The Frequency and Clinical Relevance of Multidrug Resistance Protein Expression in Patients with Lymphoma

Çoklu İlaç Direnci Protein İfadelerinin Lenfomalardaki Sıklık ve Klinik Önemi

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Abstract

Objective: Multidrug resistance is a cause of treatment failure in patients with malignant lymphoma; however, the frequency and clinical relevance of multidrug resistance protein expression are unclear. The present study aimed to investigate expression of the most common multidrug resistance proteins in a group of lymphoma patients.

Material and Methods: The study included 44 previously untreated lymphoma patients (non-Hodgkin's lymphoma [n = 21], non-malignant lymphadenopathy [n = 13], and Hodgkin’s lymphoma [n = 10]). MDR1, MRP, and LRP expression was assessed via quantitative PCR of lymph node biopsy specimens.

Results: In the non-Hodgkin's lymphoma group MDR1 was positive in 23.8% (5/21) of the patients, MRP was positive in 57.14% (12/21), and LRP was positive in 90.47% (19/21). In the non-malignant lymphadenopathy group, MDR1 was positive in 46.15% (6/13) of the patients, MRP was positive in 84.61% (11/13), and LRP was positive in 100% (13/13). In the Hodgkin's lymphoma group MDR1 was positive in 50% (5/10) of the patients, MRP was positive in 50% (5/10), and LRP was positive in 80% (8/10). MDR1, MRP, and LRP expression did not differ between the 3 groups. Furthermore, MDR1, MRP, and LRP expression wasn’t associated with tumor stage, response to first-line therapy, the erythrocyte sedimentation rate, or C reactive protein, beta 2 microglobulin, serum lactate dehydrogenase, and albumin levels. Additionally, survival time in the MDR1- and MRP-positive, and MDR1- and MRP-negative patients did not differ (comparison of LRP was not possible due to the small number of LRP-negative patients).

Conclusion: According to the present findings, future studies should investigate alternative pathways of multidrug resistance in order to arrive at a better understanding of treatment failure in lymphoma patients.

Key Words: Multidrug resistance, non-Hodgkin’s lymphoma, Hodgkin’s lymphoma, Survival
Introduction

Multidrug resistance (MDR) refers to the resistance of tumor cells to chemotherapeutic agents of varying chemical structure and mechanisms of action [1]. Numerous mechanisms are involved in drug resistance; among them, drug efflux transporters are one of the most intensively studied and most prevalent [2,3]. Common MDR proteins include permeability related glycoprotein (P-gp), multidrug resistance-associated protein (MRP), and lung resistance-related protein (LRP) [4,5]. P-gp, also referred to as P-170, is a product of the MDR1 gene and is an ATP-dependent pump capable of expelling drugs from cancer cells [6]. MRP is structurally similar to P-gp and is a member of the same transmembrane transporter superfamily [7]. LRP is a 110-kDa protein identified in a P-gp-negative MDR lung cancer cell line and functions as a major vault protein in humans [8].

Overexpression of MDR increases efflux of most lymphoma regimens from cells [9-11]. The predictive and prognostic value of MDR expression has previously been reported for multiple myeloma (MM) [12], acute myeloid leukemia (AML) [13], acute lymphoblastic leukemia (ALL) [14], and adult T-cell leukemia [15]. The MDR phenotype is also the major cause of treatment failure in patients with malignant lymphoma; however, findings regarding expression of the MDR1 gene/P-gp in malignant lymphoma patients are inconsistent [16-18].

Due to the non-clarity surrounding the frequency and clinical relevance of multidrug resistance protein expression, the present study aimed to investigate the expression of the most common MDR proteins in a group of previously untreated patients with lymphoma—specifically, whether or not these 3 multidrug resistance proteins were expressed and their impact on clinical outcome.

Materials and Methods

The study included 44 previously untreated patients that were diagnosed between 2005 and 2007. The patients were divided into 3 groups, according to pathology results. Group 1 included 21 patients diagnosed a diffuse large B-cell lymphoma (DLBCL) (n = 9), T-cell non-Hodgkin's lymphoma (NHL) (n = 8), and mantle cell lymphoma (MCL) (n = 4). Group 2 included 13 patients diagnosed as reactive lymphadenopathy (LAP) (n = 5), granulomatous inflammation (n = 5), dermatopathic LAP (n = 1), benign mixed tumor (n = 1) and Kikuchi's disease (n = 1). Group 3 included 10 patients diagnosed as Hodgkin's lymphoma (HL).

