P53 Expression In Chronic Lymphocytic Leukaemia

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Summary:

Background: *Chronic lymphoctic leukaemia (CLL) is an acuired clonal Lymphoroliferative disorder characterized by a highly variable clincal course . the biological mechanisms underlying such varibility remain largely unclear and the issue of identifying in CLL parameters , which bear predictive implication , is becoming of greater relevance in view of the progressive change in the mangement of this disease. Mutation or deltion of the P53 gene is the most common genetic event in human malignancy , being mutated in approximately 50% of all human tumors and repeted as well in a number of haematological malignancies*

Aim of study: *This study was conducted aiming to determine the frequency of P53 protein expression in Iraqi CLL patients by immunohistochemistry (IHC) and to evaluate its correlation with various parameters which bear prognostic value in this disease.*

Subjects & Methods: *The study included a total of 82 CLL cases in whom bone marrow biopsies were performed at diagnosis (67cases) or follow up (15cases) at the Department of Haematology , Teaching Labortory in Baghdad Medical City Teaching Hospital in the period between Jan 1987 & Dec 2001 . The clinical records , blood films, bone marrow aspirates and biopsy slides were re-examined and section from paraffin blocks were used for P53 IHCstaining .*

Results: *P53 expression was detected in 13 of the total 83 CLL patients (15.8%) with frequancy of 13.4% (967) in the newly diagnosed patients . In the follow cases the frequncy was almost double that of the newly diagnosed group reaching a frequency of 26.6% (415).*

In the newly diagnosed cases P53 expression was not correlated with age , gender , haemoglobin level , platelet count , percentage of prolymphocyte in the peripheral Blood , Binet clinical stage or with the pattern of bone marrow involvemnt . However , it was significantly correlated with total leukocyte count (P=0.03)and absolute count (P=0.04). ***Conclution:*** *This finding may indicate that P53 positivity is associated with poor prognosis , since absolute lymphocyte count had been documented to be a poor prognstic factor in CLL .*

Kez words*: Chronic Lymphocytic Leukaemia, P53*

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Introduction

Chronic Lymphatic (CLL) is an acquired clonal lymphoproliferative disorder , characterized by the accumulation of morphologically differentiated lymphocytes in the marrow with peripheral lymphocytosis It is the most common adult leukemia in the western world (2) . In Iraq it account for 7.6% of all leukemia's with an incidence of 0.19/100000(3).

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The disease is characterized by highly variable clinical course , with some patients living several years untreated without a change in their clinical status and others showing a more rapid disease progression and a signficantly shorter survival(2). The biological mechanisms underlying such variability in clinical behavior remain largely unclear. Attempts to define prognostic criteria to facilitate the proper choice of the most suitable therapeutic approach succeeded in identifying several . Among the most significant of which , are the clinical stage , the histological pattern of marrow involvement , the lymphocyte doubling time and cytogenetic abnormalities (4,5) .

More recently abnormalities of P 53 gene have been shown to be present in about 15% of CLL patients and Dohner et al 1995 found that compared to all other parameters , P53 status is the most

powerful predictor of survival (6) .

The P53 tumor suppressor gene is located on chromosome 17 P13.1 , & physiologically is involved in the cell cycle arrest and induction of apotosis in genetically damaged cell. Mutation or deletion of P53 gene may facilitate the transmission of a genetic damage and the emergence of neoplastic clones with survival advantage(7,8) . This gene encodes a P53-KD phosphoprotien that is normally present in the nucleus of the cell (Wild type P53 protein) , this protein can not usually be detected by immunohistochemistry as it has very short half life of about 20-30 minutes . In contrast , mutated P53 has a prolongrd half and becomes detectable by immunohistochemistry using anti_P53 monoclonal antibodies (9) .

To the best of our knowledge no previous report on expression in haematological malignancies have been reported from Iraq, Therefore this study was conducted aiming to determine the frequncy of P53 expression as detected by immunohistochemistry in Iraqi CLL patients and to study its correlation with various clinical, haematological & histopathological parameters especially those with documented prognostic value .

Patients , Materials &Methods:

Atotal of 82 CLL had bone marrow biopsies performed at diagnosis or during follow up at the Department of Haematology in Medical City Teaching Hospital in the period between jan 1987 and Dec 2001 including 67 newly diagnosed and 15 follow up cases .

All clinical , laboratory records were reviewed . Peripheral blood & marrow aspirates were reexamined carefully .

Blood counts have been performed using the S-Plus electronic coulter Counter (Coulter Electronics,USA),MS9 electronic cell counter (France), or by standard manual techniques, Bone marrow biopsy sections stained with Haematoxyline & eosin were also re-examined.

