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P-Glycoprotein (Pgp) Expression in Resistant or Relapsing Hodgkin's Disease

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ABSTRACT

Twenty-eight males and 8 females with resistant (10 patients) or relapsing (26 patients) Hodgkin's Disease (HD) after chemotherapy were included in this study. Their ages ranged from 16 to 55 years with a median age of 28±8.3 years. Immunohistochemical staining for detection of p-glycoprotein (Pgp) using monoclonal antibody to multidrug resistance marker, p170 glycoprotein, clone JSB-1-Bio Genex, San Roman, California, USA, was done for all patients at relapse. P-glycoprotein was also studied in tissue specimens of ten of the studied patients at diagnosis as a control group. Salvage chemotherapy was tailored according to initial regimens and duration of the first disease-free survival (DFS1). Patients relapsed after long DFS1 were retreated by the same regimen, while those with primary resistance or short DFS1 were crossed over to another non cross-resistant combination chemotherapy. In the control group, Pgp expression was found in 20% (2/10) of patients in the affected nodes at initial diagnosis compared with 90% (9/10) of them at relapse (p = 0.01). For all patients at relapse or resistance, Pgp expression was found in 29 patients (80%) and was more expressed in patients with B-symptoms, primary progressive disease or short DFS1 and histological subtypes other than lymphocyte predominance. Patients with negative Pgp expression had a better overall response (100%) compared with those with positive Pgp expression (72.4%). Pgp overexpression was found to be inversely correlated with 1-year survival. The overall response rate to salvage treatment was 77.8% with 38.9% complete response. Male patients, age above 40, lymphocyte depletion histology, B-symptoms, bulky disease, those with primary resistance or short DFS1 were found to have poor overall response rate. Variables associated with longer response duration after salvage therapy were complete response, long DFS1, negative Pgp expression, young age, female gender, absence of B-symptoms and lymphocyte predominance histology. It was concluded from this study that Pgp overexpression could be a useful prognostic factor in addition to other factors in predicting the response to salvage therapy and survival in patients with relapsing HD.

Key Words: *Multidrug resistance - MDR - P-glycoprotein - Hodgkin's disease - Relapse - Prognosis.*

INTRODUCTION

Progress in Hodgkin's disease therapy has been substantial over the last 15-20 years, with 60% of patients surviving disease-free at 10 years. However, 30% of the patients will die within 4 years of presentation due to progressive disease **[6]**. The development of drug resistance by the tumor cells is one of the major obstacles to the successful treatment of cancer. One of its important causes is the multidrug resistance (MDR), which is characterized by the ability of the tumor cells to be cross-resistant to the actions of a wide variety of structurally and functionally unrelated drugs and other xenobiotics **[5]**.

One of the principal mechanisms by which tumors develop the MDR phenotype is the expression of Pgp, an ATP-dependent drug efflux pump, which significantly lowers the intracellular concentration of cytotoxic agents. The expression of Pgp can occur spontaneously or after multiple rounds of chemotherapy. The agents involved in the multidrug resistance phenomenon are all natural products and for Hodgkin's disease, the relevant drugs are mostly anthracyclines, epipodophyllotoxins and vinca alkaloids **[20]**.

Several monoclonal antibodies (MoAb), directed against Pgp epitopes and Pgp specific nucleic acid probes, are available, respectively, for immunocytochemistry and Western blotting for the detection of the protein and Northern and slot blotting, RNase protection and polymerase chain reaction for the detection of its

mRNA. All these techniques have been worked out using in vitro MDR model systems with abundant p-glycoprotein expression [12]. Currently, immunohistochemistry is one of the most commonly used techniques to evaluate Pgp expression in tissue specimens. This technique allows correlation of expression with microanatomic features of tissues and can identify the degree of heterogeneity and range of intensity of Pgp expression within a tumor, however subjectivity is a primary disadvantage [3].

Elevated Pgp levels and/or mdr1/P - glycoprotein mRNA transcript levels have been reported in almost all types of haematological malignancies, either untreated or treated, with expression levels ranging from low to high the increased levels of Pgp has been correlated with poor response to chemotherapy and short disease-free interval and overall survival [5,26]. Others clearly indicate that Pgp is an independent factor that predict response to chemotherapy [18].

