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MDR Gene and LRP As Prognostic Indicators in Adult AML Patients

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ABSTRACT

Background: Multidrug resistance (MDR) genes, multidrug resistance 1 (MDR1) protein and lung associated resistance protein (LRP), are correlated with the outcome of treatment of acute myeloid leukemia (AML).

Objective: Our aim in this study was to evaluate the frequencies of occurrence of MDR1, and LRP in Egyptian adult AML patients, and to correlate between their expression and disease prognosis.

Patients and Methods: In this study, the expression of MDR1 protein (P-gp) & LRP were measured using flowcytometry on bone marrow samples of 46 de-novo adult AML patients. Expressions were correlated to clinical & laboratory variables, response to treatment and overall survival.

Results: MDR1 protein was found positive or over expressed in 14 patients (30.4%), 4 (28.6%) of them achieved CR where as 10 (71.4%) were refractory (p=0.034). LRP was found positive in 12 patients (26.1%), 6 of them (50%) achieved CR and 6 (50%) were refractory (p=0.861). MDR1 showed significant correlation with hemoglobin level (p=0.034), and response to therapy (p=0.034).

None of the 2 parameters had any correlation with age, gender, WBC count, organomegally, BM blasts, FAB classification, BM cellularity or overall survival.

Conclusion: Only positive expression of MDR1 represents a significant prognostic indicator in adult AML cases.

Key Words: *Adult acute myeloid leukemia – MDR – LRP.*

INTRODUCTION

Drug resistance is a major obstacle in the successful treatment and an important cause of death in acute leukemia. Such resistance may be present before beginning treatment or may develop during chemotherapy. Drug resistance that extends to structurally and functionally unrelated drugs is termed multidrug resistance (MDR) **[1,2]**.

Several molecular biological mechanisms have been identified as being associated with MDR **[3]**. P-glycoprotein (P-gp) is a product of the multidrug resistance1 gene (MDR1) and is an ATP-dependent pump capable of expelling drugs out of cancer cells **[4]**. P-gp is a transmembrane glycoprotein conferring cross-resistance to a variety of mechanistically and structurally unrelated cytotoxic drugs, such as anthracyclines, taxanes, vinca alkaloids and epipodophyllotoxins **[4]**. In addition, a 110 kDa protein has been identified in a P-gp negative MDR lung cancer cell line. This protein was termed the lung resistance protein (LRP). LRP is the human major vault protein frequently overexpressed in drug resistant cancer cells and its expression has been correlated with poor prognosis. Vault proteins are present in all eukaryotic cells, and they are highly conserved. Several clinical data have indicated that LRP expression can be of high clinical value to predict the response to chemotherapy in some tumor types such as non-small cell lung carcinoma, osteosarcoma, melanoma and neuroblastoma **[5]**.

Despite the identification of these proteins, the pathways that result in drug resistance in leukemic cells remain largely uncharacterized. While drug resistance gene expression has been studied in acute leukemia, the value of MDR1 and LRP gene expression as independent predictors of treatment success is still controversial **[4,6]**.

Our aim in this study is to evaluate the frequencies of occurrence of MDR proteins P-gp, and LRP in Egyptian adult AML patients, and to correlate them with disease prognosis and clinical and laboratory variables.

PATIENTS AND METHODS

Patients:

The present study was carried out on 46 adult patients with de novo AML, who presented to the National Cancer Institute, Cairo University, in the period between October 2009 and December 2010. After an informed consent all studied patients were subjected to thorough history taking and full clinical examination. In addition radiology examination in the form of chest X-ray, abdominal ultrasound and CT scan whenever needed were done.

Complete blood picture, bone marrow aspiration and morphological examination, liver and kidney function tests were also done.

Immunophenotyping was done by flow cytometry (Partec III from DAKO cytomation), on marrow blast cells with a panel of monoclonal antibodies, purchased from DAKO (Denmark), including FITC and PE conjugated CD13, CD33 and MPO. Specific isotype controls for FITC, PE conjugated monoclonal antibodies were used. Results were expressed as percentage of cells showing positive expression.

Diagnosis of AML was based on the presence of ≥20% blast cells in BM film according to WHO proposal **[7]**, together with MPO staining and immunophenotyping.

