

Evaluation of P53 in Relapsed and Refractory Aggressive NHL and Response to DHAP Protocol

FOUAD M. ABOU TALEB, M.D.*; IBRAHIM AMIN IBRAHIM, M.D.**; SAAD SAYED EISSA, M.D.***; JEHAN ABD EL-KADER IBRAHIM, M.D.** and AYMAN FATHY ABD EL-HALIM, M.B.B.Ch.

The Departments of Medical Oncology, Internal Medicine**, Zagazig University, Pathology***, Department N.C.I. Cairo University, Zagazig, Egypt.*

ABSTRACT

Forty patients with relapsed/refractory aggressive non Hodgkin's lymphomas (NHLs) of diffuse large cell type were included in the study. All patients were subjected to complete history taking, physical examination, routine laboratory and radiological investigations for proper staging and evaluation of p53 by immunohistochemistry. All patients were treated with DHAP protocol "cisplatin 100 mg/m² D1 C.I. over 24 hours, cytosine arabinoside 2 gm/m² over 3 hours every 12 hours D2 and dexamethasone 40 mg I.V./day for four days from D1 to D4". Evaluation was carried out after two cycles and responding patients continued for 6 cycles. Our results showed that DHAP protocol induced complete response (CR) in 37.5% of patients, partial response (PR) in 22.5% of patients and no response (NR) in 40% of patients. P53 expression by immunohistochemistry was positive in 47.5% patients of relapsed/refractory aggressive NHLs and more prevalent in old patients (≥ 60 years) poor performance status (PS) and high LDH. Also, P53 expression was a good predictor for poor response; the results showed that there was an independent association of P53 with drug resistance along with performance status and age. Only old age (≥ 60 years) high LDH, poor performance status, short time of disease duration before starting salvage therapy and refractory disease to initial protocol (s) were statistically significant in predicting poor response. Toxicity to DHAP protocol was acceptable except for hepatic toxicity which terminated the protocol in 12% of patients and delayed chemotherapy in 20% of cases due to HCV infection. In conclusion DHAP protocol provides effective short term therapy in relapsed/refractory aggressive NHLs but further advances in high dose chemoradiotherapy will be necessary for long-term survival. Assessment of P53 mutations and expression as well as other genes related to P53 such as P21 WAF1/CIP1 and MDM2 are needed for evaluation of the actual P53 gene status.

Key Words: P53 - NHL - DHAP.

INTRODUCTION

Most patients with NHLs ultimately develop resistance to chemotherapy and die of disease. So, the identification of molecular and biological

markers of drug resistance may allow the development of a prognostic index based on actual measures of drug resistance [16].

Only 40% or fewer of CHOP-treated patients are cured, therefore, many patients will require salvage treatment. Unfortunately, the therapy of patients with relapsed and refractory disease remains inadequate. Of importance is that 75-80% of recurrence occurs within 2 years [1].

Five to 10% of patients with aggressive NHLs do not respond to induction therapy, 5-15% of patients with aggressive NHLs achieve only a partial response and 20-40% of patients with aggressive NHLs who achieve initial CR subsequently develop recurrent disease and likelihood of relapsing from CR varies depending on patients initial risk profile [14].

P53 is a tumor suppressor gene found to be inactivated in most types of human cancer and it is proven that it plays an important role in control of cell cycle, maintenance of genomic stability, cell differentiation and programmed cell death (apoptosis) [17].

Forty percent of cases of aggressive NHLs were reported to have $> 5\%$ of the cells staining positively for P53 protein; this correlated with response to chemotherapy as CR occurred in 50% of cases with P53 positive tumours versus 77% of cases with P53 negative tumours [10]. Furthermore, with P53 positive tumours relapse occurred in 60% of patients in a median time of 6 months whereas, relapse in P53 negative tumours occurred in 26% of patients in a median time more than 22 months. Also, the overall

survival was 17 months in P53 positive tumours compared to more than 24 months in P53 negative tumours [5].

In this work we aimed to determine P53 expression, to evaluate response and toxicity of DHAP protocol as a promising salvage treatment and to evaluate relation between the response to DHAP and P53 expression in relapsed and refractory aggressive NHLs.

Table (1): Patient characteristics of 40 relapsed and refractory aggressive NHL.