There were few studies demonstrating prognostic importance of Pgp expression in malignant lymphomas and Hodgkin's disease. Dan et al., 1991 demonstrated Pgp expression in tumor cells from NHL patients. In cases where the mdr1 gene was detectable, clinical resistance to therapy was also predicted. However, correlation of Pgp expression with treatment intensity and response to therapy has been inconsistent. The quantitation of Pgp expression is complicated by the heterogeneity of the tumor, the difficulty of differentiating normal lymphocytes from tumor cells and by the variability of Pgp expression within a tumor [7]. Patients with recurrent or refractory lymphomas are more likely to exhibit detectable levels of Pgp [11]. An improved understanding of the mechanisms by which tumor cells develop resistance to chemotherapy may not only enhance the activity of cytotoxic therapy in advanced malignancies but may ultimately improve the impact of adjuvant therapy, potentially resulting in prolongation of disease-free intervals and survival. The ability to identify tumors with increased Pgp expression has several potential applications as the prediction of response to chemotherapy and the design of studies aimed at reversal of resistance with agents that inhibit MDR-mediated drug efflux.

The aim of this work is to study the importance of Pgp expression in patients with resistant or relapsing HD and its relation to other prognostic factors.

PATIENTS AND METHODS

This study was carried out at the Medical Oncology Departments in National Cancer Institute, Cairo and Mansoura University Hospital in the period between December 1994 and December 1996. Thirty six adult patients with resistant or relapsing Hodgkin's disease after chemotherapy were included in the study. Eligible patients were adult patients between 16-60 years old, normal liver and kidney function with performance status of 0-2.

All patients were subjected to complete history and physical examination with especial attention to clinical stage of the disease, B-symptoms, primary disease-free interval and tumor burden. Laboratory investigations included complete blood count, liver and kidney function tests, LDH, bone marrow examination. Radiological assessment involved chest X-ray, abdominal ultrasonography. CT scan chest, abdomen and bone or gallium 67 scans were done when indicated.

In all patients, lymph node biopsies were examined for histopathological confirmation and subtyping of HD and immunohistochemical staining for detection of p-glycoprotein (Pgp), using monoclonal antibody to multidrug resistance marker p170 glycoprotein, clone JSB-1-Bio Genex, San Roman, California, USA. Detection of p-glycoprotein in tissue specimens of 10 patients at diagnosis and after relapse was also done and was used as a control group. Positive Pgp staining reaction was considered positive when there was brown cell membrane reaction and/or granular cytoplasmic brown staining.

Salvage chemotherapy was given according to type of initial treatment and the duration of DFS1. Chemotherapeutic regimens as COPP (cyclophosphamide 650 mg/m2 iv days-1 and 8, vincristine 2mg iv days-1 and 8, procrbazine 100 mg/m2 and prednisone 60 mg/m2 po daily for two weeks), ABVD (Adriamycin 25 mg/m2, bleomycin 10 mg/m2, vinblastine 6 mg/m2 and dacarbazine 375 mg/m2 iv days-1 and 15) or COPP/ABV hybrid (cyclophosphamide 650 mg/m2 iv day-1, vincristine 2 mg iv day-1, procrbazine 100 mg/m2 po daily for 7 days and

prednisone 60 mg/m2 po daily for 14 days, adriamycin 35 mg/m2 day-8, bleomycin 10 mg/m2 day-8 and vinblastine 6 mg/m2 day-8) were commonly used as front line therapies for patients with advanced HD. Patients who relapsed after long DFS1 (one year or more) were retreated by the same initial regimen, while those who had short DFS1 or progressed on front-line therapy (primary resistance) were crossed-over to another non cross-resistant combination chemothrapy those who received sequential or alternating COPP and ABVD or COPP/ABV hybrid regimens were treated with IEM regimen (ifosfamide 1 gm/m2 iv infusion with appropriate doses of urometxane, etoposide 100 mg/m2 iv daily for 5 days and methotrexate 30 mg/m2 iv days 5 &10) or MOPLACE regimen (cyclophosphamide 750 mg/m2 iv day-1 etoposide 80 mg/m2 iv day-1, prednisone 60 mg/m2 po daily for 14 days, methotrexate 120 mg/m2 iv days 15 & 22, cytarabine 300 mg/m2 iv days 15 & 22, vincristine 2 mg iv days 15 & 22) on relapse or progression.

