Prognostic Assessment of P-Glycoprotein over Expression in Refractory and / or Relapsed Acute Myeloid Leukemia and Response to Cyclosporine A

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Abstract: Acute myeloid leukemia (AML) is a malignant hematopoietic neoplasm characterized by clonal proliferation of tumor cells that arise from the hematopoietic stem/progenitor population within the bone marrow. ***Objectives*** our study was enrolled to assess p-glycoprotein overexpression in refractory and /or relapsed acute myeloid leukemia and response to addition of cyclosporine A to chemotherapy. ***Patients and Methods*** this study was carried out at Hematology and Medical Oncology Unit, Internal Medicine Department, Zagazig University hospital during the period between July 2010 and July 2011. Forty patients with refractory or relapsed acute myeloid leukemia were classified into two groups, group (A**):** included 20 adult patients, their ages ranged from 18 to 60 years with median age 39 years, and they were treated with chemotherapy alone, group (B) included 20 adult patients with, their ages ranged from 20 to 61 years with median 40 years, they were treated with oral cyclosporine A in addition to the same chemotherapy protocol given in group A. All patients subjected to thorough medical history, physical examination, routine laboratory and radiological investigations and flowcytometry to assess p-glycoprotein overexpression. All patients had severe cardiac, pulmonary, hepatic, renal, neurological, metabolic disease, concomitant malignancies or uncontrolled infections were excluded from the study .***Results*** P-glycoprotein was overexpressed in 22 patients with refractory or relapsed AML (55%), when the unpaired (t) test was applied to test the significance of difference between the mean value ± S.D of percentage of bone marrow blasts and Pglycoprotein overexpression, there was not any significant difference detected (t=0.08 and *p*=0.91). Chi square test (χ^2) test was applied to test the significance of difference among different variables and P-glycoprotein overexpression. A statistically significant difference was found with cytogenetic study ($\mathbf{X^2}$ =8.5 and *P*=0.03) and response to treatment ($\mathbf{X^2}$ =8.02 and *P*=0.018). 13 patients were achieved CR (33%), 8 patients with PR (20%) and 19 patients with NR (47%) and when Chi square (χ^2) test was applied to test the significance of difference among variables associated with response to treatment, a high significant difference was found with cytogenetic study ($\mathbf{X^2}$ =33.93 and *P*= 0.001) The mean overall survival in group B was more than group A but wasn't significantly different (*P*= 0.25) also no significant difference between overall survival and P-glycoprotein overexpression (*P*= 0.15), but there was highly significant difference between overall survival and response to treatment (*P*= 0.0014), also Chi square (χ^2) test was applied to test the significance of difference among different toxicities which were occurred during therapy with patient groups, there is no significant difference was found ***conclusion*** P-glycoprotein was overexpressed in 55% of patients with refractory or relapsed acute myeloid leukemia and provide prognostic indicator for response to treatment and addition of oral cyclosporine as P-glycoprotein modulator doesn't improve response to chemotherapy or overall survival.

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Key words:- Acute myeloid leukemia (AML), P-glycoprotein (Pgp),Treatment response.

1. Introduction

Cancer is a major public health problem in many parts of the world [(1)] and advancements in early detection and cancer treatments have yielded significant progress [(2)]**.** Multidrug resistance (MDR) represents a major obstacle in successful therapy of neoplastic diseases so, despite of treatment with invasive chemotherapy, a considerable number of patients with acute myeloid leukemia die because of occurrence of resistance [(3)]**.** P-glycoprotein (Pgp) is a 170 kDa plasma protein, belongs to the ATP-binding Cassette (ABC) transporters, which are associated with several (in excess of 40) family members that share sequence and structural homology protecting cancer cells from apoptosis and they use the energy that is released when they hydrolyze ATP to derive the movement of various (exogenous and endogenous) molecules across the cell membrane [(4, 5)]**.** Since the discovery of P-glycoprotein (Pgp) there had been an enormous effort to generate clinically applicable inhibitors to restore sensitivity of cancer cells to chemotherapy [(6)] and many agents that modulate the

Pgp transporter were identified in the 1980s, including Cyclosporine A [7]. Cyclosporine A is a widely used immunosuppressant drug who's therapeutic and toxic actions are mediated through inhibition of calcineurin, a calcium and calmodulin-dependant phosphatase[8]. The clinical efficacy of Cyclosporine A as a modulator in AML might in part reflect a broad spectrum of activity against the MDR proteins expressed in AML cells[9]. In addition to broad spectrum modulation, CsA has been reported to have other effects that may be beneficial, including induction of apoptosis in at least some cell types as well as anti-angiogenic effects [10]. So our study had been enrolled to evaluate p-glycoprotein over expression in patients with refractory or relapsed acute myeloid leukemia and response to cyclosporine A in addition to chemotherapy.