Charater	No.	%	Charater	No.	%
Total	40	100.0	B.M. involvement	2	5.0
<i>Sex:</i>			Bulky disease	12	30.0
Male	18	45.0	Refractory disease	21	52.5
Female	22	55.0	No. of protocol: 1	(13)	(32.5)
<i>Age:</i>			≥ 2	(8)	(20.0)
< 60	18	45.0	Relapsed patients	19	47.5
≥ 60	22	55.0	No. of relapsed: 1	(14)	(35.0)
<i>LDH:</i>			≥ 2	(5)	(12.5)
Normal	12	30.0	Duration of the disease		
High	28	70.0	≥ 1 year	17	42.5
<i>PS:</i>			< 1 year	23	57.5
0-1	22	55.0			
≥ 2	18	45.0	Patients required radiotherapy	4	10.0
<i>Stage:</i>					
I/II	6	15.0			
III/IV	34	85.0			
<i>B-symtoms:</i>					
Absent	14	35.0			
Present	26	65.0			

Patient eligibility:

- Our patients were refractory or relapsed aggressive NHLs after at least one doxorubicin-based regimen.
- Patient who received regimens containing high dose-cytarabine or cisplatin were excluded.
- Patients with active cardiac, renal, liver disease were excluded.
- No age restriction.
- Patients with severe toxicities after the first cycle of DHAP requiring termination of the protocol were excluded.

Methods:

Each patient was subjected to the following:

Complete history with stress on presence or absence of B-symptoms, complete physical examination including assessment of all measura-

PATIENTS AND METHODS

Patients:

This study was carried out in Medical Oncology and Haematology unit, Internal Medicine Department, Zagazig University Hospital between Oct. 1998 and Dec. 1999, where 40 patients with relapsed or refractory aggressive NHLs (of diffuse large cell type) were included (Table 1).

ble disease, routine laboratory investigations including, CBC, bone marrow examination, liver function tests, serum creatinine, random blood glucose creatinine clearance, serum uric acid, serum LDH, routine radiological studies including CT abdomen and pelvis, CT chest if there is abnormalities in the plain film for proper staging.

Evaluation of P53 was performed on fine needle aspiration biopsy specimen fixed in absolute ethanol, immunoperoxidase staining was performed using anti P53 mouse IgG_{2a} monoclonal antibody (DAKO USA) and the avidin-biotin complex (ABC) staining technique [8].

All patient were treated with DHAP protocol:

"Cisplatin 100 gm/m² D1 C.I. over 24 hours, cytosine arabinoside 2 gm/m² over 3 hours every 12 hours D2 and dexamethasone 40 mg I.V./day for four days from D1 to D4" with

well hydration, Co-trimoxazole prophyl-axis against pneumocytitis caranii and dexamethasone containing eye drops, the cycle is repeated 3-4 weeks apart depending on recovery of CBC and after 2 cycles all sites of the disease were reevaluated and patients were restaged and responding patients continued for 6 cycles.

Statistical analysis:

Data were analyzed using Epi-Info version 6.02 computer package [2] and Logress 2 for multivariate logistic regression analysis. Data were expressed as number and percentage, Chi-squared (X^2) test or fisher exact test were used whenever appropriate and probability (P) was considered significant at 5% level.

RESULTS

Table (2) shows the response to DHAP protocol and P53 expression in relapsed/refractory NHLs, Figs (1 and 2).

Table (3) shows the association between P53 expression and other variables, only the age, LDH, PS and response to chemotherapy were statistically significant ($p = 0.02, 0.01, 0.028$ and 0.002 respectively).

Table (4) shows variables predicting the response to DHAP where age, LDH and PS were statistically significant in predicting the response. Response was better in young ages (< 60 years), normal LDH and good performance status (0-1) (p value = $0.018, 0.04$ and 0.04 respectively). Also, relapsed disease was associated with better response than refractory disease ($p = 0.002$) but the number of relapse was not statistically significant in predicting the response ($p > 0.05$). Refractoriness to more than one protocol was not statistically significant ($p > 0.05$). Duration of the disease before starting the protocol was highly significant as the response was much better in patients with disease duration ≥ 1 year ($p = 0.0001$) and P53 expression was statistically significant in predicting the response as response was worse in P53 +ve cases ($p = 0.002$).