Survival time was defined as the period (months) from diagnosis to death or data analysis. MDR1, MRP and LRP expression was assessed via quantitative PCR of lymph node biopsy specimens. Only biopsy specimens clearly proven to be malignant based on flow cytometric and pathological analysis were included in the study. Peripheral blood and lymph node biopsy specimens were obtained following provision of informed consent by the patients and approval of the study protocol by the Eskişehir Osmangazi University, School of Medicine Institutional Review Board.
The patients were treated with CVP, CHOP, Hyper-CVAD, IMVP16, DHAP, ESHAP, ABVD, and BEACOPP polychemotherapy. Patients with DLBCL and MCL also received rituximab. In all, 4 patients required radiotherapy and 4 other patients received additional peripheral stem cell transplantation (3 autologous and 1 allogeneic with reduced intensity conditioning). Response to chemotherapy was assessed according to standard criteria [19]. Tumor stage, the erythrocyte sedimentation rate, and C reactive protein, beta 2 microglobulin, serum LDH, and albumin levels were also recorded.

Detection of multidrug resistance

Lymph node biopsy specimens obtained in the surgical suite were immediately taken to the laboratory in Eppendorf tubes containing RPMI1640 medium. The specimens were preserved in liquid nitrogen at −80 °C and thawed at 4 °C before RNA isolation. An mRNA Isolation Kit II (Tissue) (Roche) was used to obtain RNA from the tissue samples using a MagNA Pure instrument. cDNA was obtained using a Transcriptor First Strand cDNA Synthesis Kit (Roche). MDR1, MRP, LRP, and beta actin primary probes from TIB MOLBIOL and Light Cycler Taqman Master reaction mix were used for quantitative PCR in a Roche Light Cycler instrument. As beta actin is present in all clinical samples, it was used as an intrinsic control. Quantitative MDR1, MRP, and LRP values were divided by the beta actin value, and the positive results were used for statistical analysis. We did not set a cut-off value and considered any level of expression as positive.

Statistical analysis

All statistical analyses were performed using SPSS (PASW) v.18.0 for Windows software. Distribution of the variables was determined using the Shapiro Wilks test. Parametric tests were used to analyze normally distributed data and non-parametric tests were used for data not normally distributed. The chi-square test was used for analysis of cross tables. Correlations between variables were determined by calculating Spearman’s correlation coefficients. The independent samples t-test and ANOVA were used to compare group means of normally distributed variables. Tukey’s post hoc test was used to determine different groups in ANOVA. The Kaplan Meier test was used to compare survival time between ≥2 groups. The log rank test was used to determine differences between mean survival times. P < 0.05 was considered statistically significant. Data are expressed as mean ± standard deviation (SD).

Results

Mean age was 53.2 ± 17.5 years in the NHL patients (group 1), 41.7 ± 15.1 years in the non-malignant LAP patients (group 2), and 37.6 ± 13.7 years in the HL patients (group 3). NHL patients were older than HL patients (P < 0.05); the difference was not significant. Mean survival time was 19.69 ± 4.33 months in group 1, 42.46 ± 5.85 months in group 2, and 49.50 ± 4.69 months in group 3. NHL patients had shorter survival than HL patients (P < 0.01), but survival time did not differ between the reactive LAP and lymphoma patients (P > 0.05) (Figure).

MDR1, MRP, and LRP positivity in the 3 groups is shown in Table 1. The frequency of MDR1, MRP, and LRP positivity was significantly different between the 3 groups (P > 0.05). While examining survival time in each group separately, comparison was not possible in some groups due to the small number of negative patients (MRP
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and LRP in group 2, and LRP in groups 1 and 3). When the other groups were compared (MDR1 and MRP in group 1 and 3, and MDR1 in group 2) a significant difference in survival time between the MDR1- and MRP-positive, and the MDR1- and MRP-negative patients was not observed. In all, 5 patients in group 1 were positive for all 3 MDR proteins, 7 were positive for MRP and LRP, and 7 were positive only for LRP. In total, 6 patients in group 2 were positive for all 3 MDR proteins, 5 were positive for MRP and LRP, and 2 were positive only for LRP. Positivity for all 3 MDR proteins was observed in 3 patients in group 3 patients, whereas 1 patient was positive for MRP and LRP, 1 patient was positive only for MDR1, and 3 patients were positive only for LRP. There weren't any significant associations between MDR1, MRP, and LRP expression, and tumor stage, response to first-line therapy, the erythrocyte sedimentation rate, or C reactive protein, beta 2 micro-globulin, serum LDH and serum albumin levels.

Discussion

In the present study expression of MDR1, MRP, and LRP was determined via quantitative real-time PCR (RT-PCR) in patients with lymphoma and non-malignant diseases, and the association between the expression of the 3 MDR proteins, and clinical and laboratory parameters was evaluated. To the best of our knowledge this is the first study to evaluate the frequency and clinical relevance of MDR proteins in patients with malignant lymphomas (both Hodgkin's and non-Hodgkin's) and non-malignant diseases. In contrast to AML, studies on lymphomas and MDR are few in number and have generally focused on P-gp expression, and most included a heterogeneous patient population. Furthermore, the methods used to determine MDR expression in such studies varied widely [20]. As such, comparison of the published data is difficult. Moreover, these studies included a small number of patients, as did the present study, and therefore definitive conclusions cannot be reached.