2. Patients and methods:-

This study was carried out at Hematology and Medical Oncology Unit, Internal Medicine Department, Zagazig University Hospital during the period between July 2010 and July 2011. It was included 40 patients with refractory or relapsed acute myeloid leukemia and was classified into two groups. Group (A) included 20 adult patients, their ages ranged from 18 to 60 years with median age 39 years and they were treated with chemotherapy alone (Ara-C 1 gm/ m^2/12 hour, 3 hours Intravenous infusion from day 1 to day 3 and Novantron 10 mg/ m^2 intravenous infusion from day 3 to day 5) [11]. Group (B) included 20 adult patients their ages ranged from 20 to 61 years with median age 40 years, they were treated with oral Cyclosporine A (5 mg/kg/d orally for 5 successive days) in addition to the chemotherapy given in the same protocol as in group A . Well informed consent was obtained , also the protocol of therapy was reviewed and accepted by our institutional board. All patients had severe cardiac, pulmonary, hepatic, renal, neurological, metabolic disease, concomitant malignancies or uncontrolled infections were excluded from the study and all patients were subjected to complete clinical history and physical examination, routine laboratory investigations includes (complete blood picture, liver & kidney functions, PT, PTT/ INR and ESR). Virology studies including (HBs Ag, HCV Ab, HIV Ab), bone marrow aspiration and biopsy with immunophenotyping and Cytogenetic study, routine radiology (chest X-ray and CT chest if indicated, pelvi-abdominal ultrasonography and CT abdomen & pelvis if indicated and echocardiography). while detection of P-glycoprotein expression level was done by anti-P-glycoprotein monoclonal antibody which was used to detect the mean fluorescence intensity (MFI) of Pgp on blast cells using Becton Dickinson FAC scan flowcytometery and the intensity of staining of mean fluorescence index (MFI) was used in the detection of the Pgp expression level which represents the ratio between the mean fluorescence intensity of cells stained with the specific antibody and that of cells stained with the isotype matched control antibody[12] and the response to treatment was evaluated according to revised recommendations of the international working group for standardization of response criteria, the complete remission (CR), when the cellularity of the bone marrow (BM) after regeneration was near normal with <5% blast cells, the peripheral blood recovered completely, and no extra-medullary leukemic infiltrates were present. When the BM blast cell count remained between 5 and 25% but was reduced by at least 50% in comparison to the initial value, and the peripheral blood levels recovered completely, a patient was be considered to be in partial remission (PR) and failure to attain CR or PR will be consistent with failure or non response (NR) [13] as well as toxicity of treatment was evaluated according to WHO common toxicity Criteria.

Statistical Analysis

Data were collected, entered and checked to a SPSS version 15. Data were expressed as mean ± standard deviation in quantitative variables, number and percentage for qualitative variables, Chi square and correlation coefficient were used for analysis of data and for all above mentioned statistical test, the threshold of significance is fixed at 5% level (P-value).Kaplan-Meier used mainly in survival studies of patients and confidence intervals were calculated using Greenwood's estimate of the standard error and differences in overall survival were tested for significance using the log-rank statistic [14].

3. Results

Patient characteristics of study are showed in table (1) and P-glycoprotein was overexpressed in 22 patients with refractory or relapsed AML (55%) in figures: 1, 2, and 3).

Chi square test (χ^2) test was applied to test the significance of difference among different variables and Pgp overexpression. A statically significant difference was found with cytogenetic study ($\mathbf{X^2}$ =8.5 and *P*=0.03) and response to treatment ($\mathbf{X^2}$ =8.02 and *P*=0.018) Table (3) Figure (4).

The patient response to treatment was as follow: 13 patients achieved CR (33%), 8 patients with PR (20%) and 19 patients with NR (47%) and when Chi square (χ^2) test was applied to test the significance of difference among variables associated with response to treatment, a high significant

difference was found with cytogenetic study (X^2 =33.93 and *P*= 0.001) Table (4) Figure (5).

Kaplan-Meier method was used to estimate one year over all survival of patients of the study. The mean overall survival in group B was more than group A but there wasn't significant difference (*P*= 0.25) also no significant difference between overall survival and Pgp over expression (*P*= 0.15), but there was highly significant difference between overall survival and response to treatment (*P*= 0.0014) Tables (5, 6, 7) Figures (6, 7, 8).

Chi square (χ^2) test was applied to test the significance of difference among different toxicities which were occurred during therapy and patient groups, there was no significant difference was found (Table 8).