Assessment of response was done according to WHO criteria after 3 cycles of therapy, with repeat of those clinical, laboratory, or radiological assessments appropriate to the defined extent of the disease at presentation. Complete response (CR) is defined as the disappearance of all known lesions on two separate measurements at least 4 weeks apart. Partial response (PR) is defined as a reduction of each lesion by at least 50%. Stable disease (SD) is defined as a decrease of less than 50% or an increase of less than 25% with no new lesions and progressive disease (PD) as an increase of greater than 25% or appearance of new lesions.

Patients who achieved CR or PR after 3 cycles received 3 more cycles. Patients with partial response who entered in CR after 6 cycle another 3 cycles (total 9 cycles) were given, while those who did not respond or still in PR were shifted to other non cross-resistant regimen.

Survival and response duration analyses were done using Kaplan Meier analysis and Log rank test.

RESULTS

Of the 36 patients included in this study, twenty eight (77.8%) were males and eight (22.2%) were females, with male to female ratio of 3.5:1. Their ages ranged from 16 to 55 years with a median age of 28±8.3 years. Ten patients (27.8%) were resistant to primary chemotherapy, 15 patients (41.7%) had early relapses (short DFS1) and 11 patients (30.5%) had a late relapse.

The demographic, histopathologic and clinical characteristics of the studied patients at initial presentation and at relapse or disease progression is shown in Table (1). The most common histopathological subtype was mixed cellularity (MC) both initially (50%) and on relapse (52.8%). Progression to more aggressive histological subtypes was noticed in some patients at relapse. Two patients with lymphocyte predominance (LP), 5 with nodular sclerosis (NS) and 4 with MC had progressed to worth histological subtypes at relapse as shown in Table (2). At initial presentation clinical stage III and IV were encountered in 24 (66.7%) and 3 (8.3%) patients, while at relapse the figures were 15 (41.7%) and 5 (13.9%) respectively. Cervical lymph nodes were the most commonly involved sites initially and on relapse, where 30 (83.3%) and 31 (86.1%) patients had cervical lymph node enlargement, respectively.

The chemotherapeutic regimens used in treatment of the patients at presentation and at relapse in relation to DFS1 are shown in Table (3). The overall response rate to salvage chemotherapy regardless of the regimen used was 77.8% with 38.9% complete response. The CR rates achieved with COPP, ABVD, COPP/ABV, MOPLACE and IEM were 80% (4/5), 37.5% (3/8), 33.3% (2/6), 33.3% (4/12) and 20% (1/5), respectively, as shown in Table (4). The main side effects encountered with salvage therapies were neutropenia, nausea and vomiting, anaemia, alopecia and peripheral neuropathy. No cardiotoxicity was encountered during ABVD therapy. Grade IV neutropenia was encountered in one patient during IEM regimen which was controlled by appropriate supportive measures using G-CSF. Mucositis was a common side effect with MOPLACE regimen, as 10 out of 12 (83.4%) patients developed mucositis in different grades as shown in Table (5).

P-glycoprotein was studied in tissue specimens of initial diagnosis of 10 patients as a control group and at relapse in all patients. There was a statistically significant difference in Pgp expression in affected tissues at initial diagnosis

compared with that at relapse. Positive Pgp expression was found in only 2 out of 10 (20%) patients in the nodes at initial diagnosis, while it was positive in 9 of these patients (90%) at relapse (p = 0.01 using McNemar test) Fig. (1) showed a microscopic picture of lymph node immunohistochemically stained showing positive Pgp expression. For all patients at relapse or resistance, Pgp expression was positive in 29 patients (80%), negative in 5 patients (14%) and equivocal in 2 patients (6%) as shown in Fig. (2).

The relation between response to salvage therapy and different clinical parameters were also studied. It was found that patients who were above 40 years, female sex, had B-symptoms at relapse, positive Pgp expression or had a primary resistance or short DFS1 had a worth overall response rates compared with other groups as shown in Table (6). There was a tendency toward better response rate for those with LP subtype. The better response rate in patients salvaged with COPP regimen compared with other regimens was attributed to the long DFS1 in all COPP treated patients.