MDR1/P-gp expression in lymphomas has been previously examined. Liu et al. [21] reported that 41.7% of 24 B-cell lymphoma patients were MDR1/P-gp-positive (based on RT-PCR) before treatment. Pileri et al. [22] reported that 44% of peripheral T-cell lymphoma patients and 40% of B-cell lymphoma patients were P-gp-positive before treatment. Findings regarding the clinical importance of MDR1/P-gp in lymphomas are inconsistent. MDR1/P-gp was reported to be predictive of a poor response to induction chemotherapy in 2 studies [22,23], but not in 2 other studies [24,25]. Overall, research shows that 2%-30% of lymphomas express P-gp immunohistochemically [22-24,26], increasing to 22%-50% when RNA-based analysis methods for detecting P-gp/MDR1 are used [24,25,27]. MDR1 expression in the present study was observed in 23.8% of non-Hodgkin's lymphoma patients and in 50% of Hodgkin's lymphoma patients; these percentages are similar to those previously reported. The present study's findings are also in agreement with studies that did observe a relationship between MDR1 expression [24,25] and poor response to induction chemotherapy.

Filipits et al. [28] reported that LRP was positive in 23% and MRP1 was positive in 44% of newly diagnosed DLBCL patients. LRP expression was associated with poor response to chemotherapy and shorter survival, which suggests that LRP is a clinically relevant drug resistance factor in DLBCL. A similar predictive and prognostic value of LRP expression was reported in patients with AML [15,29], ALL [30], MM [12], and advanced ovarian cancer [11]. Ohsawa et al. [31] reported that MRP1 was positive in 63% and LRP was positive in 68% of patients with nodal DLBCL. Huang et al. [32] observed MRP and LRP expression rates of 20.5% and 12.5%, respectively, in nasal NK/T-cell lymphoma patients. It is likely that the expression of MDR varies according to lymphoma subtype. In the present study expression of MRP and LRP was observed in 57.14% and 90.47% of NHL patients, respectively (LRP expression was higher than previously reported in Filipits [28], Oshawa [31] and Huang's [32] studies), versus MRP and LRP in 50% of HL patients, respectively.

MRP expression was not observed to play an important role in the mechanism of drug resistance associated with a poor clinical outcome in previously untreated NHL patients [33]. Filipits et al. [28] reported that MRP1 expression had no impact on the outcome of chemotherapy or survival in patients with DLBCL, and similar findings have been observed in refractory lymphoma patients in whom MRP expression was not determined via quantitative PCR [34]. Previous studies on AML [14,29], ALL [30], and advanced ovarian cancer [11] also failed to observe any predictive or prognostic significance of MRP1 expression. Additionally, in the present study MRP and LRP expressions had no correlation with response to induction chemotherapy or survival in lymphoma patients with mixed histopathology.

In the present study there weren’t any significant correlations between MDR1, MRP, and LRP expression, and tumor stage, response to first-line therapy, the erythrocyte sedimentation rate, or C reactive protein, beta 2 micro-globulin, and serum LDH and albumin levels in the NHL and HL patients. Mixed histopathology and differences in
treatment between the patients make it impossible to correlate the findings with clinical outcome, which is a limitation of the present study that warrants additional research. Anti-CD20 monoclonal antibody (rituximab) has become the standard therapy for aggressive NHL and has dramatically improved treatment outcome [35]. Recent laboratory-based evidence shows that rituximab interacts with P-gp [36]; unfortunately, due to mixed histopathology and the small number of patients (n = 13) in the present study, we were unable to examine that interaction.

Non-malignant tissues expressed MDR proteins in the present study and the frequency of expression was similar in all the lymphoma patients. In fact, normal lymphocytes were reported to express MDRI/P-gp [37], but Kang et al. [9] posited that contamination of tumor samples with T-lymphocytes and monocytes does not significantly increase the level of MDRI expression. The similarity of MDR expression between lymphoma and reactive LAP patients observed in the present study may have been due to lack of a cut-off value and considering any level of expression as positive, or that 3 patients previously reported as reactive LAP were diagnosed as NHL following rebiopsies performed during follow-up.

In conclusion, MDR1, MRP, and LRP expression was not observed to influence overall survival in NHL and HL patients. The present findings indicate that additional studies should examine alternative pathways of MDR other than MDRI/P-gp, MRP and LRP expression (apoptosis etc.) to elucidate treatment failure in this group of patients. Furthermore, the frequencies of expression observed in the present study are noteworthy, as to the best of our knowledge this is the first study to report and compare these frequencies in HL patients and non-malignant patients.

Conflict of Interest Statement

The authors of this paper have no conflicts of interest, including specific financial interests, relationships, and/or affiliations relevant to the subject matter or materials included.

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