Table (1)

Character	No.	%
Age(in years):		
Range(18-61)		
Median (41)	40	100
Sex:		
Male	18	45
Female	22	55
Clinical presentation:		
CNS infiltration	3	7.5
Pallor	8	20
Fever	10	25
Gum hypertrophy	6	15
Purpura	6	15
Lymphadenopathy	1	2.5
Spleenomegaly	1	2.5
Hepatomegaly	3	7.5
Chloroma	2	5
Virology:		
Hcv Ab +ve	14	35
HBsAg +ve	3	7.5
Hcv/Hbv -ve	23	57
Bone Marrow (B.M) Blasts (%):		
Range (48-95)		
$\bar{X}$± SD (74.87±13.85)	40	100
Immunophenotyping:		
+ve myeloid markers	40	100
FAB classification:		
M0	3	7.5
M1	4	10
M2	6	15
M4	10	25
M5	17	45
Cytogenetic study:		
Unfavorable	13	33
Intermediate	11	27
Favorable	8	20
Unknown	8	20

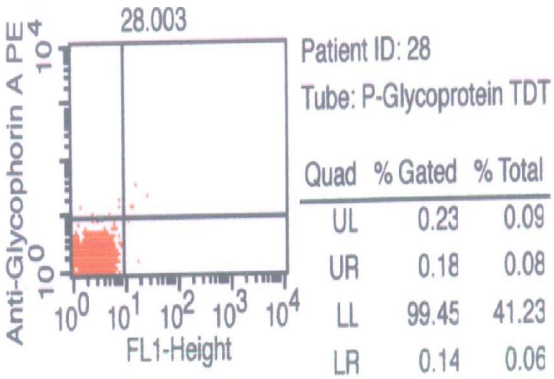

Figure (1): Histogram of patient showing –ve Pgp expression.

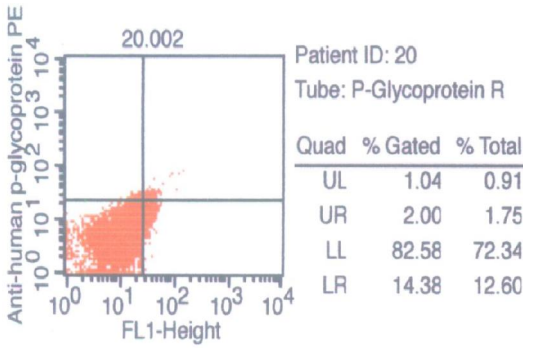

Figure (2): Histogram of patient showing +ve Pgp expression.

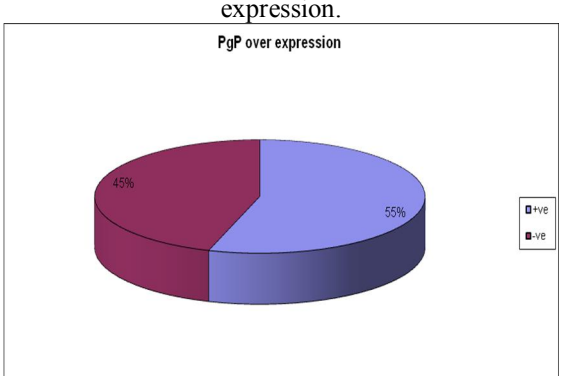

Figure (3): Distribution of cases according to Pgp over expression.

Table (2): Comparison between PgP overexpression and percentage of Bone Marrow blasts:

	Pgp -ve	Pgp +ve	T	*P*
B.M blasts $\bar{X}$± SD	71.77±11.79	74.95±15.61	0.08	0.91

Table (3): Comparison between PgP overexpression with FAB classification, cytogenetic study and response to treatment:

	Pgp –ve		Pgp +ve		X^2	*P*
	No.	%	% No.			
FAB:						
M0	1	33.3	2	66.7		
M1	2	50	2	50		
M2	4	66.6	2	33.3	3.92	0.41
M4	6	60	4	40		
M5	5	29.4	12	70.5		
Cytogenetic:						
Unfavorable	2	15.3	11	84.7		
Intermediate	5	45.4	6	54.6	8.5	0.03*
Favorable	6	75	2	25		
Unknown	5	62.5	3	37.5		
Response:						
CR	10	76.9	3	23.1		
PR	2	25	6	75	8.02	0.018*
NR	6	31.5	13	68.4		

Significant*

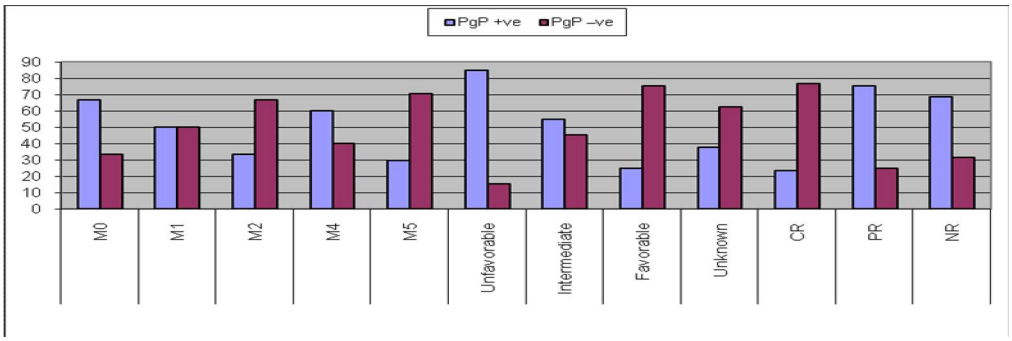

Figure (4): Comparison between Pgp overexpression with FAB classification, cytogenetic study and response to treatment