Of the 46 newly diagnosed AML patients enrolled in this study, forty-one patients received the standard AML induction chemotherapy protocols applied at the NCI, Cairo University which are differentiated according to age and the subtype of AML. Four patients with acute promyelocytic leukemia received adriamycin and vesanoid. Thirty-three patients younger than 55 years received ARA-C plus adriamycin (3&7) protocol. For the 4 patients aged above 55 years, three of them received ARA-C plus adriamycin (2&5) protocol while one patient received oral vepside capsule. Response to induction therapy was assessed between days 21 & 28 after induction therapy. Patients achieving complete remission (CR) received consolidation therapy. Patients who did not achieve CR are considered refractory cases.

Methods:

Detection of MDR1 and LRP expression by flow cytometry (Partec III from DAKO cytomation) was done on lysed whole blood using anti-human MDR1 and LRP (FITC) monoclonal antibodies, purchased from DAKO (Denemark). Irrelevant monoclonal antibodies of the same isotypes and protein concentration were used as negative controls.

For interpretation of the results, the mean fluorescence index ratio (MFIR) was used, 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, the case was considered over expressing or positive for P-gp at a ratio of ≥1.1 **[8]**, and was considered positive for LRP when the ratio exceeds ≥0.3.

Statistical methods:

Data was analyzed using SPSSwin statistical package version 17 (SPSS Inc., Chicago, IL). Numerical data were expressed as mean and standard deviation or median and range as appropriate. 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). Survival analysis was done using Kaplan-Meier method and comparison between two survival curves was done using log-rank test. A p-value<0.05 was considered significant.

RESULTS

Forty six consecutive patients with de novo acute myeloid leukemia, who presented to the National Cancer Institute, Cairo University, in the period between October 2009 and December 2010, were included in this study. They were 33 males and 13 females, their ages ranged from 18 to 65 with a median of 37.5 years.

The clinico-hematological findings, treatment status and number of deaths of the 46 adult AML patients included in this study are presented in (Table 1).

The expressions of LRP & MDR were studied in the 46 patients. In these patients, we found 9 (19.5%) patients with simultaneous activity of MDR & LRP, 29 (63%) without activity of both, 5 (11%) with MDR activity only, and 3 patients (6.5%) with LRP activity only. Therefore, 17 (37%) patients had functional activity of one or both proteins.

Table (2) represents the hematological parameters of 46 adult AML patients in relation to positive and negative expression of MDR and LRP. There was no statistically significant difference between patients with negative and those with positive MDR and LRP expression as regards the hematological parameters except for the Hb (p=0.034).

Table (3) sums the positive and negative LRP-MDR1 co-expression in relation to other hematological parameters with no significant differences (p>0.05).

Of the newly diagnosed 46 adult AML patients, 24 (52.2%) achieved complete response, 5 (10.9%) died early during the study, while 17 patients (37%) failed to achieve response.

Among those who have positive MDR expression, 4/14 (28.8%) achieved CR compared to 20/32 (62.5%) with MDR negative expression (p=0.034).

Regarding the complete response rate in relation to positive and negative LRP expressions, 6 out of 12 (50%) patients who have positive LRP expression achieved CR, compared to18/34 (52.9%) of those who have negative LRP expression (p=0.86).

Finally, we analyzed the coexpression of LRP and MDR status in relation to clinical outcome (Table 4). Response to induction chemotherapy was best (CR rate 62%) in patients lacking expression of both genes, intermediate (CR rate 44.4%) in patients expressing both genes and worst in those with expression of either of these two genes, (CR rate 25%). Although our results showed that MDR expression is significantly associated with CR rate, contribution of the combined activity of MDR and LRP in the CR rate did not achieve statistical significance (p=0.079).

Table (5) represents the impact of MDR1 expression on response to therapy showing that –ve MDR1 expression is associated with a higher CR rate (p=0.034).

Table (1): Clinical and Laboratory Characteristics of 46 adult AML patients.

