Prognostic Value of Cyclin E and Cyclin Dependent Kinase Inhibitor (P27) Gene Expression in Non – Hodgkin's Lymphoma

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Abstract: The eukaryotic cell cycle is controlled by protein kinase complexes composed of cyclins and cyclin dependent Kinases (Cdks). The activity of Cdks is regulated by binding of positive effectors, the cyclins, and by association-dissociation of inhibitory subunits, designated cyclin dependent kinase inhibitors (CKIs). Cyclins, Cdks, and CKIs are frequently altered in human cancer. P27 is a CKI that regulates progression from G1 into S phase and appears to play a role in both cell growth and differentiation. Cyclin E, in conjunction with its kinase partner Cdk2, regulates many aspects of cell division. Many human cancers express high levels of cyclin E, and this is thought to directly contribute to cell transformation and tumor aggressiveness. The aim of this study was to study the prognostic value of p27 and cyclin E protein expression levels in relation to the staging of NHL, laboratory data, clinical manifestations and to predict patient's survival. The patients were subjected to full work-up for diagnosis of NHL and Western blot analysis for detection of p27 and cyclin E protein expression.40 newly diagnosed patients suffering from non-Hodgkin's lymphoma (NHL) in different stages and twenty healthy subjects were the subject matter of this study. Our results showed over expression of cyclin E and down regulation of p27, which was significantly associated with advanced staging of the disease. There was a positive correlation between age and over expression of cyclin E and inverse correlation with p27 expression. Over expression of cyclin E and down regulation of p27 were significantly associated with laboratory and clinical findings, delayed remission, increased relapse and increased death rate. Conclusion: p27 and cyclin E expression are significant, independent prognostic factors and reliable molecular markers in predicting recurrence and selection of patients for adjuvant therapy in malignant lymphoma.

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1. Introduction:

Non-Hodgkin's lymphoma (NHL) is a group of closely related B and T-cell cancers of the lymphatic system. The incidence of NHL is rising, particularly in countries of the industrialized world. The incidence of NHL in the United States has increased by 5% over the past 15 years. The cause of this increased incidence is not fully understood, but several risk factors have been blamed including: exposure to chemicals, viral infections, organ transplantation and blood transfusion, family history, and lifestyle factors [(1)].

In general, the incidence of NHL is 50% higher in men than in women [(2)]. The cancer can develop in people at all ages, including children, although it is most common in those aging from 45 – 60 years.

The eukaryotic cell cycle is controlled by protein kinase complexes composed of cyclins and cyclin dependent Kinases (Cdks). The activity of Cdks is regulated by binding of positive effectors, the cyclins, and by association-dissociation of inhibitory subunits, designated cyclin dependent kinase inhibitors (CKIs) [(3)].

Two families of CKIs have been identified; INK4 and Cip/Kip families. The INK4 Family members including $P15^{INK4B}$, $p16^{INK4A}$, $p18^{INK4C}$, and $p19^{INK4D}$ bind to and inhibit Cyclin-D-dependant kinases (cdk4 and cdk6).The Cip/Kip family members, including $p21^{CIP1}$, $p27^{KIP1}$, and $p57^{KIP2}$ preferentially inhibit cdk2 [(4)].

Cyclins, Cdks, and CKIs are frequently altered in human cancer. $P27^{KIP1}$ is a CKI that regulates progression from G1 into S phase by inhibiting a variety of Cyclin-Cdk complexes. P27 appears to play a role in both cell growth and differentiation [(5)].

Cyclin E, in conjunction with its kinase partner Cdk2, regulates many aspects of cell division [(6)]. Cyclin E-Cdk2 exerts its cell cycle regulatory activities by phosphorylating substrates involved in G1 progression, S-phase entry and centrosome duplication, as well as a kinase- independent function involved in exiting quiescence [(7)]. Many human cancers express high levels of cyclin E, and this is

thought to directly contribute to cell transformation and tumor aggressiveness [8].

P27Kip1 protein level changes during cell cycle progression, accumulating when cells progress through G1 and sharply decreasing just before cells enter S phase. Additionally, p27Kip1 protein levels rise when cells exit the cell cycle to G0, and decreases when cells enter the cell cycle again [9]. These alternations in p27Kip1 levels are caused by regulation at the protein degradation level [10].