Regarding the relation of Pgp expression and different clinical parameters at relapse, it was found that patients with negative Pgp expression had a better overall response (100%) than patients with Pgp positive expression (72.4%) and that the density of Pgp expression was inversely related to the response rate.

There was a direct correlation between Pgp expression and stage of disease at relapse, where all patients with stage IV disease, 93.3% of stage III patients and 62.5% of stage II had positive Pgp expression. P-glycoprotein was less expressed in patients with LP compared with other histological subtypes as shown in Table (7). Twenty-five percent (1/4) of patients with LP, 100% (4/4) with NS, 84.2% (16/17) with MC and 80% (4/5) with lymphocyte depletion (LD) showed Pgp expression. Also, Pgp was more expressed in patients with B symptoms at relapse and those who had primary refractory disease or short DFS1.

After one year follow up, 8 patients were alive in remission, 13 were alive with active disease, five were dead (disease related) and status was unknown in 10 patients. There was an inverse relation between Pgp expression and second disease free survival (DFS2). Patients, who had long DFS2 (lived for one year or more), had less Pgp expression (50%) compared to those who did not achieve CR or had active disease (100%) or dead (80%) as shown in Table (7).

The median response duration among all studied patients was 3 months. Variables associated with longer median response duration were complete response, long DFS1, negative Pgp expression, female gender, negative B symptoms at relapse as well as LP histology.

Using Kaplan Meier survival analysis and log rank test, patients with positive Pgp expression at relapse, positive B symptoms, bulky disease, short DFS1 or those who did not respond to salvage chemotherapy had less survival probability than those with negative expression, negative B symptoms, long DFS1 and those who responded to treatment. However, the response to salvage therapy was the only statistically significant variable (Table 8). One year survival for all patients was 84% and 1.5 years survival was 72%.

Table (1): Clinical characteristics of 36 patients with relapse or resistant HD at presentation and at relapse or disease progression after chemotherapy.

Characteristic	Initially Number (%)	Relapse or resistant Number (%)
Histopathology:		
L.P	6 (16.7)	4 (11.1)
N.S	7 (19.4)	4 (11.1)
M.C	18 (50)	19 (52.8)
L.D	-	5 (13.9)
Unknown	5 (13.9)	4 (11.1)
Stage of disease:		
II	9 (25)	16 (44.4)
III	24 (66.7)	15 (41.7)
IV	3 (8.3)	5 (13.9)
Disease sites:		
Cervical	30 (83.3)	31 (86.1)
Axillary	14 (38.9)	10 (27.8)
Hilar/mediastinal	3 (8.4)	2 (5.6)
Inquinal	16 (44.4)	4 (11.1)
Paraaortic	2 (5.6)	3 (8.3)
Spleen	13 (36.1)	13 (36.1)
Liver	1 (2.8)	2 (5.6)
Bone marrow	2 (5.6)	3 (8.3)
Bulky disease	10 (27.8)	2 (5.6)
Positive B-symptoms	26 (72.2)	27 (75)
High ESR >50 mm/1st hour	29 (80.6)	31 (86)
Anaemia	6 (16.7)	10 (27.8)

Table (2): Histologic changes from presentation to relapse or resistance in 36 HD patients.

Initial history	Total	Relapse histology				Unknown
		LP	NS	MC	LD	
LP	6	4	1	1	0	0
NS	7	0	2	4	1	0
MC	18	0	0	14	4	0
LD	0	0	0	0	0	0
Unkown	5		1			4
Total	36	4	4	19	5	4

Table (3): Chemotherapy regimens used in treatment of 36 patients in the study.

Initial chemotherapy	No. of patients	Salvage chemotherapy	No. of patients	DFS*
COPP	13	COPP	5	> 1 year
		ABVD	8	< 1 year or resistant
COPP/ABV	16	COPP/ABV	6	> 1 year
		IMVP-16	1	< 1 year or resistant
		MOPLACE	9	< 1 year or resistant
COPP (first line) then ABVD (first relapse salvage)	7	MOPLACE (second relapse)	3	< 1 year or resistant
		IMVP-16 (second relapse)	4	< 1 year or resistant

*DFS: Disease free survival.