Parameter	Median	Range
Age (years)	37.5	18-65
BM Blasts (%)	75	18-95
TLC (x10^9/L)	58	4.3-470
Plt. (x10^9/L)	36	5-34
Hb (g/dL)	7.3	3.1-11
Parameter	Number (No.)	Percent (%)
Gender:		
Female	13	28.26
Male	33	71.74
FAB classification:		
M0	2	4.3
M1	16	34.8
M2	18	39.1
M3	5	10.9
M4	3	6.5
M5	2	4.3
Treatment Arm:		
Ara-C+ADR (3&7)	33	71.73
Ara-C+ADR (2&5)	3	6.52
ADR+Vesanoid	4	8.69
VP16	1	2.17
Early death	5	10.86

BM : Bone Marrow.
TLC : Total leucocytic count.
Plt : Platelets.
Hb : Hemoglobin.
FAB : French-American-British classification of AML.

Table (2): Hematological parameters of 46 adult AML patients in relation to MDR and LRP expression.

Parameter	MDR		LRP	
	Negative	Positive	Negative	Positive
Age (years)	39±14	35.2±11.6	37.3±14	39.4±11.4
	(20–65)*	(18–58)	(18–65)	(24–58)
	42.5**	33	33	43
BM Blasts (%)	70.1±20	69.4±20.7	71.8±19.9	65±20
	(18–91)	(30–95)	(18 –95)	(30–90)
	74	76	75.5	74.5
TLC (x10^9/L)	79.6±92.7	51.2±33.3	79.8±91.4	48.1±31.5
	(4.3–470)	(5–116)	(4.3–470)	(5–97)
	65.5	52	64	49.5
Plt. (x10^9/L)	45.2±30.2	40.9±38.1	43±31.2	46.5±36.7
	(5–134)	(8.8–134)	(5–134)	(10–134)
	37.9	27	37	33
Hb (g/dL)	6.8±1.8	8.1±2	7.1±1.8	7.3±2.4
	(3.1–11)	(4.3–11)	(3.1–11)	(4.3–11)
	6.9	8.2	7.1	7.8

* Mean±SD (range). ** Median.
No significant p-value was detected except with Hb p-value (0.034).

Table (3): LRP-MDR1 co-expression in relation to other hematological parameters in 46 adult AML patients.

Parameter	Positive co-expression	Negative co-expression
Age (years)	36.7±11.9 (24–58)* 39**	38.1±13.8 (18–65) 36
BM Blasts (%)	66.3±19.5 (30–84) 75	70.8±20.2 (18–95) 75
TLC ($x10^9$/L)	43.2±30.5 (5–97) 46	78.4±88 (4.3–470) 65.5
Plt. ($x10^9$/L)	50.4±42.8 (10–134) 34.5	42.3±29.9 (5–134) 36
Hb (g/dL)	8±2.3 (4.3–11) 8.2	7±1.8 (3.1–11) 7

* Mean±SD (range).
** Median. No significant value (p>0.05).

Table (4): LRP & MDR status in relation to response to therapy in 46 adult AML patients.

Status \ Response	CR	RF	Total
LRP^-/MDR^-	18 (62%)	11 (38%)	29
LRP^-/MDR^+ or LRP^+/MDR^-	2 (25%)	6 (75%)	8
LRP^+/MDR^+	4 (44.4%)	5 (55.6%)	9

CR: Complete remission.
RF: Refractory.

Table (5): Impact of MDR1 (P-gp) expression on response to therapy in 46 adult AML patients.

P-gp expression	CR	RF	Total	p
Positive >=1.1	4 28.6%	10 71.4%	14 100%	0.034
Negative <1.1	20 62.5%	12 37.5%	32 100%	

CR: Complete remission.
RF: Refractory.

Figs. (1,2,3) represent the impact of LRP, MDR separately and combined on overall survival. No significant differences was encountered with any (p=0.381, 0.190 and 0.714 respectively).