Deregulation of cell cycle control is a critical step in the development of human cancers and, therefore, knowledge of the expression of cell cycle regulatory proteins in tumor cells is essential for understanding tumor cell behavior and may be important for predicting prognosis of cancer patients [11].

Aim of work:

The aim of the present work is to study the expression level of the cell cycle regulators cyclin E and p27 proteins by Western blot analysis on 40 newly diagnosed patients with NHL in different disease stages and detect their correlation with the staging of NHL, laboratory data, clinical manifestation and patient's survival.

2. Subjects and Methods:

40 newly diagnosed patients suffering from NHL, were selected from the inpatients of the Oncology Unit of Tanta University Hospital and the National Cancer Institute (NCI), Cairo University. The study was designed to continue for 18 months of follow up. Twenty healthy subjects matched in age and sex, were also included, as a control group. The subjects included in this study were divided into two main groups:

Group 1: Patients suffering from NHL in different stages of the disease at presentation and after treatment. They were 25 males and 15 females, their ages ranged from 25-75 years. They were 6 patients with Mucosa Associated Lymphoma Tissue (MALT), 6 patients with Follicular lymphoma, 7 patients with Diffuse large B-cell lymphoma, 5 patients with Anaplastic large T-cell lymphoma, 5 patients with Peripheral T-cell lymphoma, 4 patients with B-cell Burkitt like lymphoma, 4 patients with Mantle cell lymphoma and 3 patients with Marginal zone lymphoma (nodal).

They were divided into four subgroups according to Ann Arbor staging system:

Group Ia: Included 7 patients in stage I suffering from one cervical or axillary lymph node enlargement and discovered accidentally during routine examination of other diseases.

Group Ib: included 8 patients in stage II suffering from both cervical and axillary lymph nodes enlargement with or without splenomegaly or B symptoms (night sweating, unexplained fever and unexplained loss of more than 10% of the body weight in the last 6 months).

Group Ic: included 15 patients in stage III suffering from both cervical and axillary or inguinal or abdominal lymph nodes enlargement, ascitis, pleural effusions or B symptoms.

Group Id: included 10 patients in stage IV suffering from both, cervical and axillary or inguinal or abdominal lymph nodes enlargement, spenomegaly, B symptoms and bone marrow infiltration with or without hepatomegaly or ascitis or pleural effusion.

Group II: normal control group; twenty healthy subjects selected from hospital staff. They were 12 males and 8 females, their ages ranged from 25-60 years.

*Patients with exclusion criteria were out of our study; including patients with inflammatory disease, malignant disease other than NHL, and previous exposure to chemotherapy.

*The Standard CHOP treatment protocol was adopted to our patients: C-Cyclophosphmide 750 mg/m^2 I.V for 1 day. H-Doxorubicin (Adriamycin) 50 mg/m^2 I.V for 1 day. O-Vincristine (Oncovin) 1.4 mg/m^2 I.V for 1 day; and -Prednisone 100 mg daily for 5 days.

This protocol was given one time every 3 weeks for 6 doses.

According to response to treatment, patients of group Ic and Id were further subdivided into Icc, Idc (cured) and Icn, Idn (non-cured), respectively.

*Patients were in complete remission when complete absence of symptoms and signs occurred and laboratory investigation returned to normal levels, while its persistence indicated non-cure.

Patients' group was subjected to the following:

A) Full work–up for diagnosis of NHL:(1)Detailed history and clinical investigation searching for important signs of prognostic significance as night sweating, unexplained fever, unexplained loss of more than 10% of the body weight in the last 6 months, lymphadenopathy, organomegaly, ascitis and pleural effusion.(2)Radiological examination including chest x-ray, CT scan chest, pelvi-abdominal CT scan and ultra-sonography.(3)Laboratory investigation: four ml venous blood was collected under complete aseptic technique, delivered into two tubes; one containing EDTA for CBC and the other was plain for LDH and uric acid assessment. BM

aspiration for cytochemistry and immunophenotyping was done.

B)10 ml venous blood was collected into heparinized tubes for mononuclear cell separation, and protein extraction for detection of Cyclin E and p27 by size separation of the proteins in the mixture on polyacrylamide gel electrophoresis (PAGE). The separated proteins were then transferred to poly vinylidenediflouride (PVDF) membrane (Bio-Rad Laboratory). Detection of the protein under investigation by its specific antibody and determination of its size relative to standard protein of known size was performed.