Table (5) study of the simultaneous effect of these factors, by logistic regression analysis, showed that only P53, PS and age can be considered as the predicting for the association. The rest of variables could not add significantly to the model.

Table (6) shows the toxicity to DHAP protocol.

Table (2): Response to DHAP protocol and P53 expression in 40 relapsed and refractory aggressive NHL patients.

Charater	No.	%
<i>Response:</i>		
CR	15	37.5
CR after 2 cycles	(9)	(60.0)
CR after 6 cycles	(6)	(40.0)
PR	9	22.5
NR	16	40.0
<i>P53 expression:</i>		
+ve	19	47.5
-ve	21	52.5

Table (3): Various parameters in 40 relapsed and refractory aggressive NHL patients is relation to P53 expression.

	Total No. examined		P53 +ve		<i>p</i> -value
	No.	No.	%		
<i>Sex:</i>					
Male	18	9	50.0		0.77
Female	22	10	45.5		
<i>Age:</i>					
< 60	18	5	27.8		0.02
≥ 60	22	14	63.6		
<i>LDH:</i>					
Normal	12	2	16.7		0.01
Elevated	28	17	60.7		
<i>PS:</i>					
0-1	22	7	31.8		0.028
≥ 2	18	12	66.7		
<i>Stage:</i>					
I,II	6	1	16.7		0.1
III,IV	34	18	52.9		
<i>B-sympoms:</i>					
Absent	14	5	35.7		0.27
Present	26	14	53.8		
<i>B.M involvement</i>					
No B.M. involvement	2	1	50.0		0.94
Bulky disease	38	18	47.4		
<i>Bulky disease</i>					
Non-bulky disease	12	8	66.7		0.11
Refractory	28	11	39.3		
<i>Refractory</i>					
Relapsed	21	11	52.4		0.51
Refractory to:	19	8	42.1		
<i>Refractory to:</i>					
One protocol	13	6	46.2		0.46
≥ 2	8	5	62.5		
<i>No. of relapse:</i>					
1	14	7	50.0		0.24
$\ddagger 2$	5	1	20.0		
<i>Duration of disease:</i>					
≥ 1 year	17	8	47.1		0.96
< 1 year	23	11	47.8		
<i>Response:</i>					
CR	15	2	13.3		0.002
PR	9	5	55.5		
NR	16	12	75.0		

Table (4): Different parameters in 40 relapsed and refractory aggressive NHL patients in relation to response to DHAP protocol.

	Total	CR		PR		NR		p-value
		No.	%	No.	%	No.	%	
<i>Sex:</i>								
Male	18	6	33.3	4	22.2	8	44.4	0.85
Female	22	9	40.9	5	22.7	8	36.4	
<i>Age:</i>								
< 60	18	11	61.1	3	16.7	4	22.2	0.018
≥ 60	22	4	18.2	6	27.3	12	54.5	
<i>LDH:</i>								
Normal	12	8	66.3	1	8.3	3	25.0	0.04
High	28	7	25.0	8	28.6	13	46.4	
<i>PS:</i>								
0-1	22	12	54.5	4	18.2	6	27.3	0.04
≥ 2	18	3	16.67	5	27.78	10	55.56	
<i>Stage:</i>								
I,II	6	3	50.0	1	16.7	2	33.3	0.78
III,IV	34	12	35.3	8	23.5	14	41.2	
<i>B-sympoms:</i>								
Absent	14	5	35.7	3	21.4	6	42.9	0.96
Present	26	10	38.46	6	23.08	10	38.46	
<i>B.M involvement</i>								
No B.M. involvement	2	0	0.0	0	0.0	2	100.0	0.2
Bulky disease	38	15	39.5	9	23.7	14	36.8	
<i>Refractory disease</i>								
Refractory disease	12	3	25.0	2	16.7	7	58.3	0.23
Relapsed disease	28	12	42.9	7	25.0	9	32.1	
<i>Refractory to:</i>								
One protocol	21	7	33.3	1	4.8	13	61.9	0.002
≥ 2	19	8	42.1	8	42.1	3	15.8	
<i>No. of relapse 1:</i>								
1	13	6	46.2	0	0.0	7	53.8	0.15
≥ 2	8	1	12.5	1	12.5	6	75.0	
<i>Duration of disease:</i>								
≥ 1 year	14	6	42.9	5	35.7	3	21.4	0.4
< 1 year	5	2	40.0	3	60.0	0	0.0	
<i>P53 expression:</i>								
+ve	17	8	47.1	8	47.1	1	5.9	0.001
-ve	23	7	30.45	1	4.35	15	65.2	
<i>P53 expression:</i>								
+ve	19	2	10.5	5	26.3	12	63.2	0.002
-ve	21	13	61.9	4	19.05	4	19.05	

Table (5): Logistic regression analysis for response to DHAP protocol.