Based on the above re-evaluation, the following data were documented for each of the patients:Age, sex, haemoglobin, total leukocytes count, absolute lymphocyte count. Percentage of prolymphocytes, platelets count and the bone marrow findings , specially the percentage of the lymphocytes in marrow aspirates and the pattern of infiltration in the biopsy whether diffuse, interstitial, nodular or mixed (10) Clinical staging was performed using the Binet et al, 1981 clinical staging system(ll) All included patients were then evaluated for P53 expression by immunohistochemistry using monoclonal P53 protein antibody Do-7 (Dako-Denemark) with Avidin -Biotin complex detection system. The Do-7 antibody reacts with both the wild and the mutant protein. The result of the staining was analyzed according to three independent variables: 1- the pattern of positivity whether diffuse, regional or focal. 2- The intensity, graded as weak, moderate & strong and 3- The extent or the percentage of the positive cells /HPF which was interpreted as high extent when there was more than 10% positive cells & low (5-10% positive cells). Cases were considered negative when there was complete absence of the staining or less than 5% positive cells (12,13)

Results

The median age for the newly diagnosed and the follow up CLL cases were 58 & 60 years with Male to Female ratio of 2.1:1 & 1.5:1 respectively.

Clinically the most predominant Binet prognostic stage in both groups was stage C, constituting 62.7% & 73.7% of the cases respectively. The most frequent histological pattern of marrow infiltration was the diffuse one in both the newly diagnosed and the follow up cases(73.1% & 80% respectively) No significant differences were found between the newly diagnosed and the follow up cases in relation to Hb level, total leukocyte count, absolute lymphocyte count, percent of prolymphocytes, platelets count and the percent of marrow lymphocytes (Table -1-).

P53 expression was detected in 13 of the total 82 CLL patients . The frequency of P53 positivity in the newly diagnosed patients was 13.4% (9/67), While in the follow up cases the frequency of positivity was almost double that in the newly diagnosed group reaching a level of 26.6% (4/15),however, the findings was not statistically significant (P=0.15). In general the most frequent pattern of P53 positivity was the regional predominantly with strong intensity and high extent.

In the newly diagnosed group no significant differences were demonstrated between P53 positive and negative groups in relation to age(p=0.573), Sex (P=0.425), Clinical stage (p=0.111), Hb level (p=0.098), peripheral prolymphocytes (p=0.628), marrow lymphocytes percent (p=0.941) and the pattern of marrow infiltration (p=0.090). On the other hand the median total leukocytes count & the absolute lymphocyte count were significantly higher in P53 positive group (p=0.03 & 0.041 respectively) Table 1 &2.

Table (1): Main haematological parameters in newly diagnosed and follow up CLL cases

		Hemoglobin (g/dl)	Total Leucocyte Count ($x10^9$/L)	Absolute Lymphcyte Count ($x10^9$/L)	% Pro-lymphocytes	% Bone marrow lymphcytes
Newly diagnosed	Mean ±SD	9.6128 ±31.784	134.507 ±140.333	120.433 ±128.997	5.6757 ±7.6632	83.1045 ±11.3326
	median	9.6000	83.700	76.167	2.0000	85.0000
Follow up	Mean ±SD	8.8333 ±2.6177	112.318 ±97.978	110.981 ±86.278	4.0000 ±2.5820	87.4000 ±10.2316
	median	8.0000	79.300	75.335	4.0000	90.0000
Asymp. Sig. (2-tailed) Mann-Whitney U test		0.384	0.598	0.924	0.909	0.110

Table (2): Clinical Stage and marrow infiltration pattern in P53 positive and negative newly diagnosed and follow up CLL cases

	Newly diagnosed CLL NO:67		Follow up CLL No: 15	
	P53 Positive No:9	P53 Negative No: 58	P53 Positive No:4	P53 Negative No:11
Clinical Satge				
A	4 (44.4%)	10 (17.2%)		3 (27.3%)
B	2 (22.2%)	9 (15.5%)		1 (9.1%)
C	3 (33.3%)	39 (67.2%)	4 (100%)	7 (63.6%)
Pattern of Marrow infiltration				
Diffuse	5 (55.5%)	44 (75.9%)	4 (100%)	8 (72.7%)
Interstitial	4 (44.4%)	7 (12.1%)		2 (18.2%)
Nodular		1 (1.7%)		
Mixed		6 (10.3%)		1 (9.1%)

Table (3): Main Haematological Parameters in P53 positive & negative newly diagnosed CLL cases

		Hemoglobin (g/dl)	Total Leucocyte Count ($x10^9$/L)	Absolute Lymphcyte Count ($x10^9$/L)	% Pro-lymphocytes	% Bone marrow lymphcytes
P53 positive	Mean ±SD	11.1333 ±1.5859	183.811 ±111.009	167.816 ±107.157	2.6667 ±1.5055	84.4444 ±7.6503
	median	12.7000	164.000	134.500	2.0000	90.0000
P53 negative	Mean ±SD	9.3769 ±3.3049	126.856 ±143.631	113.080 ±131.325	6.2581 ±8.2420	82.8966 ±11.8391
	median	9.0000	81.650	70.765	2.0000	85.0000
Asymp. Sig. (2-tailed) Mann-Whitney U test		0.098	0.032	0.041	0.628	0.941

