the rest 16 (80%)cases were p53 positive .A significant correlation was found between p53 and Ki-67 expression where($r=0.608, p=0.001$) .
conclusion : it is suggested that immunohistochemical studies of p53 and Ki-67 expression in tumor tissue including HL and NHL can help in monitoring of patients at risk, and the markers may aid in controlling the progression of lymphoma and detect the degree of aggressiveness of the disease to give suitable treatment and management of patients .

Key Words: *Hodgkin's lymphoma (HL), Non-Hodgkin'slymphoma(NHL), Ki-67*

Introduction:

Hodgkin lymphoma (HL), which accounts for approximately 30% of all lymphomas, is composed of two different entities: the rare lymphocyte predominant Hodgkin lymphoma and the more frequent classical Hodgkin lymphoma (cHL) which represent approximately 95% of all Hodgkin lymphomas (1, 2).There has been accumulating evidence that Hodgkin and Reed-Sternberg (H/RS) cells, the presumed neoplastic-cell population in cHL , are characterized by a profound disturbance of the cell cycle and apoptosis regulation.(3)

Most of the studies on the Ki-67 and its prognostic value were performed in the last decade, mainly in malignancies (4). It is a nuclear and nucleolar protein antigen present in all proliferating cells during the active part of the cell cycle: G1, S, G2, and mitosis. Its expression is evaluated immunohistochemically which makes it an excellent marker for determining the so-called growth fraction of a given cell population(5).

Canioni D et al., 1994 (6) stated that Ki-67 with other marker could be used in association with clinical parameters to identify newly diagnosed cHL with favorable or unfavorable prognosis and to establish better treatment for risk groups.

A high proliferation rate has been shown to be associated with adverse clinical outcome in a variety of ma-

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lignant hematological disorders, including non-Hodgkin's lymphomas (NHL) .Ki-67 is used as prognostic factor in NHL and it differentiate indolent from aggressive disease (7) ,(8).High Ki-67 antigen expression has been repeatedly described in Hodgkin's and Reed–Sternberg cells(9). Hall 1988 (4) and Hussainand Harris2006 (10),made clinical classification of non-Hodgkin's lymphomas into high- and low-grade lymphomas which was shown to be mirrored by differences in Ki-67 staining.

One of the major pathways in controlling cell cycle is P53 tumor suppressor, the p53 gene may block the progression of cell growth cycle and trigger apoptosis in response to DNA damage(11). It encoding p53 protein which is involved in regulating a series of pathways including apoptosis, DNA repair, transcription, cell cycle control and genomic stability(11). The mutation of p53 gene causes a loss of tumor-suppressor function, promotes cellular proliferation and inhibits apoptosis(5). The biological activities of p53 are attributed to its ability to arrest the cell cycle at G1 or G2 phase, to induce apoptosis and to maintain genomic stability by modulating DNA repair, replication and recombination (12).

Aim of this study was to clarify Ki-67 expression level and its correlation with p53 expression in Hodgkin's and Non-Hodgkin's lymphoma.

Materials and Methods:

Patients and samples:

A total of 28 of patients newly diagnosed by biopsies to have HL and NHL at Baghdad medical city and private laboratories. Four to five micron section were prepared from paraffin embedded lymphoma tissues , one section was stained with haematoxylin and eosin for histopathololgic review.

Two sections had been prepared to be stained immunohistochemically with p53 and ki 67monoclonal antibody using primary monoclonal mouse anti-human p53 protein (clone Do 7 from Dako,Carpinteriacalif.) and primary monoclonal mouse anti human ki67 antibody from BIO GenexCompany.USA, method used according to Dakocytomation immunohistochemistry detection kit ,USA and examined for Ki-67 and p53 protein expression .

To determine the signal specificity , negative control slides prepared by omission of the primary antibody were included .

All the slides were examined by the light microscope ,a random selection of the fields was used. Positive ki 67 and p53 results gave nuclear dark brownish color .

The results of p53 positivity in each specimen were analyzed according to(15):

