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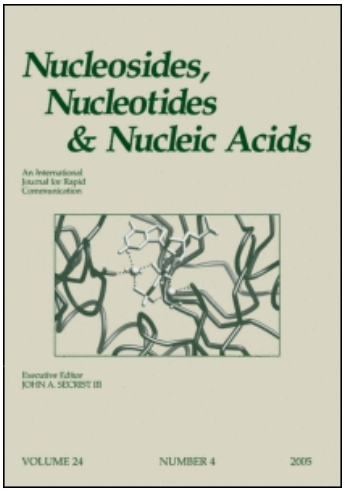

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Expression of the *MDR1* and *MRP* Genes in Patients with Lymphoma with Primary Bone Marrow Involvement

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Expression of the *MDR1* and *MRP* Genes in Patients with Lymphoma with Primary Bone Marrow Involvement

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ABSTRACT

Expression of *MDR1* and *MRP* genes in patients with low-grade and high-grade non-Hodgkin's lymphomas with primary bone marrow involvement before and after chemotherapy was investigated. The data demonstrate that overexpression of *MDR1* and *MRP* genes in hematological malignancies elevates in patients after chemotherapy and correlates with poor clinic prognosis and more frequent recurrences of the malignancies.

Key Words: Multi-drug resistance; Lymphoma; Chemotherapy.

INTRODUCTION

The standard treatment schemes of malignant hematological diseases evoke good response in the majority of the patients. However, in many cases the remission is short and it is frequently followed by a relapse of the disease because tumor cells became resistant to anticancer drugs.[1,2] The multi-drug resistance (MDR) phenotype is characterized by resistance of tumor cells to a variety of structurally unrelated cytotoxic

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drugs and correlates with some adverse prognostic variables such as disease progression and failure of cancer chemotherapy.[3]

In more than 90% cases multi-drug resistance occurs due to overexpression of the P-glycoprotein encoded by the *MDR1* gene. P-glycoprotein functions as transmembrane ATP-dependent pump, removing different toxic substances from the cell. The second important type of multi-drug resistance is associated with overexpression of multi-drug resistance associated protein (MRP), which acts similar to P-glycoprotein.[4]

Patients with low-grade non-Hodgkin lymphomas (NHL) generally experience a slowly progressive time course and may benefit from treatment with cytotoxic drugs. Patients with high-grade NHL usually show fast progression of the disease and require more aggressive therapy.[5,6]

The role of *MDR1* and *MRP* gene expression in development of multi-drug resistance syndrome in patients with non-Hodgkin lymphoma is still unclear. However it is demonstrated, that P-glycoprotein (P-gp)-positive patients may have a poor prognosis compared to P-gp-negative patients.[7] The aim of the present study was to determine the level of expression of the *MDR1* and *MRP* genes expression in patients with non-Hodgkin lymphomas before and after chemotherapy.

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MATERIALS AND METHODS

62 patients with NHL were admitted to the study. 29 of the patients were suffering from low-grade lymphomas and 33 from high-grade lymphomas. 24 patients were newly-diagnosed and the others had already received chemotherapy.

Patients involved in the study had the leukocyte count $> 12 \times 10^9/1$. Therefore contamination with non-malignant lymphocytes was minimal. Blood samples from patients with NHL were collected in tubes with 1 M sodium citrate, pH 7.0. Within a