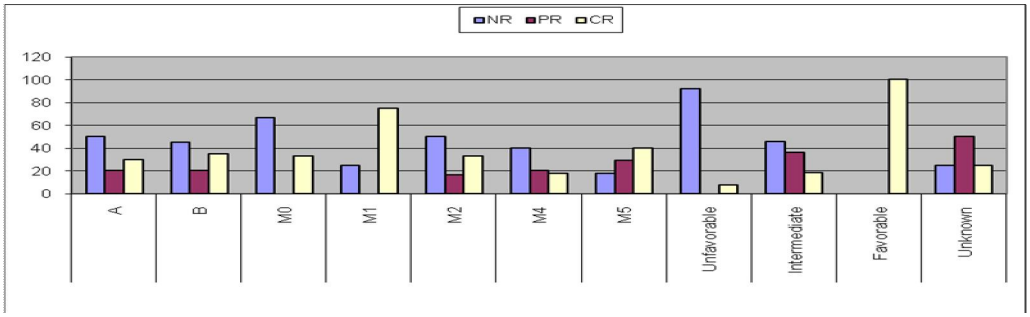

Figure (5): Comparison between treatment response with studied groups, FAB classification and cytogenetic study.

Table (4): Comparison between response to treatment with studied group, FAB classification and cytogenetic study:

	CR		PR		NR		X2	*P*
	NO.	%	NO.	%	NO.	%		
Patient group:								
A	6	30	4	20	10	50	0.13	0.93
B	7	35	4	20	9	45		
FAB:								
M0	1	33.3	0	0	2	66.6		
M1	3	75	0	0	1	25	6.62	0.57
M2	2	33.3	1	16.7	3	50		
M4	4	40	2	20	4	40		
M5	3	17.6	5	29.4	9	52.9		
Cytogenetic:								
Unfavorable	1	7.7	0	0	12	92.3		
Intermediate	2	18.2	4	36.4	5	45.5	33.93	0.001**
Favorable	8	100	0	0	0	0		
Unknown	2	25	4	50	2	25		

Table (5): Comparison between overall survival and patient groups:

	Group A		Group B		Log rank	
	No.	%	No.	%		
Censored	6	30	10	50		
Event	14	70	10	50		
Mean survival ±SD	4.90 ± 0.46		6.83 ± 0.88		1.3	0.25
95% confidence interval	4.00 – 5.81		5.10 – 8.57			

Table (6): Comparison between overall survival and Pgp over expression:

	Pgp –ve		Pgp +ve		Log rank	
	No.	%	No.	%		
Censored	9	50	7	31.82		
Event	9	50	15	68.18		
Mean survival ± SD	7.31 ± 0.87		5.08 ± 0.59		2.04	0.15
95% confidence interval	5.60 – 9.01		3.92- 6.23			

Table (7):Comparison between overall survival and response to treatment:

	CR		PR		NR		Log rank	
	No.	%	No.	%	No.	%		
Censored	5	83.33	1	25	0	0		
Event	1	16.76	3	75	10	100		
Mean survival ± SD	7.33 ± 0.54		5.25 ± 1.09		3.5 ± 0.40		13.3	0.0014**
95 % confidence interval	6.27 – 8.40		3.11 – 7.39		2.7 – 4.29			

** Highly significant

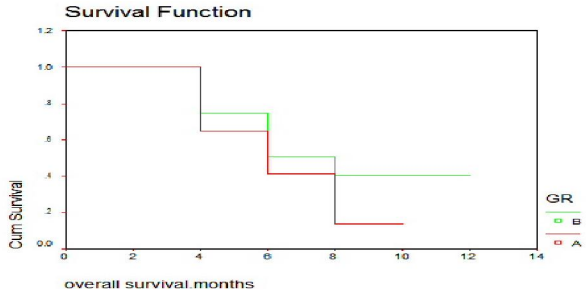

Figure (6): comparison between overall survival and patient groups

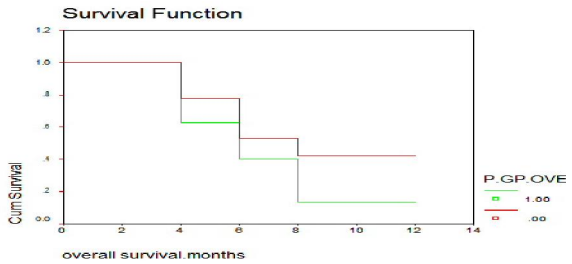

Figure (7): Comparison between overall survival and PgP over expression.