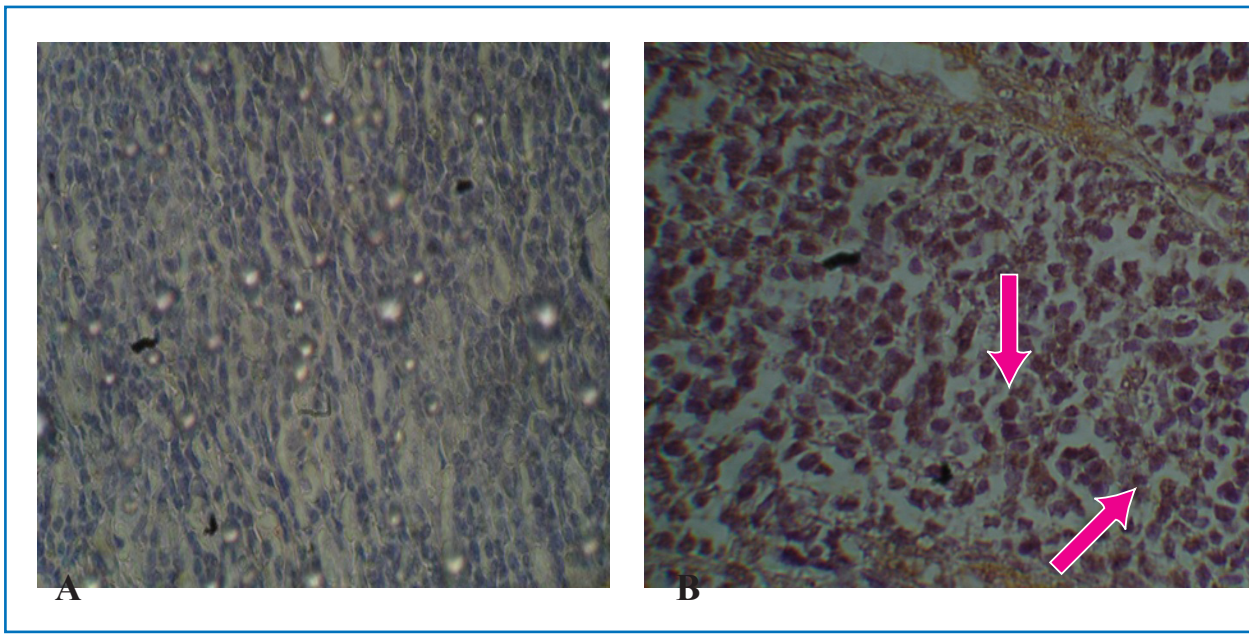
Negative (Score 0) :None of the cells revealed positivity ,
Weak or mild : Staining (5%-< 10%)positive of tumor cells (score +1)
Moderate : Staining (< 25%) (score +2) .
Strong : Staining (< 25% -<50%) (score +3) .
Highly strong (over 50%) (score +4).

Frequency of Ki-67 was counted by dividing number of positive nuclear stained cells by total number of positive and negative immunostained nuclei . The Ki-67 frequency was defined as the percentage of Ki-67-positive tumor cells in representative areas .

Statistical analysis:

Chi – square test was used for tables with frequencies and percentages. Values were considered statistically significant when $p<0.05$.

Results:



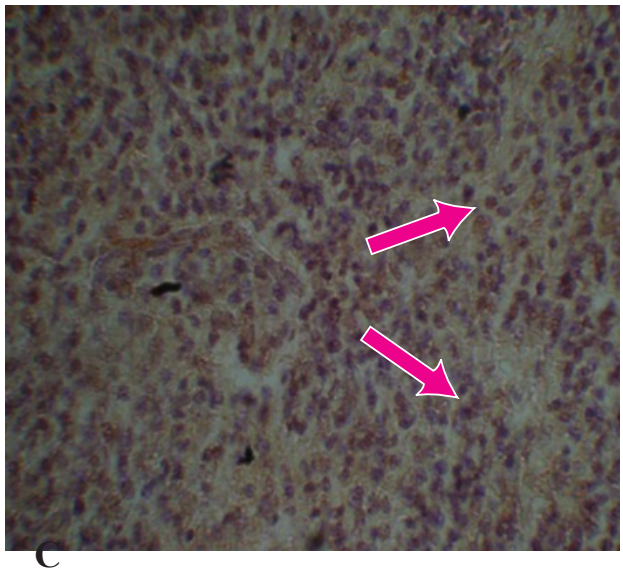


Figure (1) Immunostaining of Hodgkin's lymphoma for Ki-67 Protein
A - Negative control
B- Nuclear Ki-67 protein immunostaining
C- P53 immunostaining of nucleus at the tip of the arrow, Original magnification x 400
Immunohistologic staining revealed nuclear localization of Ki-67 protein.

Table (1) Ki 67 index in patients with Hodgkin's lymphoma and Non Hodgkin's lymphoma .

		Ki-67 positive expression			Chi-square	df	Sig.
		Ki-67 negative	Ki-67 positive	Total			
		Count (%)	Count (%)				
gender	Male	16(76.2%)	5(23.8%)	21 (75%)	0.933	1	0.334
	Female	4(57.1%)	3(42.9%)	7 (25%)			
Type	HL	8(53.3%)	7(46.7%)	15 (53.6%)	5.184	1	0.023*
	NHL	12(92.3%)	1(7.7%)	13 (46.4%)			
Histopathological type	Mixed cellularity	10(62.5%)	6(37.5%)	16 (57.1%)	1.458	1	0.227
	intermediate grade	10(83.3%)	2(16.7%)	12 (42.9%)			
	Total	20(71.4%)	8(28.6%)				

**p< 0.05*

The clinical and pathological features in Table 1 shows that 75% of patients were males and 25% were females. The Ki67 expression was 23.8% in males and 42.9 % in females. There was no significant difference between gender and Ki67immunohistochemical expression (p>0.05).