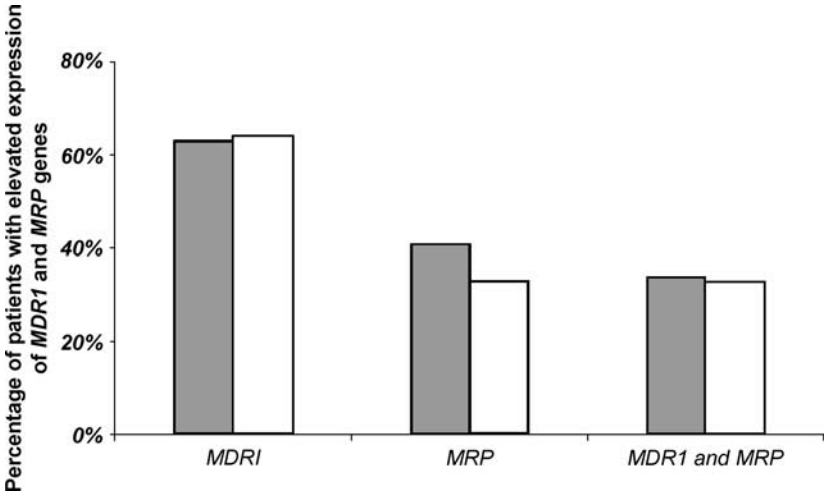

Figure 1. The frequency of elevated expression of *MDR1* and *MRP* genes in patients with high-grade and low-grade NHL. Black bars – low-grade lymphomas; open bars – high-grade lymphomas.

few hours of sampling, the mononuclear cells were separated by Ficol–paque (Pharmacia, Sweden) centrifugation at 480 g for 30 min at room temperature as recommended by the manufacture. The mononuclear cells present in the interphase were recovered and washed twice with phosphate buffer saline PBS.[8,9] Total RNA was isolated from the mononuclear cells by phenol-SDS method and levels of *MDR1* and *MRP* genes expression was assayed by reverse transcription–polymerase chain reaction method using the expression level of the gene encoding ribosomal protein L30 (RPL) as internal standard. The following primers were used: *MDR1* sense 5′-CCC ATC ATT GCA ATA GCA GG 3′, *MDR1* antisense 5′-GTT CAA ACT TCT GCT CCT GA 3′, (product length 157 base pairs), *MRP* sense 5′-GGA AAC CAT CCA CGA CCC TAA TCC CT 3′, *MRP* antisense 5′-CCA CCT CCT CAT TCG CAT CCA CCT TG 3′ (product length 296 base pairs), *RPL30* sense 5′-ATG GTG GCT GCA AAG AAG AC 3′, *RPL30* antisense 5′-GTT TAC TTC TCA CCA GTC TG 3′, (product length 350 base pairs).

Cellular suspensions from 19 blood donors were used to analyze *MDR1* and *MRP* expression in normal non-malignant lymphocytes.

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RESULTS AND DISCUSSION

To determine the frequency of *MDR1* and *MRP* elevated expressions we used the expression levels in donors as a control. Thus, according to the fact that even in control group we detected expression of both genes, the overexpression was standardized as two-time increase in comparison to the average level of the control group.

Enhanced expression of *MDR1* and *MRP* genes was detected in 64% and 33% of the samples from patients with high-grade lymphoma respectively. In 33% of the samples enhanced expression of both genes was observed. 63% of the samples from

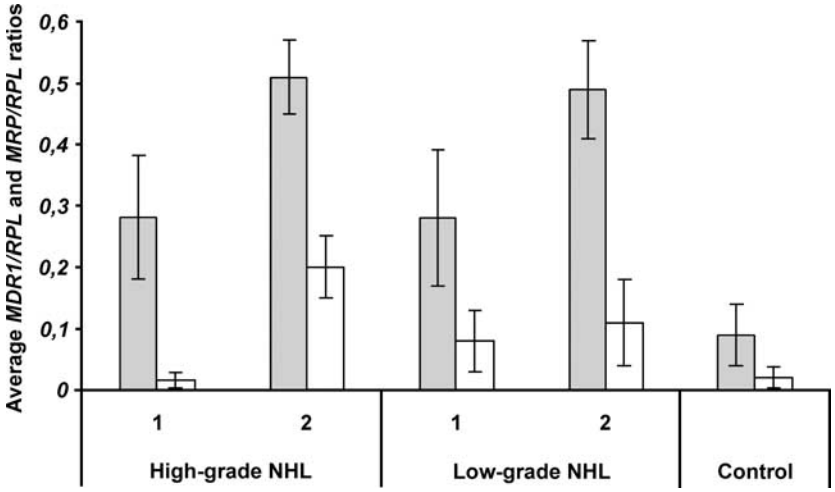

Figure 2. Comparison of the *MDR1/RPL* (grey bars) and *MRP/RPL* (open bars) ratios between newly-diagnosed patients (1), patients after chemotherapy (2), and control group (3).

patients with low-grade lymphoma demonstrated high levels of *MDR1* gene expression and 41% of samples had elevated *MRP* gene expression. Enhanced expression of both genes was detected in 34% patients (Fig. 1).