Table (4): Responses to salvage chemotherapy in 36 HD patient in the study.

Response*	COPP N (%)	ABVD N (%)	COPP/ABV N (%)	IEM N (%)	MOPLACE N (%)	Total N (%)
CR	4 (80)	3 (37.5)	2 (33.3)	1 (20)	4 (33.3)	14 (38.9)
PR	1 (20)	3 (37.5)	3 (50)	2 (40)	5 (41.7)	14 (38.9)
PD	0	2 (25)	1 (16.7)	2 (40)	3 (25)	8 (22.2)
Total	5	8	6	5	12	36

*CR: Complete response, PR: Partial response, PD: Progressive disease.

Table (5): Most common toxicity after salvage treatment in 36 patients in the study.

Toxicity: grade*	COPP N (%)	ABVD N (%)	COPP/ABV N (%)	IEM N (%)	MOPLACE N (%)
Neutropenia:					
II	1 (20)	3 (42.9)	4 (66.7)	3 (50)	4 (33.3)
III	2 (40)	1 (14.3)	2 (33.3)	2 (33.3)	0
IV	0	0	0	1 (16.6)	0
Anaemia:					
II	3 (60)	2 (28.6)	3 (50)	4 (66.7)	5 (41.7)
III	0	1 (14.3)	1 (16.7)	1 (16.7)	0
Vomiting:					
II	2 (40)	5 (35.7)	4 (66.7)	4 (66.7)	7 (58.3)
III	1 (20)	1 (14.3)	2 (33.3)	2 33.3)	2 (16.7)
Alopecia:					
II	3 (60)	1 (14.3)	1 (16.7)	4 (66.7)	6 (50)
III	2 (40)	3 (42.9)	5 (83.3)	2 (33.3)	2 (16.7)
Mucositis:					
II	0	2 (28.6)	3 (50)	4 (66.7)	5 (41.7)
III	0	0	0	0	3 (25)
IV	0	0	0	0	2 (16.7)
Peripheral neuropathy:					
II	2 (40)	0	2 (33.3)	0	3 (25)
III	1 (20)	0	0	0	0

*According to WHO crieteria.

Table (6): The relation between the response to salvage treatment and different prognostic factors and chemotherapy regimen.

Parameter/response	CR N (%)	PR N (%)	NR N (%)	Total
Age:				
< 40 year	11 (37.9)	12 (41.4)	6 (20.7)	29
> 40 year	3 (42.8)	2 (28.6)	2 (28.6)	7
Gender:				
Males	9 (32)	12 (43)	7 (25)	28
Females	5 (62.5)	2 (25)	1 (12.5)	8
Stage:				
II	7 (44)	5 (31)	4 (25)	16
III	6 (40)	6 (40)	3 (20)	15
IV	1 (20)	3 (60)	1 (20)	5
Histopathology:				4
LP	3 (75)	1 (25)	-	
NS	1 (25)	2 (50)	1 (25)	4
MC	6 (31.6)	8 (42.1)	5 (26.3)	19
LD	2 (40)	1 (20)	2 (40)	5
Unknown	2 (50)	2 (50)	-	4
DFS1:				
Resistant or < 1y	8 (32)	10 (40)	7 (28)	25
> 1 year	6 (54.5)	4 (36.4)	1 (9.1)	11
B symptoms:				
Present	7 (29.6)	12 (44.5)	8 (25.9)	27
Absent	5 (55.5)	4 (44.5)	-	9
Pgp expression:				
Positive	8 (27.6)	13 (44.8)	8 (27.6)	29
Negative	5 (100)	-	-	5
Equivocal	1 (50)	1 (50)	-	2
Degree of expression:				
±	1 (50)	1 (50)	-	2
+	1 (20)	4 (80)	-	5
++	5 (38.5)	5 (38.5)	3 (23)	13
+++	2 (18)	4 (36.4)	5 (45.6)	11
Tumor size:				
Bulky	-	-	2 (100)	2
Non bulky	20 (59)	8 (24)	6 (17)	34

LP : Lymphocyte predominance, NS : Nodular sclerosis, MC: Mixed cellularity
LD: Lymphocyte depletion DFS1: First disease free survival.

