ORIGINAL ARTICLE

Anticancer Drug Resistance in Plasma Cell Myeloma Patients Having Lung Resistance Related Protein Emergence

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ABSTRACT

Background: Lung related resistance protein (LRP) is a central part of the multidrug resistance (MDR) phenotype involved in cell resistance toward xenobiotics or chemotherapy. Expression of the LRP in plasma cells from multiple myeloma (MM) patients is associated with resistance to various anticancer drugs, including melphalan and, therefore, may affect the clinical outcome in MM. [1] To determine the clinical significance of LRP, we have compared LRP expression in bone marrow plasma cells with clinical and laboratory parameters including age, sex, monoclonal immunoglobulin (Ig), calcium (Ca), creatinine (Cr), lactate dehydrogenase (LDH), β2-microglobulin (β2-M), interleukin-6 (IL-6), p53 deletion and stage of disease according to conventional durie and salmon staging system, response to chemotherapy and survival of patients.

Methods: The current research meant to establish the role of LRP in drug resistance in plasma cell myeloma (PCM) patients in 244 subjects who were diagnosed as MM, seen at several cancer hospitals in Pakistan. These subjects were measured for LRP expression assessed by Human Lung Resistance Related Protein (LRP) ELISA (Elabscience Biotechnology Inc. USA) [2] applies to the in vitro quantitative determination of Human LRP concentrations in serum, plasma and other biological fluids.

Results: LRP levels were negative in 63 patients and positive in 176 patients. Correlation between LRP expression and other disease parameters i.e. age, sex, monoclonal Ig, Ca, Cr, β2-M, IL-6, p53 deletion and stage of disease according to conventional durie and salmon staging system. There was no correlation between LRP expression and age, sex, monoclonal Ig, Ca, Cr, β2-M, IL-6, and stage of disease according to conventional durie and salmon staging system. However, LRP expression was more frequently observed in patients with a p53 deletion than in those without such a deletion (P – value 0.01). The response to induction chemotherapy was successful in 69 patients out of 176 LRP positive patients; response was unsuccessful in 107 patients out of 176 LRP positive patients. Kaplan-Meier analysis revealed that patients with LRP expression had a shorter survival median, 18 months than those without LRP expression, having median, 45 months. These data show that LRP expression is an important marker for clinical drug resistance and predicts a poor outcome in MM.

Key words: Lung related resistance protein (LRP), multidrug resistance (MDR), multiple myeloma (MM), durie and salmon staging system, monoclonal immunoglobulin (Ig), calcium (Ca), creatinine (Cr), lactate dehydrogenase (LDH), β2-microglobulin (β2-M), interleukin-6 (IL-6), p53, and plasma cell myeloma (PCM).

INTRODUCTION

Chemotherapy (alkylating agents) is the bastion of management for MM. Melphalan, like other alkylating agents, exerts its cytotoxic effect through the covalent linkage of alkyl groups to DNA. Resistance against alkylating agents includes both cellular and extracellular factors. In cell line studies resistance to melphalan has been attributed to a decreased drug uptake caused by alterations in either the number or the affinity of membrane bound proteins[3]. An alternative explanation may be an increased cellular detoxification by glutathione S-transferases. So far, studies in hematological malignancies such as MM have failed to show a role of these laboratory findings in clinical specimens. But resistance to anticancer drugs remains a major problem in the clinical management of MM patients. Initially responsive tumors relapse and develop resistance to a broad spectrum of drugs known as MDR. Consequently, a metastatic cancer finally becomes refractory to cytotoxic drugs and is typically incurable by chemotherapy. Thus, intrinsic or acquired drug resistance is primarily responsible for the failure of current treatment regimens in particular cancer. MDR is one important type of drug resistance that is clinically relevant in several solid tumors and leukemias i.e., Testicular cancer, Wilms' tumor, Rhabdomyosarcoma, Neuroblastoma, Ewing's sarcoma, Acute myeloid leukemia, Small cell lung cancer, Ovarian carcinoma, Breast carcinoma, Bladder carcinoma, Head and neck carcinoma, Soft tissue sarcoma, Osteosarcoma, Endometrial carcinoma, Gastric carcinoma, Esophageal carcinoma, Squamous cell Adenocarcinoma, Colo-rectal carcinoma, Pancreatic carcinoma, Renal carcinoma, Melanoma, Non-small cell lung cancer, Squamous cell carcinoma, Adenocarcinoma, Mesothelioma, Pheochromocytoma, Thyroid carcinoma, Hepatocellular carcinoma, Adrenal carcinoma, Ggastric cancer, Ovarian carcinoma, Acute lymphoblastic leukemia B cell origin, and Acute lymphoblastic leukemia T cell origin etc. MDR1/P-glycoprotein, (which purposes as an effervescence controlled drug efflux pump for real aquaphobic composite) countenance arises with a range of different frequencies in PCM and is coupled with scientific drug resistance to anthracyclines, vinca alkaloids & epipodophyllotoxins[4]