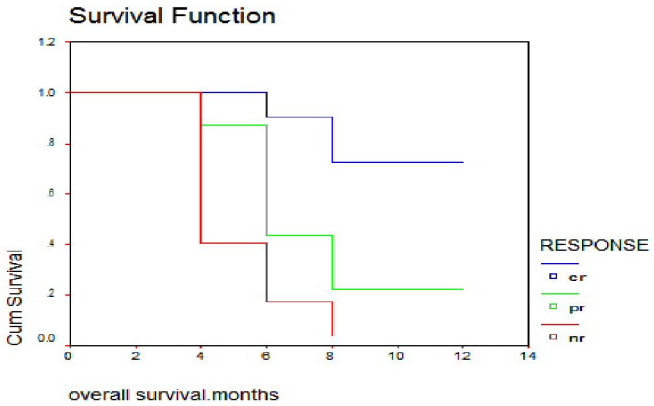

Figure (8):comparison between overall survival and response to treatment.

Table (8): Comparison between patient groups as regard common toxicity criteria :

Toxicity & grading	Group A No.	%	Group B No.	%	X_2	*P*
***Hematological:*Neutropenia**						
II	1	5	0	0		
III	4	20	4	20	1.32	0.72
IV	15	75	16	80		
HB %						
I	1	5	0	0		
II	3	15	4	20	1.03	0.59
III	12	60	13	65		
IV	4	20	3	15		
Thrombocytopenia						
III	2	10	3	15		
IV	18	90	17	85	0.22	0.63
Gastrointestinal:						
Nausea/vomiting						
I	3	15	2	10		
II	8	40	5	25	4.69	0.19
III	7	35	13	65		
IV	2	10	0	0		
Diarrhea						
0	9	45	9	45		
I	3	15	4	20	1.14	0.76
II	1	5	0	0		
III	7	35	7	35		
Constipation						
0	10	50	8	40		
I	1	5	1	5		
II	4	20	2	10	5.89	0.21
III	3	15	9	45		
IV	2	10	0	0		
Liver:						
AST/ ALT						
0	15	75	13	65		
I	4	20	4	20	1.14	0.56
II	1	5	3	15		
Bilirubin						
0	18	90	14	70		
I	1	5	3	15	2.5	0.28
II	1	5	3	15		
Clinical status						
No coma	20	100	20	100	0.0	1
Cardiac:						
Rhythm						
0	17	85	20	100	3.2	0.07
I	3	15	0	0		
Ejection fraction						
0	20	100	19	95		
I	0	0	1	5	fisher	1.0
Renal:						
Creatinine						
0	18	90	17	85		
I	2	10	3	15	fisher	1.0
Infection:						
I	4	20	4	20		
II	4	20	8	40	2.1	0.34
III	12	60	8	40		
Stomatitis:						
0	3	15	6	30		
I	4	20	7	35	4.15	0.24
II	7	35	5	25		
III	6	30	2	10		
Alopecia:						
I	1	5	1	5		
II	4	20	6	30	0.54	0.76
III	15	75	13	65		

4. Discussion

Acute myeloid leukemia (AML) is a malignant hematopoietic neoplasm characterized by clonal proliferation of tumor cells that arise from the hematopoietic stem/progenitor population within the bone marrow [15].

AML is the most common acute leukemia in adults and accounts for approximately 69 percent of cases in this group, AML accounts for less than 1% of all cancers and 29% of all leukemia, approximately 12,950 new cases of AML are diagnosed annually in the United States[1].

In NCI, Cairo University, AML accounts for approximately 41.5% of newly diagnosed cases with acute leukemia registered in the time period between January 2002 and December 2003[16]. The incidence of AML increases with age, and is most frequently observed in older adults, the median age at diagnosis was 67 years of age [17] but in our study, the patient median age was 41 years and the range was 18-61 years and in both patient groups the median age was nearly equal. The incidence of AML is higher in males than in females with male to female ratio of 1.1:1.0[1] but in our study, males were 45% of patients and females were 55% with female to male ratio of 1.2:1.1.

This difference of demographic data might be attributed to difference in selection criteria as in our study the patients were refractory or relapsed AML cases not de novo cases.

The clinical signs and symptoms of AML are diverse and nonspecific, but they are usually directly attributable to the leukemic infiltration of the bone marrow, with resultant cytopenia[18].

Fever was the most common clinical manifestation of patients in our study followed by pallor, purpuric eruption and gum hypertrophy and this is consistent with data published by Weinblatt [19] who noted that fever and manifestations of bone marrow failure represent the most common initial clinical presentation followed by manifestations of extra-medullary involvement.

The FAB morphologic classification names the AML according to the normal marrow elements that they most closely resemble [20].

M5 was the most common in our study (45%) followed by M4 (25%), in Bassan *et al.* [21], M1 was the commonest (27%) followed by M2 (22%), in List *et al.* [22], M2 was the commonest (27%) followed by M4 (23%) and this difference might be attributed to difference in selection criteria.

Karyotype analysis is a key component of the initial evaluation of a patient with AML[23]. In our study, Cytogenetic analysis of patients revealed that 33% of them were with unfavorable cytogenetics , 27% were with intermediate cytogenetics, 20% were with favorable cytogenetics and 20% were unknown and this reflect the aggressiveness of the disease from the start and explain the poor response of these patients to initial chemotherapy, furthermore, patients with normal karyotyping must be classified into favorable or unfavorable cytogenetic according to e.g. NPM1/FLT3mutations in order to give more accurate data.