Informed consent was taken from every patient and control before enrollment in the study and the research was approved by the Ethical committee of Tanta University.

Statistical methods:

Data was analyzed using SPSSwin statistical package version 17 (SPSS Inc., Chicago, IL). Numerical data were expressed as mean and standard deviation. Qualitative data were expressed as frequency and percentage. Chi-square test (Fisher's exact test) was used to examine the relation between qualitative variables. For quantitative data, comparison between two groups was done using Mann-Whitney test (non-parametric t-test). Comparison between 3 groups was done using Kruskal-Wallis test (nonparametric ANOVA). A *p*-value < 0.05 was considered significant and of < 0.001 was considered highly significant.

3. Results:

NHL Patients were divided according to Ann Arbor staging system, as shown in Tables 1 and 2.

Stage I (Group Ia): Seven patients, 4 males (57.1%) and 3 were females (49.9%). Their age ranged from 32 to 59 with mean of 51.14 ± 10.22 years. Stage II (Group Ib): Eight patients, 6 males (75%) and 2 females (25%). Their age ranged from 25 to 62 with mean of 49.87 ± 12.94 years. Stage III (Group Ic): Fifteen patients, 9 males (60%) and 6 females (40%). Their ages ranged from 38 - 75 with mean of 57.6 ± 8.77 years. (Group Id): Ten patients, 6 males (60%) and 4 females (40%).Their ages ranged from 48 - 75 with mean of 60.4 ± 7.5 years. As regards the control group, their ages ranged from 25 to 60 years with a mean of 49.95+ 6.81 years. There was a high statistically significant difference between the expression of p27 and cyclin E in patients and controls (p =0.0001 for both). Figure (1) demonstrates Western blot analysis of cyclin E and p27 in patients compared to controls.

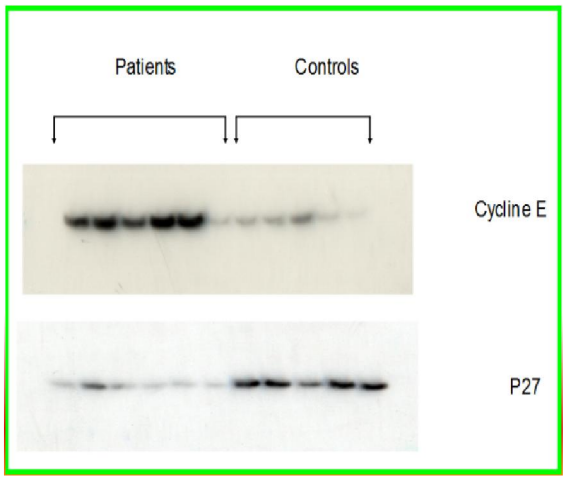

Fig. (1): Cyclin E and P27 protein expression in NHL cases by Western Blot analysis in comparison to controls.

Table (1): Distribution of patients according to age

	Age (years)			
	G Ia	**G Ib**	**G Ic**	**G Id**
Range	32 – 59	25 – 62	38 – 75	48 – 75
Mean	51.14	49.87	57.60	60.40
± SD	10.22	12.94	8.77	7.50
	p-value = 0.079			

Table (2): Relation between sex and the stage of NHL

		Sex		
		Male	**Female**	**Total**
G Ia	**N**	4	3	7
	%	57.1	42.9	100
G Ib	**N**	6	2	8
	%	75	25	100
G Ic	**N**	9	6	15
	%	60	40	100
G Id	**N**	6	4	10
	%	60	40	100
Total	**N**	25	15	40
	%	62.5	37.5	100
	p-value = 0.876			

Down regulation of p27 and over expression of Cyclin E was significantly correlated with advancement of the stage of NHL with *p*-value of 0.001 as shown in tables 3 and 4.