Table (7): Correlation between Pgp expression and different clinical characteristics in 36 patients in the study.

Characteristics	Positive Pgp N (%)	Negative Pgp N (%)	Equivocal Pgp N (%)	Total
Stage: II	10 (62.5)	4 (25)	2 (12.5)	16
III	14 (93.3)	1 (0.7)	0	15
IV	5 (100)	0	0	5
Histopathology: LP	1 (25)	3 (75)		4
NS	4 (100)	0	0	4
MC	16 (84.2)	1 (5.3)	2 (10.5)	19
LD	4 (80)	1 (20)	0	5
Unknown	4 (100)	0	0	4
**DFS:* Resistant & < 1 year	22 (88)	1 (4)	2 (8)	25
More than 1 year	7 (63.3)	4 (36.7)	0	11
B symptoms: Positive	25 (92.6)	1 (3.7)	1 (3.7)	27
Negative	4 (44.4)	4 (44.4)	1 (11.2)	9
Tumor burden: Bulky	1 (50)	1 (50)	0	2
Non bulky	28 (82.3)	4 (11.8)	2 (5.9)	34
Status: Alive in remission	4 (50)	3 (37.5)	1 (12.5)	8
Alive active disease	13 (100)	0	0	13
Dead	4 (80)	1 (20)	0	5
Unknown	8 (70)	1 (20)	1 (10)	10

*DFS: Disease free survival.

Table (8): Survival probability in relation to different clinical parameters.

Parameter	Survival probability (%) at 12 months	*p* value*
Sex:		NS**
Males	87	
Females	80	
Age:		NS
<40 year	82	
>40 year	82	
B-symptoms:		NS
Positive	75	
Negative	100	
DFS1:		NS
<1year	83	
>1 year	85	
Pgp:		NS
Positive	79	
Negative	100	
Response:		<0.001
Complete response	100	
Partial response	87	
Non rsponders	45	

* *p* value was calculated using log rank test.
** NS: Not statistically significant.

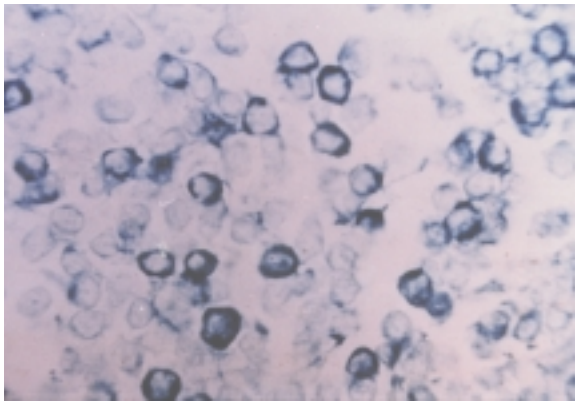

Fig. (1): Microscopic picture of lymph node showing positive Pgp expression.

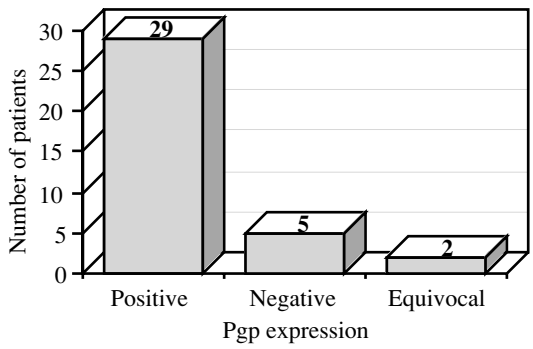

Fig. (2): P-glycoprotein (Pgp) at relapse in 36 patients.

DISCUSSION

The past 20 years have seen considerable progress in the treatment of a variety of malignancies. Despite these advances, the majority of patients with cancer relapse after primary treatment and become refractory to subsequent chemotherapy. The major cause of lack of response to subsequent treatment is the development of MDR. So far, three separate forms of MDR have been characterized in more details: classical MDR phenotype, atypical MDR and non-Pgp MDR. The classical MDR phenotype is caused by enhanced cellular drug efflux due to increased activity of a transmembrane-bound Pgp. P-glycoprotein is encoded by the so called mdr genes and in humans, two Pgp isoforms mdr1 and mdr3) have been identified, of which, in transfection experiments, only the mdr1 gene product (referred to as P-glycoprotein) confers drug resistance **[19]**.