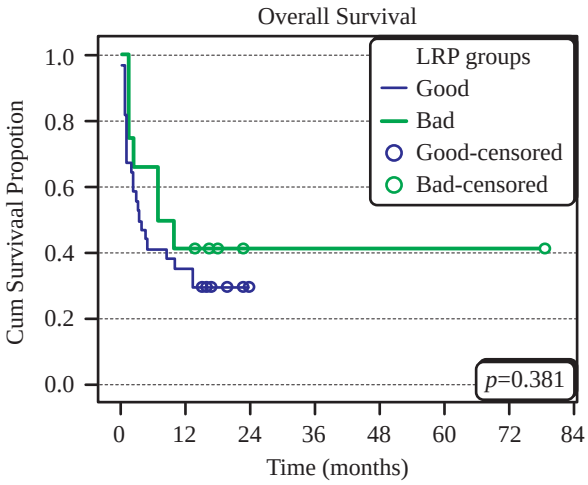

Fig. (1): Overall survival in relation to LRP expression in 46 AML patients.

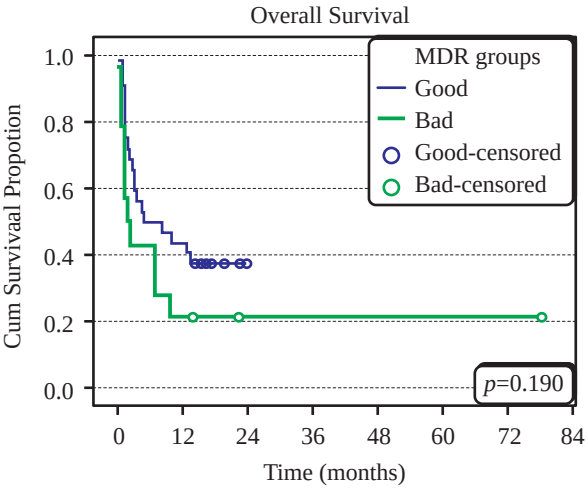

Fig. (2): Overall survival in relation to MDR expression in 46 AML patients.

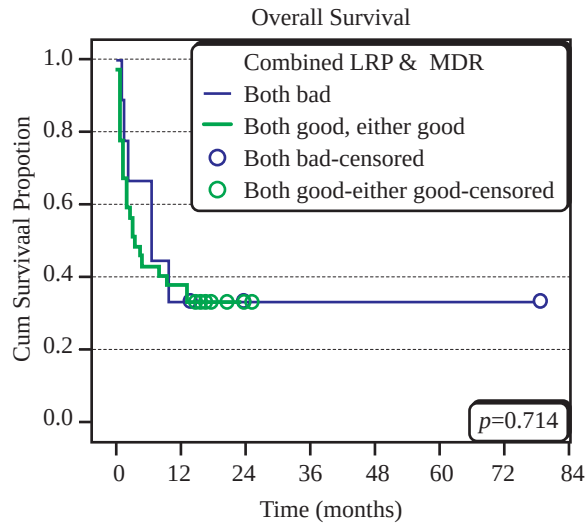

Fig. (3): Overall survival in relation to combined LRP and MDR expression in 46 AML patients.

DISCUSSION

Drug resistance is a multifactorial phenomenon and several mechanisms have been recognized for clinical resistance to chemotherapy in solid tumors as well as in hematologic malignancies. The two important mechanisms of drug resistance in leukemia are expression of drug resistance genes and activation of anti-apoptotic mechanism **[4]**.

With the advent of better chemotherapy and supportive therapy care in the past decade, clinical outcome has improved considerably for adult patients with both acute myeloid leukemia (AML) as well as acute lymphoblastic leukemia (ALL). However, leukemic cells from adults are intrinsically more resistant to drugs commonly used in induction chemotherapy as compared to those from pediatric patients **[6]**. Unfavorable karyotype, poor treatment tolerance and over expression of multi drug resistant genes in adults could account for this difference **[9]**.

Studies on the treatment of adult AML have shown only modest improvements over the last 2 decades, with the actual cure rate still ranging between 15% and 40%. The resistance of tumor cells to chemotherapeutic drugs is a major limitation in cancer treatment. MDR phenotype is the most frequently studied mechanism for intrinsic drug resistance, yet the prognostic role of P-gp and other MDR-associated proteins in adult AML is still largely unknown **[4]**.

Our aim in this study is to evaluate the frequencies of occurrence of MDR agents P-gp (MDR1), and LRP in Egyptian adult acute myeloid leukemia patients, and to correlate them with disease prognosis and clinical and laboratory variables.