Table (3): Relation between p27 expression and the stage of NHL

Age (years)	P27	
	Normal expression	Down regulation
Mean	47.15	59.70
±SD	11.18	6.76
P. value = 0.000*		

Table (4): Relation between cyclin E expression and stage of NHL

		P27		
		Normal expression	Down regulation	Total
G Ia	N	6	1	7
	%	85.7	14.3	100
G Ib	N	6	2	8
	%	75	25	100
G Ic	N	1	14	15
	%	6.7	93.3	100
G Id	N	0	10	10
	%	0	100	100
Total	N	13	27	40
	%	32.5	67.5	100
	p-value = 0.001*			

Table (5): P27 expression in relation to age

Age (years)	P27	
	Normal expression	Down regulation
Mean	47.15	59.70
±SD	11.18	6.76
P. value = 0.000*		

Table (6): Cyclin E expression in relation to age

Age (years)	Cyclin E	
	Over expression	Normal expression
Mean	62.77	49.77
±SD	5.41	9.52
P. value = 0.000*		

As regards the relation of expression of p27 and cyclin E with sex, no statistical significance could be detected between p27 down regulation or cylin E over expression and sex (p value = 0.138 and 0.251 respectively). However, there was a highly significant association between down regulation of p27 and over expression of cyclin E and increasing age with a p value of 0.0001 for each, as shown in tables 5 and 6. Elevated LDH and reduction in Hb were highly significant with down regulation of p27 with a p value of 0.0001. Thrombocytopenia was associated with down regulation of p27 (P=0.002). There was no significant relation between p27 down regulation and WDCs count with a p value of 0.097, nor with hyperuricemia with a p value of 0.065 (Table 7).

Table (7): P27 expression in relation to Laboratory results

		P27				*P.* value
		Normal expression		Down regulation		
		N	%	N	%	
LDH	N	12	92.3	6	22.2	0.000*
	↑	1	7.7	21	77.8	
HB	N	12	92.3	9	33.3	0.000*
	↓	1	7.7	18	66.7	
Platelet	N	13	100	14	51.9	0.002*
	↓	0	0	13	48.1	
WBCs	N	13	100	22	81.5	0.097
	↓	0	0	5	18.5	
Uric acid	N	13	100	21	77.8	0.065
	↑	0	0	6	22.2	

Elevated LDH and reduced Hb were highly significant with over expression of cyclin E with a p value of 0.0001. Cyclin E over expression was associated with leucopenia (P=0.008), thrombocytopenia (P=0.0001) and hyperuricemia (P = 0.003) (Table 8).

Table (8): Cyclin E expression in relation to laboratory results

		Cyclin E				*p.* value
		Over expression		Normal expression		
		N	%	N	%	
LDH	N	1	5.6	17	77.3	0.000*
	↑	17	94.4	5	22.7	
HB	N	4	22.2	17	77.3	0.001*
	↑	14	77.8	5	22.7	
Platelet	N	6	33.3	21	95.5	0.000*
	↑	12	66.7	1	4.5	
WBCs	N	13	72.2	22	100	0.008*
	↑	5	27.8	0	0	
Uric acid	N	12	66.7	22	100	0.003*
	↑	6	33.3	0	0	

Down regulation of p27 was significantly associated with B symptoms with P value 0.0001. Significant relations were found between down regulation of p27 and the presence of respiratory manifestations, bleeding manifestations, bone ache and splenomegaly with p values of 0.047; 0.047, 0.002, 0.033 and 0.001 respectively. No significant relation was found between p27 down regulation and the presence of hepatomegaly (P=0.065), abdominal manifestations (P=0.13) or neurological manifestation (P=0.097) (Table 9).

Table (9): P27 expression in relation to clinical data

		P27				*P.* Value
		Normal expression		Down regulation		
		N	%	N	%	
Asymptomatic	**Presence**	6	46.2	0	0	0.000*
	Absence	7	53.8	27	100	
B symptoms	**Presence**	7	53.8	27	100	0.000*
	Absence	6	46.2	0	0	
Respiratory	**Presence**	0	0	7	25.9	0.047*
	Absence	13	100	20	74.1	
Bleeding	**Presence**	0	0	13	48.1	0.002*
	Absence	13	100	14	51.6	
Bone ache	**Presence**	1	7.7	11	40.7	0.003*
	Absence	12	92.3	16	59.3	
splenomegaly	**Presence**	2	15.4	21	77.8	0.000*
	Absence	11	84.6	6	22.2	
Hepatomegaly	**Presence**	0	0	6	22.2	0.065
	Absence	13	100	21	77.8	
Abdominal	**Presence**	3	23.1	13	48.1	0.130
	Absence	10	76.9	14	51.9	
CNS	**Presence**	0	0	5	18.5	0.097
	Absence	13	100	22	81.5	

Over expression of cyclin E was significantly associated with B symptoms (*P* =0.004), the presence of respiratory manifestation (*P*=0.024), bleeding manifestations (*P*=0.0001), bone aches (*P*=0.013), the presence of hepatomegaly (*P*=0.003), abdominal manifestations (*P*=0.014) and neurological manifestations (*P*= 0.008) (Table 10).