A second form of pleiotropic drug resistance, atypical MDR, is associated with quantitative or qualitative alterations in topoisomerase II, nuclear enzyme that actively participates in the lethal action of cytotoxic drugs. Atypical MDR cells do not overexpress Pgp and are unaltered in their ability to accumulate drugs **[2]**. The third form of MDR (non-Pgp MDR) strongly resemble the classical MDR, as far as cross-resistance patterns are concerned, however, without expression of the Pgp molecule **[27]**. Of these cellular mechanisms of drug resistance the best understood and most intensively studied is the classical MDR. Enhanced drug efflux and reduced intracellular drug accumulation are characteristic of cells that overexpress Pgp **[22]**.

Clinical data indicate that classical MDR may be involved in the development of drug resistance, especially in some haematological malignancies, such as acute myelocytic leukaemia (AML), non-Hodgkin's lymphoma (NHL) and multiple myelomas (MM). Ma et al., 1987 were the first who reported P-glycoprotein expression in two adult patients with AML by immunocytochemical assay using the MoAb C219, developed by. Ling's group. P-glycoprotein staining could not be detected in leukemic cells at first clinical admission. However, as the patients relapsed after 3 or 4 courses of combined chemotherapy containing daunorubicin, C219 positive leukemic cells appeared in the peripheral blood. The proportion of positive staining

cells and the intensity of staining increased as the disease progressed [16]. The study of Ma et al., had been initiated by Bell et al., who had successfully applied the same MoAb earlier in an ovarian carcinoma study and ushered in a period of large scale screening for Pgp expression in all kinds of haematological malignancies [4]. Several studies reported that Pgp expression is a reliable marker of resistance in induction treatment, early death and shorter disease-free survival in patients with de novo AML and VAD-refractory multiple myeloma patients [21,23].

P-glycoprotein expression has been also demonstrated in malignant lymphomas. Miller et al. (1991) detected overexpression of Pgp in 2.4% of untreated patients with HD or NHL and in 64% of patients examined in relapse. Expression of mdr1 was found in 48% (11/23) of patients with recurrent lymphoma and there was a statistically significant difference in the response rates to salvage chemotherapy in patients with positive mdr1 expression compared with those who had negative mdr1 expression (30% vs 90%) ($p < 0.05$). The survival after recurrence curves significantly favoured mdr1 negative recurrent lymphoma [8]. Also, Yuen & Skic 1994 found that mdr1 expression was relatively low in untreated lymphoma patients (10%-20%) but increased in patients with recurrent disease (50-70%). These results are in agreement with our results where, Pgp immunostaining was found in only 10% of tissues at initial presentation in HD patients compared with 90% in affected tissues of these patients at relapse ($p = 0.01$). Also, patients with negative Pgp expression had a better overall response in comparison to those with positive Pgp expression (100% vs 72.4%).

Careful analysis of the literature on mdr1 expression in lymphomas shows the existence of disturbing discrepancies as regard its clinical value. Several studies reported little or no correlation between the mdr1 expression and either treatment intensity or response to therapy [11,18]. Contrary to our results, Sun and coworkers could not identify the clinical impact of Pgp expression in malignant lymphoma because no relation was observed between Pgp expression and prognosis (58.8% objective response in Pgp positive patients vs. 63% in Pgp negative patients). Moreover, their results confirmed the importance of p53 expression as a key prognostic factor and no objective response was found in patients with p53 positivity [24].