	B-coefficient	SE	Z	OR (95% CI)
P53	-0.47	0.24	2	1.61 (1.01-2.55)
PS	-0.08	0.03	2.89	1.08 (1.03-1.14)
Age	0.06	0.03	2.57	1.07 (1.02-1.12)

Table (6): Toxicity of DHAP protocol.

Toxicity	Grade	No.	%
Nausea	2	40	100.0
	3	22	55.0
	Total*	40	100.0
Vomiting	1	9	22.5
	2	3	7.5
	3	9	22.5
Neutropenia	4	3	7.5
	Total*	24	60.0
	2	3	7.5
Anaemia	3	9	22.5
	4	7	17.5
	Total*	19	47.5
Thrombocytopenia	1	5	12.5
	2	2	5.0
	3	1	2.5
Hepatic enzymes affection	Total*	8	20.0
	1	9	22.5
	2	3	7.5
Clinical	4	5	12.5
	Total*	17	42.5
	3	3	7.5
Renal (serum creatinine)	4	3	7.5
	Total*	6	15.0
	1	2	5.0
Diarrhea	2	3	7.5
	3	2	5.0
	4	1	2.5
Sensory neural hearing loss	Total*	8	20.0
	1	5	12.5
	2	2	5.0
Sensory peripheral neuritis	3	3	7.5
	Total*	10	25.0
	2	4	10.0
	2	6	12.5

* Total was the number showing the manifestation out of the whole group.

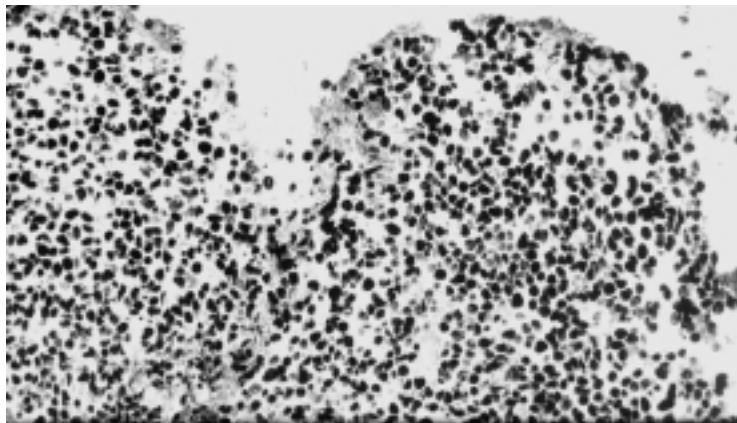


Fig. (1): Cell block preparation of FNA biopsy of lymph node. NHL, large cell type, immunoperoxidase stained, showing high expression of mutated P53 protein product in the nuclei of the tumour cell, magnification x 1250.

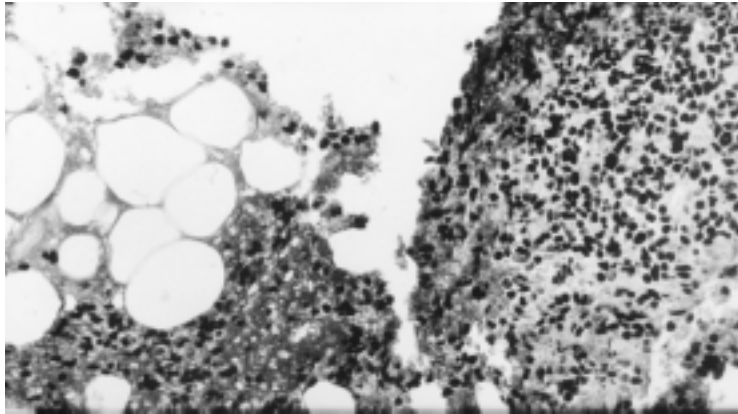


Fig. (2): Cell block preparation of FNA biopsy of lymph node. NHL, large cell type, immunoperoxidase stained, showing high expression of mutated P53 protein product in the nuclei of the tumour cell, magnification x 1250.