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Parallel clinical experiments to conquer these conflicts through alloy chemotherapy accompanied by confrontation-qualifiers have been executed. The particular work advocated so as to, as a minimum in a few long-sufferings, intonation of P-glycoprotein do arise and was correlated by surpass results. Nevertheless, the on the whole retort speeds were small and of dumpy period in these researches. Multidrug resistance associated protein (MRP), one more significant thing engrossed in MDR, is too uttered in MM, although its scientific significance lingers to be concluded. Current data proposes that modifications in programmed cell deaths are engrossed in the drug resistance of MM. Fas arbitrated apoptosis may be significant in MM subjects, in general reduced endurance is observed in patients with P53 deletion in comparison with subjects had no deletions. Initially LRP noticed in patients without P-gp MDR cell line in lung cancer. LRP is considered as vital fraction of the human individual chief vault (crypts) protein intricate. In recent time's lung related proteins is under gone the process of genetic copy and recognized as an (human p110) foremost vault (crypts) protein[5]. The crypts as cellular organelle, which leads to intervene transport inside the cell for different extensive diversity of substrates. Vaults are RNA constituent parts which contain important crypts proteins along with some inconsequential crypt proteins and undersized RNA. Small quantity of vaults is present nuclear cell membrane and a major fraction is located inside the cytoplasm. These vaults thought to intervene transport inside the cell as well as nuclear cytoplasm. Tumor cell lines of lung resistant proteins linked with doxorubicin resistance, as well as with cisplatin, carboplatin, vincristine, of fastidious attention for melphalan in MM. In large intestine, alveolar lung tissue, tissue macrophages, proximal tubules in kidney adrenal gland cortex lung resistant proteins are physiologically (normally) present, and its presence remain under study process. In blood plasma cells expression of LRP is to be determined that LRP may had any analytical implication in subjects who had MM, and also their response to treatment associated with chemotherapy and patient continued existence[6].

MATERIAL AND METHODS

244 patients not taking treatment (147 males, 97 females) diagnosed cases of MM were included in study after getting informed and written consent according to international guiding principle. Initial chemotherapy received by all subjects. Regimens based on Melphalan [prednisone, melphalan or vincristine, melphalan (M), cyclophosphamide, prednisone (VMCP)] were intravenously administered in 227 patients. Out of which 17 subjects taking treatment including vincristine, doxorubicin, dexamethasone (VAD), after that getting melphalan in greater doses. 7 patients were died during chemotherapy. Results of this initial chemotherapy were evaluated on the basis of customary criteria.

These subjects were measured for LRP expression assessed by Human Lung Resistance Related Protein (LRP) ELISA (Elabscience Biotechnology Inc. USA) [2] applies to the in vitro quantitative determination of Human LRP concentrations in serum, plasma and other biological fluids. This ELISA kit uses the Sandwich-ELISA principle. The micro ELISA plate provided in this kit has been pre-coated with an antibody specific to Human LRP. Standards or samples are added to the micro ELISA plate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for Human LRP and Avidin-Horseradish Peroxidase (HRP) conjugate are added successively to each micro plate well and incubated. Free components are washed away. The substrate solution is added to each well. Only those wells that contain Human LRP, biotinylated detection antibody and Avidin-HRP conjugate will appear blue in color. The enzyme-substrate reaction is terminated by the addition of stop solution and the color turns yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450nm±2nm. The OD value is proportional to the concentration of Human LRP. You can calculate the concentration of Human LRP in the samples by comparing the OD of the samples to the standard curve.

Statistical analysis: Co-relation among laboratory indices and clinically in patients with LRP were evaluated by one of these test like Fisher's exact and chi-squared. Endurance possibilities were considered with the method of product limit as suggested by Kaplan-Meier estimator. The period among disease diagnosis moment of death is survival time. With the help of log-rank test two groups were analyzed on the basis of endurance division.

RESULTS

Presence of LRP appearance of earlier patients not taking treatment was resolute by Human Lung Resistance Related Protein (LRP) ELISA. LRP levels were depressing in 63 patients and positive in 176 patients. There was no correlation between presence of LRP expression and age, sex, monoclonal Ig, Ca, Cr, β2-M, IL-6, and stage of disease according to conventional durie and salmon staging system. On the other hand, LRP appearance was much commonly present in subjects with a deletion of p53 which was perceive by Fluorescence in situ hybridization than in those without such a deletion (P – value 0.01). The results of chemotherapy administration were doing well in 69 patients out of 176 LRP positive patients; response was unsuccessful in 107 patients out of 176 LRP positive patients. The protocol of treating subjects was uniformly circulated between LRP= + ve and LRP= -ve patients. When LRP positivity percentile is increased the treatment results rate were reduced. Subjects having presence LRP expression had reduced endurance median as suggested by Kaplan-Meier, 72 weeks or 18 months as compare with those no LRP expression, comprise median, 3 years 9 months. This dissimilarity in endurance was noticed in group of subjects treated with induction chemotherapy, and also noticed in group of patients getting treatment melphalan in high-doses. These data analyses show that expressions of LRP are an imperative marker for drug resistance clinically and envisage an unfortunate result in MM.