Development of resistance to chemotherapy is common during disease progression. Apart from selection of the MDR phenotype by repeated chemotherapeutic treatment, it remains possible that chemotherapeutic agents might themselves directly induce Pgp expression at the transcriptional level. Evidence is accumulating that, at least in vitro in rodent and human cell lines, the mdr1/P-glycoprotein promoter can be activated by chemical stress-inducing agents, including cytotoxic and cytostatic drugs, like vincristine, etoposide, daunorubicin, doxorubicin, colchicines and hydroxyurea [14]. Furthermore, Webb et al. 1998 reported that mdr1 is involved in drug resistance only in patients treated with Pgp transportable drugs. Increased mdr1 expression was detected in 79% of patients with CLL/NHL when compared with normal controls and the group of patients treated with high cumulative doses of Pgp transportable drugs had a higher frequency (100%) of increased mdr1 expression compared to the untreated or treated with non Pgp transportable drugs $p < 0.05$, ANOVA. Moreover, Chin et al. 1992, showed that the promoter of the human mdr1/P-glycoprotein gene can be activated by the ras-1 oncogene and the p53 tumor suppressor gene products. So, Pgp expression could be activated by chemotherapy and the frequent occurrence of mutations, during tumor progression, which is observed in many tumors including malignant lymphomas and Hodgkin's disease.

Relapsing HD patients with positive Pgp expression may have also one or more of poor prognostic factors. It has long been known that systemic symptoms are associated with more unfavourable prognosis [6,25]. Also, B - symptoms on relapse affect the outcome of salvage treatment and is considered to be poor prognostic factor for relapsing patients [15]. Patients with negative B symptoms on relapse in this study had better overall response to salvage treatment than those with positive B symptoms (100% vs 74.1%). Moreover, Pgp was more expressed in patients with B-symptoms (92.6% vs 44.4%).

Although, the overall response to salvage treatment were nearly similar between all stages, the CR rates after salvage treatment for

stage II (44%) and stage III (40) were better than for stage IV (20%). This is agreeable to the prognostic importance of extent of disease on relapse and its impact on the outcome of salvage treatment [13]. Also, in this study, there was a direct correlation between Pgp expression and stage of disease at relapse, where all patients with stage IV disease, 93.3% of stage III and 62.5% of stage II patients had positive Pgp expression. P-glycoprotein was also less expressed in patients with LP compared with other histological subtypes. Anastasi et al., 1989 reported that lymphocyte predominance has a better prognosis than nodular sclerosis which is better than mixed cellularity and lymphocyte depletion is the most aggressive subtype. In this study the overall response for salvage treatment for patients with LP was better than the other subtypes (100% vs 75%) and those with LD subtype had the worst response. This relation is agreeable to the good prognosis of LP and poor prognosis of the LD subtypes reported by other [6,13].

In this study, Pgp was more expressed in patients who were resistant to front line treatment or those with short primary disease-free interval. Patients with negative Pgp expression had a better overall response (100%) compared with those positive Pgp expression (72.4%) and that the density of Pgp expression was inversely related to the response rate. These results are in harmony with other studies that clearly indicated that Pgp expression and response to chemotherapy are independent variables and suggesting that Pgp may be a marker of cancer cell behaviour [11,18]. There is still a need for additional clinical trials that correlate the antitumor response with the presence of MDR at presentation and at relapse as well as a need to identify new drugs which inhibit MDR expression and to define their role and efficacy.

In survival analysis; patients with positive Pgp expression on relapse, age > 40 years, no response to salvage treatment had less survival probability than those with negative expression, age < 40 years and patients who were responding to salvage treatment. However, only the response status was statistically significant. Patients who were still alive and in remission after one year of follow-up had lower Pgp expression in their tissue of relapse (50%) than those who were either alive with active disease (100%) or dead (80%). This is agreeable to Bridget who reported short progression free survival and overall survival for patients with increased level of Pgp expression at relapse [5].

It was concluded from this study that Pgp expression is related to the tumour behaviour and could be a useful prognostic factor in addition to other factors in predicting the poor response to salvage therapy and short progression free survival in patients with resistant and relapsing HD.

Further studies with recruitment of a larger number are needed to further confirm our results before implementation of Pgp expression as a definite and useful prognostic parameter in relapsing HD patients. The ability to identify tumors with increased Pgp expression has several potential applications as the prediction of response to chemotherapy and the design of studies aimed at reversal of resistance with agents that inhibit MDR-mediated drug efflux. Recently, reversal of MDR by non-cytotoxic agents such as verapamil, cyclosporin A and PSC 833 (Valsopar) was explored in acute leukaemia and multiple myeloma. Preliminary results from clinical trials indicate that reversal of MDR is possible and that may lead to alterations of the plasma pharmacokinetics of the cytotoxic agents, in addition to P-glycoprotein inhibition in tumor cells [23].

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