According to the type of lymphoma 53.6 % were HL from which 46.7 % of them showed positive Ki- 67 expression. NHL represent 46.4 % of all cases, they were only 7.7 % positive Ki-67 expression. There was significant correlation between the type of lymphoma and Ki67 expression (p<0.05).

The histopathologcal type was appeared to be mixed cellularity 57.1% and 37.5% of them showed positive Ki-67 expression while 42. 9 % were intermediate grade and only 16.7 % showed positive Ki-67 expression.There was no significant association between thehistopathologcaltype and Ki67 expression (p>0.05) .

Table (2) Association between p53 and Ki-67 expression in lymphoma patients

P53	Ki-67 positive(%)	Ki-67 negative(%)	Total
P53 positive	8(33.3%)	16(66.6%)	24 (100%)
P53 negative	0(0%)	4(100%)	4 (100%)
Total	8	20	28

Table (3)Association between p53 and Ki-67 expression in lymphoma patients according to scoring system

		Ki-67 negative		Ki-67 positive		Chi-square	df	Sig
		Count	%	Count	%			
p 53 Score	Negative	4	100.0%	0	.0%	11.667	4	0.020*
	Score 1	7	100.0%	0	.0%			
	Score 2	8	66.7%	4	33.3%			
	Score 3	1	33.3%	2	66.7%			
	Score 4	0	.0%	2	100.0%			
	Total	20	71.4%	8	28.6%			

** The Chi-square statistic is significant at the 0.05 level.*
Correlation coefficient(r)= 0.608.

Table 2 shows that 4 cases were p53 and ki-67 negative. The rest 24 cases were p53 positive including 8 (33.3%) were ki-67 and p53 positive , 16 (66.6%) were ki-67 negative. Table (3) showed the results with more details, that, the majority of ki-67 negative cases and p53 positive were 7 in score 1, 8 in score 2 and 1 in score3.

Eight cases (28.6 %) were Ki-67 and p53 positive, while 20 cases (71.4 %) were negative for Ki-67. There is a significant association between Ki-67 and p53 expression where p is<0.05 and r= 0.608.

Disscussion:

Non-Hodgkin's lymphomas represent a group of lymphoid neoplasms that are varied in their manner of presentation, response to therapy and prognosis. A proper clinical evaluation, histopathologic and immunohistochemical assessment may aid in the diagnosis and help in management of the disease at an early stage.(1, 14)

Previous studies have shown that in HL , NHL and others neoplasms, tumoral progression, treatment response, and outcome are related to the expression of different oncogenic and tumor suppressor proteins (15).

In this study 46.7 % of those with HL showed positive Ki- 67 expression, and only7.7 % of NHL cases were with Ki-67 positive expression.

The results showed a significant association between the type of lymphoma and Ki67 expression (p<0.05) which were in accordance with (16, 15). Also Broyde et al ., 2009(7), stated that Ki-67 expression differs by type of lymphoma and its significantly correlated with clinical coarse and outcome and stated that high expression of Ki-67 indicate bad prognosis .

High expression of Ki-67 could lead to aggressiveness of lymphoma as recorded by(7) , others stated that high proliferation rate was associated with adverse clinical outcome in a variety of malignant hematological disorders, including Hodgkin's and non-Hodgkin's lymphomas where high Ki-67 antigen expression has been repeatedly described in Hodgkin's and Reed–Sternberg cells (17) . The Ki-67 index was higher in advanced stage tumors; hence a higher Ki-67 index points toward the aggressive behavior

and poorer clinical outcomes (18).

Regarding to histopathologcal type , Ki67 positive expression was appeared to be 37.5% and16.7 % of mixed cellularity and intermediate grade respectively ,these results indicate that there were no significant association between the histopathologcal type and Ki67 expression (p>0.05) this is in agreement with Kanavarous et al (19).

Wang et al., 2006 (20) , concluded that overexpression of Ki-67 may be an unfavorable prognostic factor, others found overall survival was significantly reduced in those patients with a high Ki-67-associated proliferative index compared with those with a low proliferative index. Xu G, et al 2010(21,22).

Regarding low expression of Ki-67 in this study in NHL patients and intermediate grade histopathologic type , these results may indicate that those patients were with long survival rate and this may agreed with (8).