There was a significant inverse relation between the expression of cyclin E and p27 in NHL patients with p value of 0.004 (Table 11).

After the follow up period, when the fate of the patients was correlated with the level of expression of p27 and cyclin E, the following results were obtained:

Group Ia (Stage I): Significant relation associated between good prognosis and the normal expression of P27 and vice versa with a *p* value of 0.004. Over expression of cyclin E associated significantly with bad prognosis and vice versa with a *p* value of 0.013.

Group Ib (Stage II): A borderline significance could be detected between prognosis and the expression of p27 and cyclin E with a *p* value of 0.061 and 0.058 respectively.

Group Ic (Stage III): Significant relation associated between bad prognosis and the down regulation of p27 and vice versa with a p value of 0.045. Over expression of cyclin E associated with bad prognosis but it was of a borderline significance with a *p* value of 0.051.

Group Id (Stage IV): Significant association was found between bad prognosis and the down regulation of p27 and vice versa with a *p* value of 0.042. Over expression of cyclin E associated significantly with bad prognosis with a *p* value of 0.045.

Table (10): Cyclin E expression in relation to clinical data

		Cyclin E				*P.* value
		Over expression		Normal expression		
		N	%	N	%	
Asymptomatic	**Presence**	0	0	6	27.3	0.16*
	Absence	18	100	16	72.7	
B symptoms	**Presence**	18	100	14	63.6	0.004*
	Absence	0	0	8	36.4	
Respiratory	**Presence**	6	33.3	2	9.1	0.024*
	Absence	12	66.7	20	90.9	
Bleeding	**Presence**	12	66.7	1	4.5	0.000*
	Absence	6	33.3	21	95.5	
Bone ache	**Presence**	9	50	3	13.6	0.013*
	Absence	9	50	19	86.4	
Splenomegaly	**Presence**	17	94.4	6	27.3	0.000*
	Absence	1	5.6	16	72.7	
Hepatomegaly	**Presence**	6	33.3	0	0	0.003*
	Absence	12	66.7	22	100	
Abdominal	**Presence**	11	61.1	5	22.7	0.014*
	Absence	7	38.9	17	77.3	
CNS	**Presence**	5	27.8	0	0	0.008*
	Absence	13	72.2	22	100	

Table (11): Relation between cyclin E expression and the expression of p27 in NHL patients

		Cyclin E					
		Over expression		Normal expression		Total	
		N	%	N	%	N	%
P27	**Normal expression**	1	5.6	12	54.5	13	32.5
	Down regulation	17	94.4	10	45.5	27	67.5
Total		18	100	22	100	40	100
P* = 0.004							

Table (12): Fate according to NHL staging in relation to cyclin E and P27 expression

Groups	Fate & Expression		Remission	relapse	Died	*P* value
Group Ia	**P27**	Normal expression	6	1	0	0.044*
		Down regulation	1	3	1	
	Cyclin E	Over expression	0	3	1	0.013*
		Normal regulation	7	1	0	
Group Ib	**P27**	Normal expression	5	1	0	0.061
		Down regulation	1	3	2	
	Cyclin E	Over expression	1	3	2	0.058
		Normal regulation	5	1	0	
Group Ic	**P27**	Normal expression	0	0	1	0.045*
		Down regulation	0	0	14	
	Cyclin E	Over expression	0	0	10	0.051*
		Normal regulation	0	0	5	
Group Id	**P27**	Normal expression	0	0	0	0.042*
		Down regulation	0	0	10	
	Cyclin E	Over expression	0	0	8	0.045*
		Normal regulation	0	0	2	

Kaplan-Meier analysis of overall survival in NHL patients in relation to p27 expression shows significant short overall survival with p27 down regulation with (P=0.004) as shown in fig. 2.