In List *et al.* [22], Cytogenetic analysis of patients reveal that 34% of them were with unfavorable cytogenetics, 29% were with intermediate cytogenetics, 8% were with favorable cytogenetics and 29 % were unknown and this was approximately consistent with our data.

Although the clinical outcome of acute leukemia has been improved by recent progress in chemotherapy, it stills a difficult disease to treat. One major problem is the emergence of leukemic blast cells that are resistant to anticancer drugs and it is obvious that this resistance of leukemic blast cells to chemotherapeutic agents eventually will lead to treatment failure [24].

The overproduced P-glycoprotein that extrudes anti cancer drugs from cells is the most common mechanism of multi-drug resistance [25].

In our study Pgp was overexpressed in 55% of patients and normally expressed in 45% reflecting overexpression of MDR1 gene. In List *et al.* [22], Pgp was over expressed in 30% of patients and normally expressed in 57% and there was 13% with unknown expression level.

In Leith *et al.*[26], Pgp was overexpressed in 35% of patients and normally expressed in 65%, In Damiani *et al.* [27], Pgp was over expressed in 33% of patients and normally expressed in 67%.

P-glycoprotein expression level, in the present work didn't show any significant difference with FAB subtypes and this is consistent with data reported by Senent *et al.* [28]. In contrast with these results, many authors found that the frequency of Pgp expression is significantly correlated with certain AML subtypes. Motoji *et al.* [29] found that Pgp expression level was low in M3 subtypes and the difference in these results may be attributed to absence of AML (M3) patients in our study. There was significant relationship between P-glycoprotein over expression with poor cytogenetic of patients of our study and this is consistent with data reported by Wüchter *et al.* [30].

The development of agents able to modulate MDR mediated by Pgp and other ABC transporters remained a major goal for the past 20 years including Cyclosporine A (CSA) which was the first immune suppressor that have been shown to modulate Pgp activity and entered very early into clinical trials for reversal of MDR[31].

The main purpose of our study is to evaluate oral cyclosporine A as a Pgp modulator, so it was given in addition to chemotherapy then response was evaluated in the CsA and non CsA arms and was as

follow: 33% of patients achieved CR, 20% PR, 47% NR and there was no significant difference between patient groups (addition of cyclosporine A doesn't improve response to chemotherapy).

In List *et al.* [22], significant greater proportion of patients treated with cyclosporine A achieve CR after one course of induction treatment compared to the non CsA arm, also percentage of refractory disease in the non CsA arm was 47% compared with 31% in the CsA arm.

In Bassan *et al.* [21], infusional cyclosporine A was used with HiDAC and Idarubicin in treatment of refractory or relapsed AML patients and results was as follow: 61% of patients achieve CR, 16% achieve PR and 23% were non responders. In List *et al.* [22], infusional cyclosporine A was used with HiDAC and Daunorubicin in treatment of Patients with poor-risk acute myeloid leukemia (AML) and results was as follow: 62% of patients achieve CR,7% achieve PR and 31% were non responders. In all of these previous studies, cyclosporine A which was used as p-glycoprotein modifier was given by the intravenous route but in our current study it was used by the oral route which is available and easy administered, and this may explain the absence of significant response to cyclosporine. FAB subtypes of patients didn't affect the response to treatment and in contrast with these results Daenen *et al.* [32] who found that M0,M6 and undefined FAB subtypes was associated with poor response, also Meletis *et al.* [33] found that M0 and M1 was associated with poor response to treatment.

P-Glycoprotein is associated with poor outcome in acute myeloid leukemia [34] and its expression on leukemic blast cells at initial presentation affects the responsiveness to induction chemotherapy.it has become apparent from many studies that the remission rate is significantly lower in Pgp +ve patients than in Pgp -ve patients[35].

In our study there was significant relationship between PgP expression and response to treatment (Pgp over expression is associated with poor response) and this matched with Wüchter *et al.* [30]who found that CR after induction chemotherapy was correlated with significant lower PgP function. In contrast to these results List *et al.* [22] found that there was no significant relationship between PgP expression and response to treatment. In our study there was highly significant relationship between cytogenetics of patients and response to treatment (unfavorable cytogenetics were associated with poor response) and these results are matched with those of List *et al.* [22] and Meletis *et al.* [33]. Cytogenetics remains the most important disease related prognostic factor[36]. In our study there was highly significant relationship between cytogenetics of patients and response to treatment; unfavorable cytogenetics are associated with poor response and these results are matched with those of List *et al.* [22] and Meletis *et al.* [33].