Table:1: Characteristics of MM patients. (NS, non significant)

	Characteristics	No. of Patients	LRP – ve Patients (n)	LRP + ve Patients(n)	P – Value
	No. of Patients	244	68	176	NS
Age	>60y	198	55	143	NS
	<60y	46	15	31	NS
Sex	Male (n, %)	147 (60.25)	39	108	NS
	Female (n, %)	97 (39.75)	29	68	NS
	Hb (<10mg/dl)	233	59	174	NS
Myeloma Proteins	IgG	163	67	96	NS
	IgA	53	16	37	NS
	IgD	20	7	13	NS
	Bence jones proteins	6	2	4	NS
	IgM	1	Nil	Nil	NS
	IgE	1	Nil	Nil	NS
	Non-secretory myeloma	Nil	Nil	Nil	NS
	Calcium >10.5mg/dl	189	57	132	NS
	Creatinine>1.5mg/dl	236	68	168	NS
	B2M>2µg/ml	244	68	176	NS
	CRP>8.2mg/l	244	68	176	NS
	Deletion of p53	56	5	51	0.01
Stage	I	37	12	25	NS
	II	106	29	77	NS
	III	101	27	74	NS

Table 2: Outcome of induction and high dose chemotherapy, clinical and laboratory parameters compared with the outcome of induction chemotherapy.

	Charateristics	No of Patients	Response to chemo	No Response to chemo	P-Value
LRP	Negative	68	58	10	0.01
	Positive	176	69	107	
Age	>60y	198	101	97	NS
	<60y	46	29	17	
Sex	Male (n, %)	147 (60.25)	88	59	NS
	Female (n, %)	97 (39.75)	57	40	
Myeloma Proteins	IgG	163	107	56	NS
	IgA	53	32	21	
	IgD	20	13	7	
	Bence jones proteins	6	4	2	
	IgM	1	1	Nil	
	IgE	1	1	Nil	
	Non-secretory myeloma	Nil	Nil	Nil	
	Calcium >10.5mg/dl	189	118	71	NS
	Creatinine>1.5mg/dl	236	159	77	NS
	B2M>2µg/ml	244	163	80	NS
	CRP>8.2mg/l	244	163	81	NS
p53	Normal	188	119	69	0.01
	Deletion	56	18	38	
Stage	I	37	26	11	NS
	II	106	78	28	
	III	101	75	26	

Table: 3: Survival Kaplan-Meier Analysis

	Charateristics	No of Patients	Survival mean
LRP	Negative	68	45 months
	Positive	176	18 months

DISCUSSION

In this study, we have established the medical implication of LRP in Multiple Myeloma (MM). The association of LRP expression with deprived reaction to induction chemotherapy and in general shorter endurance of MM subjects[1,7]. LRP expression used as an analytical factor in association with results and responses of chemotherapy without complications survival and in general endurance in subjects treating with melphalan used as conventional-dose and melphalan used in high-doses, p53 deletion and LRP expression are associated with each other. Presence of p53 deletion was correlated with reduced survival rate with decreased in response for chemotherapy treatment. LRP and deletion p53 increases the likely hood that this deletion of p53 might implicate in the directive of expressions of LRP[7]. A probable collision of p53 over the drug confrontation reason has formerly been revealed for MRP and MDR1. Cases associated with of MDR1, malformed p53 has been exposed to particularly excite the

MDR1 supporter in vitro, while uncultivated form of p53 suppress its movement[24]. In cases associated with MRP, p53 in wild-type perform as a depressing the factor that regulates the transcription of MRP, and decreasing the undomesticated function of p53 that leads to increases the gene for MRP. [4] Other possible authoritarian feature associated with expression of LRP comprises β-estradiol and (TNF-a) tumor necrosis factor-a. When cell lines of colon carcinoma were initiate nucleic acids into cells enclose TNF-a and cDNA, both can decreases the stage of RNA of LRP proteins and raises the levels of RNA of MRP protein were identified. These outcomes recommend that the communication among LRP and receptor of β-estrogen is most likely associated with transporting intracellular molecules of receptor[3]. It is considered that LRP cause molecules transport in two directions among nucleus and in cytoplasm. LRP causes drug used for cancers to transport outside the cell and inside the vesicles of cytoplasm. The participation of LRP anticancer compounds in transportation is maintained by the current discovery that LRP formation is in a straight line connected to drug confrontation of cells[5,6,8].

CONCLUSION

Expression of LRP is analytical factor for reduced results to initiation of chemotherapy high doses in subjects of MM and a predictive aspect with reference to endurance of the subjects.

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