P-gp expression:

In the present study, P-gp expression was found positive in 14/46 patients (30.4% of all AML cases), 4 of them (28.6%) achieved complete remission (CR), and was found negative in 32 patients 69.6%, 20 (83.3%) of them achieved CR. In agreement with our study Huh et al. **[5]** reported P-gp mRNA expression by RT-PCR in adult and childhood acute leukemia in 25% of cases at diagnosis. Our data are also in the range of that described before by Tafuri et al 21.7% **[10]**. The percentage of expression in most of studies is widely variable. This may be attributed to the use of different techniques and methods, (different cut-off values) and pooling of heterogenous groups of patients such as AML and ALL, initial and relapse samples, and adult and pediatric cases.

LRP expression:

In our study, we found LRP was expressed in 12 (26.1%) patients, 6 of them (50%) had CR. Negative expression was present in 34 patients (73.9%), 18 (75%) of them entered CR.

Similar to our study Tafuri et al. **[10]** found that 60.5% of cases were positive for LRP, and that LRP expression had no influence on CR. Another study **[13]** found that MRP1, LRP, BCRP and GSTP1 expressions showed no significant association with response to induction chemotherapy in AML patients. Recent literature on expression of MRP1, LRP and BCRP mRNA at diagnosis has also found no significant association of these genes with response rates **[12]**.

However Pradeep et al. **[11]** found that the clinical relevance of other drug resistance genes LRP, BCRP, GSTP1, DHFR and apoptosis related genes need to be elucidated.

Double expression P-gp and LRP:

In the current study dual expression of P-gp and LRP was detected in 9 (19.6%) cases, 4 of them (44.4%) had CR, and 5 (55.6%) were refractory. Although the result is suggestive of significant difference, the sample size does not support statistical confirmation. Huh et al. **[5]** reported coexpression of MDR1 and LRP in 21.1% of cases with no significant influence on CR rate.

Correlation of P-gp and LRP with rates of CR and refractory cases:

Our results showed significant correlation between CR group and refractory group in the expression of MDR1 with (p=0.034) but showed no significant correlation with LRP (p=0.861).

In agreement with our results, earlier study **[14]** stated that only MDR1/P-gp expression and cyclosporine-inhibited efflux were significantly associated with complete remission (CR) rate (p=0.012 and 0.039 respectively). Our data are also in accordance with recent different groups who stated that MDR1 expression in AML cases could be one of the mechanisms responsible

for induction failure in adult patients **[10,11,12]**. These results can concur with a report showing that P-gp expression does not correlate with CR rates **[1]**.

In contrast to our results, Huh et al. **[5]** found that LRP expression was associated with lower CR rate, while MDR1 appeared to have statistically no significant effect on CR.

Although our data reported that MDR expression is significantly associated with CR rate, contribution of the combined activity of MDR and LRP did not achieve significance. These contradictory results might be partially caused by the relatively small patient numbers in our study. Earlier studies reported that coexpression of LRP and MDR1 might result in worst prognosis **[1,10]**.

Correlation of P-gp and LRP with clinical and laboratory parameters:

In the current study a significant correlation was detected between Hb level & MDR1 expression (*p*-value 0.034). However no correlation was detected between P-gp or LRP and other known prognostic markers such as age and WBCs count, which represent tumor cell mass, as well as BM blasts. Our results are in accordance with other researchers who revealed no relation between either P-gp or LRP and either age or WBCs count **[3]**. Moreover, Ozlem and his colleagues **[3]** supported our results; they also found no significant relation between P-gp and LRP with other prognostic markers.

In conclusion, the present study demonstrates that only positive expression of MDR1 appears to represent a significant prognostic indicator in adult AML, whereas LRP expression has no significant impact on prognosis in adult AML cases.

The expression of the tested parameters does not correlate with other known prognostic factors such as age or WBCs count.

For better understanding of the factors involved in MDR, we recommend involving alternative drug-resistance mechanisms as MRP and BCRP with MDR1 and LRP in one study. Also we recommend conducting studies on large number of uniformly treated AML patients so that the statistical studies are more conclusive.

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