DISCUSSION

P53 mutations occur with a frequency of 12.5% in lymphoid malignancies. The viral associated diseases such as adult T-cell leukemia and Burkitt's lymphoma showed higher rates of P53 mutations with frequencies of 24% and 41% respectively [11].

High grade B-NHLs have about 30% incidence of P53 mutations, whereas indolent B-NHLs rarely have P53 mutation. About 10% of T-cell NHLs have P53 mutation. Whereas, 50% of aggressive B-cell NHLs express high level P53 protein, only 20-50% of these cases had demonstrable P53 mutations [9].

In Egypt, diffuse aggressive NHLs predominate and constitute 87% of cases in adults, most of them of B-phenotype (82.6%) with over expression of P53 (34.6%) and BCL-2 (44.2%) [7].

We undertook the present study of P53 expression and response to DHAP protocol as a salvage treatment in 40 patients with relapsed/refractory aggressive NHLs to assess the relationship of this marker of drug resistance, to different clinical variables including those of international prognostic index (IPI).

The response to DHAP protocol was 37.5% CR, 22.5% stable PR (over all response is 60%) with no response and/or disease progression in 40% of patients.

These results are in agreement with the initial report by (Press et al., 1991 and Martelli et al., 1996) [6,12] of an overall response 67% and 59% respectively

Our toxicity studies were in agreement with (Press et al., 1991) [12] but in our study there was a higher incidence of hepatotoxicity due to

the higher prevalence of viral hepatitis in our patients especially HCV infection.

In our study, old age (≥ 60 years) high LDH, poor performance status (≥ 2), primary refractory disease, patients with short duration of the disease (< 1 year) and P53 expression were adverse prognostic feature. This is in agreement with (Wilson et al., 1997) [16]. Bulky disease was another poor prognostic factor that did not reach the statistical significance in our study, this may be due to the small number of patients with bulky disease. Also, duration of the disease was not studied by (Wilson et al., 1997) [16] and this variable was highly significant in our study.

In our study the incidence of P53 +ve cases of relapsed/refractory aggressive NHLs was 47.5% and the incidence was estimated by (Wilson et al., 1997) [16] to be 21%. This difference might be due to presence of cases with indolent and transformed pathology which are not included in our study and indolent B-NHLs rarely show P53 mutations [9], it may also reflect the special unfavorable features of Egyptian NHLs [3] and finally may reflect that the true incidence of P53 expression is not known [4].

In our study P53 is significantly correlated with age, PS, LDH and response to DHAP protocol but was not correlated with any of international prognostic index in the work of (Wilson et al., 1997) [16]. This may be due to difference in total number and presence of histopathology other than diffuse large cell type in our study.

Two interesting observations emerged from (Ichikawa et al., 1997) [5] study analysis. First, patients, with P53 mutations were older and far

less likely to achieve a CR. Second, P53 was an independent marker in lower risk patients per IPI criteria, whereas high risk patients per IPI had dismal out-come irrespective of their P53 status and this is in agreement with our results.

In our study P53 expression correlated significantly with drug resistance in the form of no response in 63.2% of +ve cases and only in 19% of -ve cases, this confirm the results of (Wilson et al., 1997 and Sun et al., 1998) [15,16].

None of the variables affected the time of CR including P53 and this is in agreement with (Sakai et al., 1998) [13].

Logistic regression analysis of all variables confirmed the association of P53 abnormality with drug resistance along with PS and age and this is in agreement with (Wilson et al., 1997) [16].

In conclusion DHAP protocol provides effective short term therapy in relapsed/refractory aggressive NHLs but further advances in high dose chemoradiotherapy will be necessary for long-term survival. Assessment of P53 mutations and expression as well as other genes related to P53 such as P21 WAF/CIP1 and MDM2 are needed for evaluation of the actual P53 gene status.