The average *MDR1/RPL* ratio was 0.28 in newly-diagnosed patients with high-grade NHL and 0.51 in the pretreated patients. Average *MRP/RPL* ratio was 0.015 in newly-diagnosed and 0.2 in pretreated patients with high-grade NHL respectively. *MDR1/RPL* and *MRP/RPL* average ratio in newly-diagnosed patients with low-grade NHL were 0.28 and 0.02 respectively, while in pretreated patients with low-grade NHL *MDR1/RPL* ratio was 0.49 and *MRP/RPL* ratio was 0.11. In the control group *MDR1/RPL* ratio was found to be 0.09 and *MRP/RPL* ratio was 0.02 (Fig. 2).

All patients were divided, according to the clinical data, into group with good response to therapy 14 patients with high-grade NHL and 13 patients with low-grade NHL respectively, and group with treatment failure 19 patients with high-grade NHL and 16 patients with low-grade NHL respectively. In patients with high-grade lymphoma, *MDR1/RPL* and *MRP/RPL* average ratios were 0.27 and 0.02 in group with good response to therapy and 0.57 and 0.23 in group with treatment failure. In patients with low-grade NHL, *MDR1/RPL* and *MRP/RPL* average ratios were 0.28 and 0.07 in group with good response to therapy and 0.61 and 0.14 in group with treatment failure (Fig. 3).

The data demonstrate that the post-chemotherapy patients show 2-fold and higher increase of expression of the *MDR1* and *MRP* genes as compared to patients who never received chemotherapy. Overexpression of these genes in patients with high-grade and low-grade non-Hodgkin lymphoma correlates with poor clinic prognosis. The correlations were more evident in the study of therapy response than between untreated and pretreated patients. Therefore evaluation of the expression of the *MDR1*

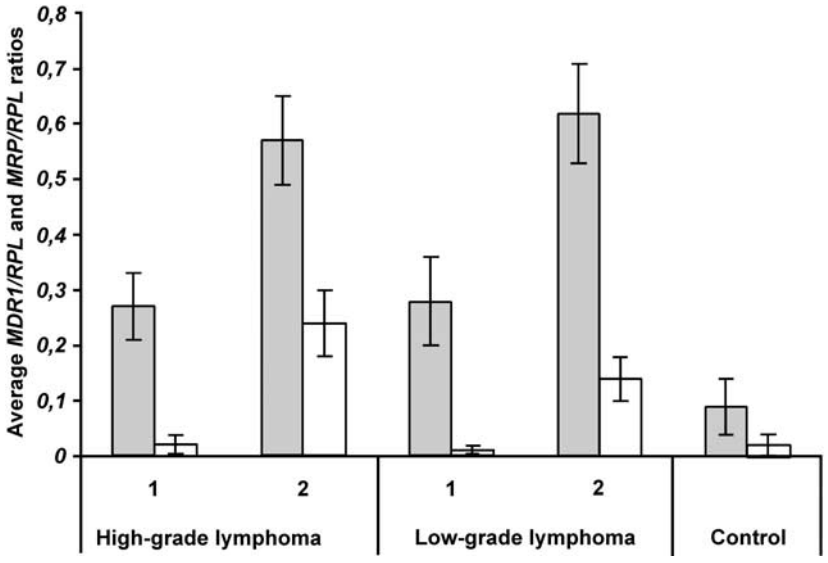

Figure 3. Comparison of the *MDR1/RPL* (grey bars) and *MRP/RPL* (open bars) ratios between patients with good response to therapy (1), patients with treatment failure (2) and control group.

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and *MRP* genes in patients with lymphoma can be used as a prognostic method for administration more aggressive protocols of chemotherapy to those patients with increased levels of *MDR1* and/or *MRP* genes.

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