Overall survival for all patients of a trial is measured from the date of entry into a study to the date of death from any cause and patients not known to have died at last follow-up are censored on the date they were known to be a live[13]. In our study the mean one year over all survival in group B was more than group A but wasn't significantly different and this was consistent with Daenen *et al.* [32] who found no significant difference between the two patient arms (CsA and non CsA) as regard overall survival, In contrast to these results List *et al.* [22] found that overall survival was significantly improved in the cyclosporine arm. PgP over expression didn't significantly affect overall survival in our study, and this is matched with results of List *et al.* [22]. In contrast to these results Meletis *et al.* [33], found that PgP over expression was associated with short duration of overall survival. There was highly significant relationship between overall survival and response to treatment in our study (patients with good response are associated with longer overall survival) and this was matched with List *et al.* [22] and as regard toxicity criteria, all toxicities were acceptable and there was no significant difference between patient groups as regard toxicity, this is may be due to short time of administration of cyclosporine A to cause a significant grades of toxicities and difference between studies might attributed to difference in selection criteria of patients.

Conclusion

P-glycoprotein was overexpressed in 55% of patients with refractory or relapsed acute myeloid leukemia and provides prognostic indicator for response to treatment and addition of oral Cyclosporine as P-glycoprotein modulator doesn't improve response to chemotherapy or overall survival.

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References

1- Jemal A, Siegel R, Brawly O and Ward E. Cancer statistics. CA Cancer J Clin. 2011; 61:212–236.
2- Gottesman MM. Mechanisms of cancer drug resistance. Annu Rev Med. 2002;53:615–627.
3- Arbelbide J, Garcia Rivello H ,Tacchi MC, Nucifora EM, Morandi A, Cardenas MP and

Fantl D. prognostic value of the expression of MDR-1 in Acute myeloid leukemia. Medicine. 2003; 63(4):277.
4- Agrawal M, Abraham J, Balis FM, Edgerly M, Stein WD, Bates S, Fojo T and Chen CC.: Increased 99mTc-sestamibiaccumulation in normal liver and drug resistant tumors after the administration of the glycoprotein inhibitor, XR9576. Clin Cancer Res. 2003; 9:650–656.
5- Baguley BC. Multidrug Resistance in Cancer. 2010; 596:1-31.
6- Coley HM. Overcoming Multidrug Resistance in Cancer: Clinical Studies of P-Glycoprotein Inhibitors. Methods MolBiol. 2010; 596: 341-58.
7- Krishna R and Mayer LD. Multidrug resistance (MDR) in cancer. Mechanisms, reversal using modulators of MDR and the role of MDR modulators in influencing the pharmacokinetics of anticancer drugs. Eur J Pharm Sci. 2000; 11:265–283.
8- Fakata KL, Elmquist WF, Swanson SA, Vorce RL, Prince C and Stemmer PM. (1998) Cyclosporine A has low potency as a calcineurin inhibitor in cells expressing high levels of P-glycoprotein. Life Sci. 1998; 62(26): 2441-8.
.9- Germann UA, Ford PJ, Shlyakhter D, Mason VS and Harding MW. Chemosensitization and drug accumulation effects of VX-710, verapamil, cyclosporin A, MS-209 and GF120918 in multidrug resistant HL60/ADR cells expressing the multidrug resistance-associated protein MRP. Anticancer Drugs.1997; 8:141–55.
10-Nakahara C, Nakamura K and Yamanaka N. Cyclosporin-A enhances docetaxel-induced apoptosis through inhibition of nuclear factor-κB activation in humangastric carcinoma cells. Clin Cancer Res. 2003;9:5409–16
11-Hiddemann W, Kreutzmann H, Ludwig WD, AulHc, Donhuijsen-Ant R, lengfelder E and Buchner Th. Mitoxantrone and high-dose cytosine arabinoside in refractory acute myeloid leukaemia. Lancet. 1985; 508.
12-Shi T, Wrin J, Reeder J, Liu D and Ring DB. High affinity monoclonal antibodies against p-glycoprotein. ClinImmunol Immunopathol. 1995; 76:44.
13-Cheson B, Bennett J, Kopecky K, Lister TA, Tallman MS, Estey EH, Larson RA, Biondi A and Head DR.) Revised recommendations of the international working group for diagnosis, standardization of response criteria, treatment outcomes, and reporting standards for therapeutic trials in AML. J ClinOncol. 2003; 21:4642-4649.
14-Levesque R. (2007) SPSS Programming and Data Management: A Guide for SPSS and SASUsers, Fourth Edition , SPSS Inc., Chicago Ill.
15-Ichikawa M and Kurokawa M. Molecular mechanisms in the development of acute myeloid leukemia. Nihon Rinsho. 2009; 67(10): 1889-93.
16-National Cancer Institute (NCI) Registry. National Cancer Institute statistics 2004. Department of Biostatistics and cancer Epidemiology, NCI Cairo. 2004; 63-66.
17-Ries L, Melbert D, Krapcho M and Miller BA. (2008) SEER Cancer Statistics Review, http://seer.cancer.gov/csr/1975_2005/, based on November 2007 SEER data submission, posted to the SEER web site.
18-Löwenberg B, Pabst T, Vellenga E, van Putten W, Graux C, Schouten HC, Schanz U, Biemond BJ and Gregor M. Cytarabine dose for acute myeloid leukemia, Dutch-Belgian Cooperative Trial Group for Hemato-Oncology (HOVON) and Swiss Group for Clinical Cancer Research (SAKK) Collaborative Group. N Engl J Med. 2011;364(11):1027-36.
19-Weinblatt ME. (2004) Acute myelocytic leukaemia. http://www.emedicine.com.
20-Schiffer C and Stone R. Acute Myeloid Leukemia in Adults, in:Robert c., Bast Jr., Denold W., Kufe., eds, Cancer Medicine e.5 Section 36.,B.C Decker inc. 2001; 124.
21-Bassan R, Lerede T, Borleri G, Chiodini B, Rossi A and Buelli M. Phase I trial with escalating doses of idarubicin and multidrug resistance reversal by short-course cyclosporin A, sequential high-dose cytosine arabinoside, and granulocyte colony-stimulating factor for adult patients with refractory acute leukemia, Haematologica. 2002; 87:257-263.
22-List AF, Kopecky KJ, Shurafa M,Doroshow JH, Karanes C and Willman CL. Benefit of cyclosporine modulation of drug resistance in patients with poor-risk acute myeloid leukemia: a Southwest Oncology Group study. Blood. 2001; 98:3212–20.
23-Schlenk RF, Benner A, Hartmann F, del Valle F, Weber C, Pralle H, Fischer JT, Gunzer U and Pezzutto A. Risk adapted post-remission therapy in acute myeloid leukemia: results of the German Multicenter AML HD93 treatment trial. *Leukem. 2003;*17(8): 1521-1528.
24-Schaich M, Soucek S, Thiede C, Ehninger G and lllmer. MDR1 and MRP1 gene expression are independent predictors for treatment outcome in adult acute myeloid leukemia. Br J Haematol. 2005; 128(3):324.
25-GauriPatwardhan, Vineet Gupta, Juowen Huang, XinGu, and Yong-Yu Liu. Direct assessment of