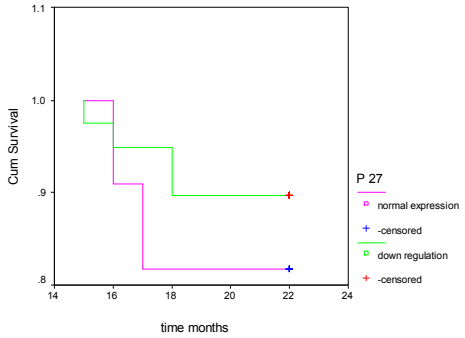

Fig. (2): Overall survival in NHL patients in relation to p27 expression at the end of the study

Kaplan-Meier analysis of overall survival in NHL patients in relation to Cyclin E expression show significant short overall survival with Cyclin E over expression with (P=0.011) as shown in fig.3.

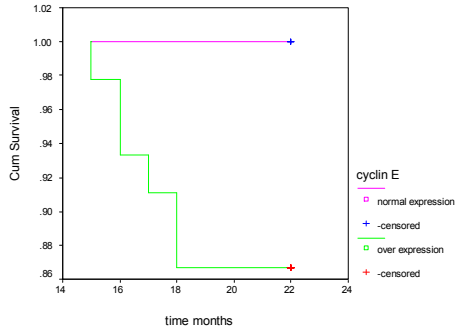

Fig (3): Overall survival in NHL patients in relation to Cyclin E expression at the end of the study

Kaplan-Meier analysis of overall survival in NHL patients in relation to Cyclin E expression shows significant short event free survival with Cyclin E over expression with (P=0.041) as shown in fig. 4.

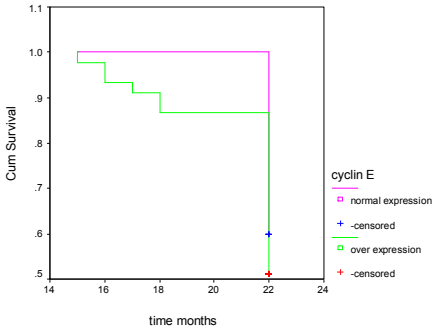

Fig. (4): Event free survival in NHL patients in relation to Cyclin E expression at the end of the study

Kaplan-Meier analysis of event free survival in NHL patients in relation to P27 expression shows significant short event free survival with P27 over expression with (P=0.03) as shown in fig.5.

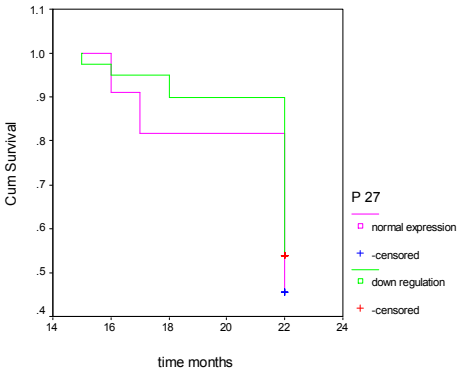

Fig. (5): Event free survival in NHL patients in relation to P27 expression at the end of the study

4. Discussion

A re-evaluation of parameters of cell cycle kinetics in view of our increasing knowledge of the molecular pathways of cell cycle control may provide more prognostic information for the management of patients with malignant lymphomas. Precise staging of NHL is prerequisite for the selection of a suitable therapeutic regiment and influence the likelihood of its success [1, 12].

The aim of the present work was to study the expression of the cell cycle regulators Cyclin E and P27 proteins by Western blot analysis on 40 newly diagnosed patients with NHL in different disease stages and to correlate their expression with the laboratory data, clinical manifestations, staging of NHL, and patient's survival.

In relation to the staging of NHL the present study showed that over expression of Cyclin E was significantly correlated with worsening of NHL outcome.

In agreement with this study **Keyomarsi *et al.*,** [13] and **Qi *et al.*,** [10] had observed a correlation between cyclin E expression, proliferation and aggressive staging. In contrast to this study **Farleya *et al.*,** [14] showed that expression of cyclin E was not associated with tumor stage.

In this study, the cyclin E expression in relation to sex was statistically not significant. On the other hand, the advancement of age of NHL patients was highly significantly associated with over expression of cyclin E. In agreement with this study **Xiangming *et al.*,** [15] showed that expression of cyclin E was related to the age of patients. In contrast, **Farleya *et al.*,** [14] showed that expression of cyclin E was not correlated with age.

As regards cyclin E expression and its relation to clinical data and laboratory findings, it was found that cyclin E over expression is significantly associated with worsening of all clinical and laboratory results. We agree in our results with **Ferreri *et al.,*** [16] who showed that high levels of cyclin E have been associated with advanced NHL manifestation.

