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Plasma phospholipid changes are associated with response to chemotherapy in non-Hodgkin lymphoma patients

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ABSTRACT

Limited studies have been performed to associate abnormal phospholipid (PL) profile and disease activity in hematological malignancies, including non-Hodgkin lymphoma (NHL). The aim of his study was to evaluate the levels of plasma PL fractions in NHL patients, in response to chemotherapy. Forty non-treated patients with NHL and 25 healthy individuals were recruited. Blood samples from patients were taken before chemotherapy, after 3 cycles and after the end of the treatment, and PL fractions were resolved by one-dimensional thin-layer chromatography. To assess potential relationship between plasma PL profile and response to therapy, patients were divided according to clinical outcome in 3 groups: complete remission (CR), stable disease (SD) and progression (PG). Despite significant differences between NHL patients and healthy controls, no differences were found at baseline among patients divided according to clinical outcome. During and after chemotherapy important alterations in PL profile were observed. Levels of total PLs and all PL fractions decreased in patients with PG while in patients who responded to therapy (CR, SD) PLs significantly increased. Results of our study suggest that changes of total PLs and PL fractions during the therapy are associated with the effects of therapy and clinical outcome in patients with NHL.

1. Introduction

Lipids are a diverse class of molecules with the essential role in cellular structure, energy storage and signaling. *De novo* biosynthesis of fatty acids (FA) and cholesterol is restricted to liver, adipose and lactating breast tissue, while other tissues uptake lipids from the bloodstream. Development and progression of most tumors is accompanied not only by *de novo* synthesis of FA and cholesterol, but also by an elevated uptake of dietary lipids from the circulation. As a consequence, tumor affects systemic lipid homeostasis, which then reflects as the change in lipid profile of body fluids [1]. Phospholipids (PLs) are key components of cellular membranes and important bioactive molecules. Characteristics of tumor tissue compared with normal tissue are elevated levels of cell membrane phosphatidylcholine (PC) and phosphatidyletanolamine (PE). Increased FA saturation of PLs in cancer cell membranes affects their fluidity and signal transduction, which alters the resistence of cancer cells to chemotherapy and their anti-oxidant capacity [1].

It is known that metabolism of lipids is frequently altered in various diseases, but recent evidence indicates that lipid-related genes may link cancer with inflammatory and metabolic diseases [2]. Lipidomic studies have demonstrated that a cancer risk [3], existence [4] and progression [5] may be correlated with altered levels of particular tissue and plasma PL and fatty acids. The most recent data from a large cohort study found an association between the risk of three frequent type of cancer and plasma levels of LPC (C18:0) and PC (C30:0) [3]. Differences in expression pattern of tissue/plasma PL level showed good prognostic performance in distinguishing breast cancer from benign tumors as well as uterine fibroids from cervical cancer [4].

Non-Hodgkin lymphomas (NHL), a heterogeneous group of hematologic malignancies, has been rapidly increasing over the past few decades. Etiology is still unclear although some autoim-

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mune disorders, infectious agents and lifestyle factors including diet, appear to play a role in pathogenesis of NHL [6]. Identification of altered PL in human lymphoma and animal models revealed the link between lipid signature and specific oncogenes, such as v-mic avian myelocytomatosis viral oncogene (MYC) [7]. Survival rate of the most common subtypes of NHL, follicular lymphoma and diffuse large B-cell lymphoma, have been improved in European region as a result of early diagnosis and advances in treatment [8].

We have previously reported significantly lower levels of total cholesterol, HDL-cholesterol and total PL in serum of patients with NHL [9]. Moreover, altered plasma FA profile in NHL patients was linked to the clinical stage and aggressiveness of the disease [10], as well as to response to chemotherapy and clinical outcome [11]. Nevertheless, PL fractions and their possible relation to clinical outcome in NHL patients have not been investigated so far. Thus, the aim of the present study was to determine plasma PL profiles in non-treated NHL patients and to follow the changes during the therapy, in order to resolve potential associations between PL profile and clinical outcome/response to therapy in NHL patients.