P-glycoprotein efflux to determine tumor response to chemotherapy. Biochem Pharmacol. 2010; 80(1): 72–79.
26-Leith CP, Kopecky KJ, Eijdems L, Weick J, Grever MR and Willman CL. Frequency and Clinical Significance of the Expression of the Multidrug Resistance Proteins MDR1/P-Glycoprotein, MRP1 and LRP in Acute Myeloid Leukemia. A Southwest Oncology Group Study, Blood. 1999; 49:1086-1099.
27-Damiani D, Tiribelli M, Calistri E,Geromin A, Chiarvesio A and Fanin R. The prognostic value of P-glycoprotein (ABCB) and breast cancer resistance protein (ABCG2) in adults with de novo acute myeloid leukemia with normal karyotype, Haematologica. 2006; 91:825-828
28-Senent L, Jarque I, Martin G, Sempere A, González-García Y, Gomis F and Sanz MA. P-glycoprotein expression and prognostic value in acute myeloid leukemia, Haematologica. 1998; 83:783.
29-Motoji T, Motomura S and Wang YH. Multidrug resistance and a strategy to overcome it .Int J Haematol. 2000; 72:418.
30-Wüchter C, Karawajew L, Ruppert V,Schrappe M,Harbott J and Schoch C. clinical significance of p-glycoprotein expression and function for response to induction chemotherapy, relapse rate and overall survival in acute leukemia. Haematologica. 2000; 85:711.
31-Morjani Hand Madoulet C. Immunosuppressors as multidrug resistance reversalagents. Methods MolBiol. 2010; 596:433-46.
32-Daenen S, van der holt B, Verhoef GEG, Löwenberg B, Huijgens PC and Verdonck LF. addition of CsA to the combination of mitoxantrone and etoposide to overcome resistance to chemotherapy in refractory or relapsed acute myeloid leukemia: a randomized phase II trial from HOVON,the Dutch-Belgian Hemato-Oncology working group for adults, leukemia research. 2004; 28:1057-1067.
33-Meletis J, Tsimberidou AM, Paterakis G, Androutsos G and Symeonidis A. Evaluayion of the clinical relevance of the expression and function of p-glycoprotein,multi drug resistance protein and lung resistance protein in patients with primary acute myeloid leukemia, leukemia research. 2002; 26:143-154.
34-Benderra Z, Faussat AM, Sayada L, Perrot JY, Tang R, Chaoui D, Morjani H, Marzac C, Marie JP and Legrand O. MRP3, BCRP, and P-glycoprotein activities are prognostic factors in adult acute myeloid leukemia. Clin Cancer Res. 2005; 1;11(21):7764-72.
35-Norgaard J and Hokland P. Biology of multiple drug resistance in acute leukemia .Int J Haematol. 2005;72:290.
36-Dühner H, Estey EH, Amadori S, Appelbaum FR, Büchner T, Burnett AK, Dombret H, Fenaux P, Grimwade D and Larson RA. Diagnosis and management of acute myeloid leukemia in adult. Blood. 2010; 115:453-474.

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