As we know that p53 is a transcription factor that induces the expression of genes involved in cell cycle arrest or apoptosis in response to a variety of toxic or oncogenic stimuli, the final cellular response depending on the biologic context (16).

In this study 71.4% of all the patients were positive for P53 and negative for Ki-67 expression, this results could be explained according to (23) when they evaluate two tumor biomarkers, MIB-1(Ki-67) and p53 act as potential risk factors in diffuse large cell lymphoma, they suggested that p53 is a dependent risk factor and they stated that the 2-year survival rate was significantly lower with p53 over expression than among those with negative p53 expression .The increase in p53 expression possible reflect an attempt of p53 in excess to induce cell cycle arrest.

A group of 28.6 % of all patients were positive for p53 and Ki -67 and a significant correlation between p53 and Ki-67 expression was found (r=0.608). this is in accordance with (24), they study deregulated expression of cell –cycle and apoptosis- related proteins, that may play roles in the pathogenesis of HL.

They demonstrate that overexpression of p53 and Ki-67 strongly modulate tumor response to chemo and radiotherapy. Our results were in accordance with (25), they concluded that the expression of p53 and Ki-67 correlated directly with each other. But not in accordance with(19).

This study shows that 14.3% of cases under study were both p53 and Ki-67 negative expression, this is in agreement with (23) in which they correlate the negativity of those biomarker with response to chemotherapy which was inversely related to MIB-1 (Ki-67)expressitivity.

In a conclusion ,we suggest that immunohistochemical studies of p53 and Ki-67 expression in tumor tissue including HL and NHL will help in monitoring of patients at risk marker and will aid in controlling the progression of lymphoma and detect the degree of aggressiveness of the disease to give suitable treatment.

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تعبير البروتين Ki-67 والبروتين P53 في مرضى الهوجكن واللاهوجكن ليمفوما باستخدام تقنية المناعة النسيجية الكيمياوية

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الخلاصة:

خلفية الدراسة: ان مرض الهوجكن واللاهوجكن ليمفوما يتصف بانه نتاج لما يحدث من اضطراب او خلل في دورة الخلية و تنظيم عملية استماتة خلايا الورم الليمفاوي .فقد وجد ان نسبة ظهور المعلم Ki-67 و p53المميز للخلايا المنقسمة يترافق مع الحالة المرضية المتقدمة في مرضى الهوجكن واللاهوجكن ليمفوما .

هدف الدراسة: استخدام تقنية المناعة النسيجية الكيمياوية للتقصى عن التعبير المناعي لمعلم انقسام الخلاياKi-67 ومدى التوافق الاحصائي بينه وبين بروتين السيطرة p53في مرضى الهوجكن واللاهوجكن ليمفوما .

طرق العمل: شملت هذه الدراسة ثمانية وعشرون مريضا من مرضى الهوجكن واللاهوجكن ليمفوما باستخدام طرائق التصبيغ المناعي الخلوي لغرض فحص نسبة التعبير البروتين Ki-67 و P53.

النتائج: سجلت هذه الدراسة ان 46.7% من الحالات اضهرت تعبيرا موجبا لبروتين Ki-67في مرض الهوجكن ليمفوما . و نسبة 7.7 % في مرضى اللاهوجكن ليمفوما . كما اظهرت الدراسة ان 57.1% من الحالات تحت الدراسة من نوع mixed cellularity وان 37.5% منها اظهرت تعبيرا موجبا للبروتين Ki-67 , بينما كانت 42.9% من الحالات المدروسة من نوع Intermediate grade وان% 16.7 منها اظهرت تعبيرا موجبا لنفس البروتين . و لم يكن هناك فرق معنويبين نوع النسيج المرضي وتعبير البروتين Ki-67 .

سجلت الدراسة ان هناك اربع حالات ذات تعبير سالب للبروتين p53 و اربعة وعشرين حالة ذات تعبير موجب للبروتين نفسه .عند استخراج الفرق المعنوي الاحصائي (p value) بين p53وki-67 تبين ان نسبة التعبير الموجب لكلا النوعين هو 28.6% بينما بلغ التعبير السالب للبروتين Ki-67 لوحده 71.4% وان اربعة منها هي سالبة للبروتين p53 و ستة عشر حالة موجبة لنفس البروتين وبلغت قيمة (p) اقل من 0.05 كما ان هناك توافق معنوي قوي فيما بين p53 و Ki-67حيث بلغت قيمة (0.608 (r.

الاستنتاجات: ان الدراسة النسيجية المناعية لكلا البروتينين p53 وKi-67 في نسيج الورم الليمفاوي قد تساعد في متابعة مرضى الهوجكن واللاهوجكن ليمفوما لمعرفة شدة وتقدم المرض واعطاء العلاج المناسب .