REFERENCES

- 1- Cheson B.D.: Treatment strategies for intermediate and high grade NHLs. In Educational program book, ISH EHA, combined haematology congress, Amsterdam, 4-8 Jul. 1998. Lowenberg B., Degosl, Willemze R., Mc. Arthur. J. and Bojuszji (eds.) European Haematology Association, p. 128-139, 1998.
- 2- Dean A.G., Dean J.A., Coulmbier D. and Brendel K.A.: Epi-info version 6.02: A word proessing, data base and statistics program for epidemiology on microcomputer. Center for Disease Control, Atlanta, Georgia, USA, 1994.
- 3- El-Bolkainy M.N.: Non-Hodgkin's lymphomas In: Topographic pathology of cancer, El-Bolkainy M.N. (ed) 1st ed, NCI Cairo University, p. 175-181, 1998.
- 4- Ferreira C.G., Tolis C. and Giaccone G.: P53 and chemosensitivity. *Annals of Oncology*, 10: 101-107, 1999.
- 5- Ichikawa A., Kinoshita T. and Watanabe T.: Mutations of P53 gene as a prognostic factor in aggressive B-cell lymphoma *N. Engl. J. Med.*, 337: 529-536, 1997.
- 6- Martelli M., Vignetti M. and Zinazoni P.L.: High dose chemotherapy followed by autologous BMT versus DHAP protocol in aggressive NHLs with partial response to front line chemotherapy: A prospective randomized Italian multi-center study *J. Clin. Oncol.*, 14 (2): 539-551, 1996.
- 7- Mokhatar N., Khald H., Anwar N., El-Houseiny S., Taha H., Murad M., Gd El-mawal H., El-Bolkainy N., Jaffe E., Magrath I. and Kingma D.: Biologic profile of NHL in Egypt, relation to response to BECOP regimen cancer *Mol. Biol.*, 1: 255-263, 1994.
- 8- Nadji M. & Morales A.R.: "Immunoperoxidase" In: the technique and its pit falls. *Lab. Med.*, 14: 767. In: Tumour makers, 2nd, ed. 1998, Eissa S. and Shoman S. (eds). Rub. Chapman Hall, p. 3-11, 1983.
- 9- Nakamura H., Said J.W., Miller C.W. and Koefler H.P.: Mutation and expression of P53 in acquired immunodeficiency syndrome related lymphoma. *Blood*, 82: 920-926, 1993.
- 10- Navaratman S., William G.I. and Pettigrew N.: Expression of P53 predicts treatment failure in aggressive NHLs leukemia and lymphoma, 29 (1-2): 139-148, 1998.
- 11- Newcomb E.W.: "p53 gene mutation in lymphoid diseases and their possible relevance to drug resistance" *Leukemia and lymphoma*, 17 (3-4): 211-218, 1995.
- 12- Press O.W., Livingston R. and Mortimer J.: Treatment of relapsed NHLs with dexamethasone, high dose cytrabine and cisplatin before bone marrow transplantation *J. clin. Oncol.*, 9 (3): 423-431, 1991.
- 13- Sakai A., Oda K., Asooku H. and Shintaku S.: Expression of P53 and PCNA do not correlate with international index or early response to chemotherapy in NHLs *Am. J. Haematol.*, 58 (1): 42-48, 1998.
- 14- Shipp M.A., Mauch P.M. and Harris N.L.: "Non-Hodgkin's lymphomas". In: *Cancer principles and practice of oncology*. Devita V.T., Hellman T.S. and Rosenberg S.A. (eds) 5th ed, lipincott Raven Publishers, Philadelphia, p. 2163-2171, 1997.
- 15- Sun R.X., Coste J., Segara C. and Rousset T.:

- MDR rearrangement and P-glycoprotein expression are not independent prognostic factor like P53 protein in malignant lymphoma. *Clinical and laboratory Haematology*, 20 (2): 87-94, 1998.
- 16- Wilson W.H., Teruya-Feldstein J.T., Fest T., Harris C. and Raffeld M.: Relationship of P53 BCL-2 and tumor proliferation to clinical drug resistance in non-Hodgkin's lymphoma *Blood*, 89 (2): 601-609, 1997.
- 17- Yin Y., Tainsky M.A., Farideh Z.B. and Wahl G.M.: Wild-type P53 restores cells cycle control and inhibit gene amplification in cell with mutant P53 alleles. *Cell*, 70: 937-943, 1993.