Regarding the fate of the patients in relation to expression of cyclin E, our study demonstrated that over expression of cyclin E associated significantly with bad prognosis as delayed response to treatment, high rate of relapse and high incidence of mortality. The overall survival using Kaplan-Meier analysis in NHL patients in relation to cyclin E expression, showed significant short overall survival with Cyclin E over expression.

Event free survival showed significantly shorter event free survival with cyclin E over expressed patients. We agree in this with **Hayashi *et al.,*** [17] and **Keyomarsi *et al.,*** [13] who reported that all patients with a high level of cyclinE died. Also, **Keyomarsi *et al.,*** [13] and **Porter *et al.,*** [18] reported that the overall survival and the event-free survival were significantly longer among patients with low levels of cyclin E than among patients whose tumors had high levels of this protein. However, **Porter *et al.,*** [18] reported that high expression of cyclin E protein was not statistically significantly associated with either overall survival or event-free survival.

As regards p27 expression; down regulation of p27 in NHL patients was significantly associated with advanced staging of the disease. In agreement with our study, is the study of **Yasui *et al.,*** [19], who reported that down regulation of p27 expression significantly correlated with advanced stage, depth of tumor invasion and lymph node metastasis. These findings coincide also with those reported by **Xiangming *et al.,*** [15]; **Chiarle *et al.,*** [20]; **Khoo *et al.,*** [21]; **Moreira *et al.,*** [22] and **Porter *et al.,*** [18]. However, **Xiangming *et al.,*** [15]; **Gelen *et al.,*** [23] and **Filipits *et al.,*** [24] reported that p27 expression did not correlate with any of the clinic-pathological parameters examined including stage of the tumors, but **Xiangming *et al.,*** [15] reported that down regulation of p27 protein expression is age related and age has been reported as an important prognostic factor for malignant lymphomas.

Other authors as **Gelen *et al.,*** [23]; and **Filipits *et al.,*** [24] investigated the relationship between the expression of p27 and a series of clinico-pathological parameters including age and did not correlate p27 expression with any of the clinic-patholgical parameters examined.

Regarding the fate of the patients in relation to expression of p27, our study demonstrated that p27 down regulation is associated significantly with bad prognosis as delayed response to the treatment, high rate of relapse and high incidence of mortality.

Analysis of the overall survival in NHL patients in relation to p27 expression showed significant short overall survival with p27 down regulation. Analysis of event free survival also showed significant short event free survival with p27 down regulation.

In agreement with our results, are the results of **Yasui *et al.,*** [19], **Erlanson *et al.,*** [25] and **Moller *et al.,*** [26], who reported that reduced expression of p27 had been associated with aggressive tumor growth and predicted poor survival of patients. **Chiarle *et al.,*** [20] reported that total or partial loss of p27 appears to be a consistent feature of mantle cell lymphoma (MCL) and one that differentiates MCL from low-and even high-grade lymphomas, because p27 expression appears to correlate inversely with the cell proliferation index and the overall survival times.

Moreover, **Gelen *et al.,*** [23] reported that the presence of recurrence and relapse was more frequent in tumors with low p27. **Boudova *et al.,*** [3] reported that loss of p27 expression is a negative prognostic factor in the majority of B-Cell lymphomas. Also **Paik *et al.,*** [27] reported that p27 down regulation has been seated to be indicative of a poor prognosis in malignant lymphoma. **Porter *et al.,*** [18] reported that the expression of p27 protein was statistically significantly associated with overall survival and with event-free survival, and lower expression associated with poorer survival.

This work revealed that when p27 down regulation is accompanied with cyclin E overexpression this indicates advancing stage of the disease, delayed response to the treatment, increased possibility of relapse and recurrence and finally increased risk of death as indicated by short overall and event free survival among the patients. Several other authors reported that a high cyclin E level was shown to be a prognostic marker for poor prognosis, particularly when correlated with a low p27 level [10, 16, 18, 28, 29]

Conclusion:

P27 and cyclin E expression are important prognostic markers for lymphomas and, when combined with serum LDH levels, distinct prognostic subgroups of NHL patients can be defined. Modulation of p27 and cyclin E expression may be a potential therapeutic strategy to improve clinical outcome in patients with NHL in the future.

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