Table (4): Prevalence pf P53 abnormalities in CLL in different studies utilizing different methods

Author (year)	Total No. of cases	% of P53 abnormalities	Methods of detection
Gaidano et al (1991)	40	15%	PCR-SSCP
El-Rouby et al (1994)	53	15%	PCR
Wattle E et al	81	11%	SSCP
Lepelley P et al (1994)	40	10%	IHP
Dohner H et al (1995)	100	17%	FISH & SSCP
Paydas et al (1995)	15	20%	IHC & SSCP
Lens D et al (1997)	49	30%	IHC & SSCP
Dordone L et al (1998)	181	15%	IHC & PCR
Current study	67	13.4%	IHC

PCR: *Polymerase chain reaction*

SSCP: *Single Strand Conformational Polymorphism*

IHC: *Immunohistochemistry*

FISH: *Fluorescent In Situ hybridization*

DICUSSION

Over the last decade, a variety of studies all over the world have focused on the role of P53 tumor suppressor gene in the pathogenesis of human malignancies and its relationship with various pathological and clinical parameters especially survival and drug resistance. P53 was found to be the most frequently altered gene in human cancer, being mutated in approximately 50% of all human cancers (14). This gene is known to be altered in a wide variety of hematological malignancies with the highest frequency being reported in prolymphocytic leukaemia (53%) and Burkitts lymphoma (41%) (15). Among the haematlogical malignancies evaluated in relevance to P53 was CLL and it has been suggested by several studies from western countries that p53 mutation contributes to the biological behaviour of CLL and is often associated with disease progression or transformation (6,16) However, no studies on P53 in CLL or other haematological malignancies have been reported from our country & it appears that such studies are now overdue. Thus the current study attempted to address the immunohistochemical expression of P53 in CLL Iraqi patients. The frequency of P53 expression in newly diagnosed Iraqi CLL patients studied was 13.4%. This frequency lies within the range of 10-30% demonstrated by various methods from Western countries (6,16-19) Table -4-.

An interesting observation was that the frequency of P53 positivity in the follow up cases was almost double that in the newly diagnosed group (26.6% Vs 13.4%) despite the fact that there was no significant differences in age , sex, clinical stage, basic laboratory data nor the histological pattern of marrow infiltration in the two groups. Although this interesting observation did not prove to be statistically significant, which is mostly due the small number of cases in the follow up group, yet this observation may indicate that CLL patients may acquire new P53 defect as their disease progress. The latter is favored by the fact that all the four P53 positive follow up cases (100%) were Binet stage C and showing diffuse infiltration pattern, while only 33.3% and 55.6% of newly diagnosed P53 positive patients were Binet stage C with diffuse infiltration pattern respectively (table-2-). Binet stage C and diffuse marrow infiltration in CLL are well known to be associated with poor prognosis (4). However, the prognostic significance of Binet clinical staging and the histological pattern of marrow infiltration pattern are not invariable and that there is definite heterogeneity due to differences in

disease biological behavior in different patients and therefore, cellular markers as alteration in P53 may help in identifying these CLL subcategories as different entities. The current study has revealed that P53 positivity was significantly associated with higher absolute lymphocyte count and consequently higher total leukocyte count. This association is consistent with findings of other workers (6,20). Since a high lymphocyte count in CLL has a documented poor prognostic value (4,21), P53 positivity therefore, may be implicated as a prognostic factor in this disease. Moreover, the above association further support the concept that CLL is an accumulative lymphoproliferative disease because it may favour the proposition that the malignant lymphocytes acquire P53 abnormalities and later on fail to undergo apoptosis leading to their accumulation. Therefore, P53 detection may show prognostic significance not only at the diagnosis of the disease but also during its course and treatment. Many studies on P53 expression in CLL have shown the poorer response to therapy in p53 positive patients compared to those who are negative (6,16). The explanation of this observation is related to the fact that the antineoplastic therapy acts predominantly through the induction of apoptosis in malignancies (22). This susceptibility to apoptosis is an important determinant of response to therapy and certainly central to this response are the proteins that modulate apoptosis including P53 (16,20). Therefore, wt P53 protein is important for inducing response of tumor to antineoplastic therapy. Therefore, short survival in P53 positive CLL is more likely to be due to treatment resistance rather than tumor aggressiveness only, hence, evaluation of P53 expression may give some indication about the susceptibility of the patient to chemotherapy as well as the disease progression . Accuracy of the clinical evaluation in CLL might then be improved with the inclusion of P53 detection as an additional prognostic factor.

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