2. Material and methods

2.1. Study design and patients

The study included 40 adult patients (21 male and 19 female) with a histologically confirmed NHL, aged 19–75 years, median 56 years. The patients were recruited from the Department of Hematology Clinical Hospital Center Zemun, Belgrade, from March 2011 to December 2013. All of these patients had no other malignant disease or a serious chronic disease and did not receive any therapy, which could affect lipid metabolism in the 3 months prior to entering the study. None of them had any indications of cachexia. Control group consisted of 25 healthy adult individuals (11 male and 14 female), median 55 years.

Histological diagnosis of NHL was made according to the Revised European-American Lymphoma Classification/World Health Organization Classification [12] after lymph node biopsy or biopsy of the primary extranodal site.

Clinical staging was determined by the criteria of the Ann Arbor Conference [13]: clinical stage (CS) I – 4 patients, CS II —11 patients, CS III—11 patients and CS IV —14 patients. Follow-up time ranged from 1.5-3.5 years. According to NHL histology [14], patients were divided into three groups: group I – the patients with indolent *i.e.* low risk NHL (n = 15), group A – the patients with aggressive *i.e.* intermediate risk NHL (n = 17) and group VA – a very aggressive disease *i.e.* high risk NHL (n = 8). Three patients dropped out during the study due to the chemotherapy intolerance or death.

Most of the patients (n = 23) were treated with CHOP (cyclophosphamide, doxorubicin, vincristine and prednisone), 7 with other anthracycline-containing regimens, 4 patients with CVP (cyclophosphamide, vincristine and prednisone) and 6 with fludarabine-based regimens. Rituximab was added in 30 patients. Clinical outcome, determined as complete remission (CR), stable disease (SD) or progression of the disease (PG) were assessed 3 weeks after completed chemotherapy.

All study participants provided written informed consent, which was approved by the Ethical Review Boards of the participating institutions in accordance with the principles of the Declaration of Helsinki.

2.2. Analytical methods

Venous blood samples were drawn after an overnight fast, prior to starting chemotherapy (baseline), after third cycle of chemotherapy regimen (middle) and after the completion of therapy (end). Serum triglyceride, total cholesterol and HDL cholesterol concentrations were assayed by the automated enzymatic methods (Roche, Basel, Switzerland). LDL cholesterol was estimated using the Friedewald formula [15]. The total PL concentration was determined by the Zilversmit method [16]. Plasma lipids were extracted with a chloroform–methanol mixture (2:1 v/v) as we previously described [17]. The PL fraction was isolated from the extracted lipids by one-dimensional thin-layer chromatography in a neutral solvent system (petrol ether –diethyl ether – acetic acid; 87:12:1 v/v/v) on Silica Gel GF plates (Merck, Darmstadt, Germany). Four fractions of PL were detected in plasma: lysophosphatidylcholine (LPC), sphingomyelin (SM), phosphatidylcholine (PC) and phosphatidylethanolamine (PE).

2.3. Statistical analysis

All the results are expressed as mean ± SD. Normality was tested using the Kolmogorov-Smirnov test. Since all variables showed normal distribution, one-way ANOVA, followed by the Tukey post *hoc* test, and the Student *t*-test for the comparisons between two groups were used. In addition, paired Student *t*-test was performed to follow the time course changes of the parameters in the same patient at the beginning, in the middle and after the chemotherapy. The level of significance was set at $p \leq 0.05$.

3. Results

Blood lipid and PL profile of patients with NHL, before, in the middle and after chemotherapy are presented in Table 1. Untreated NHL patients have significantly lower concentrations of plasma lipid and PL fractions, when compared with healthy individuals, with an exception of triglycerides which was similar in both groups. During and after chemotherapy regimen, all measured lipids gradually increased in NHL patients, but remained significantly lower when compared with the control group, except for HDL-C level which almost achieved value of the controls.

When the patients were divided according to aggressiveness of NHL into indolent (I), aggressive (A), and very aggressive (VA) NHL, we found a significantly lower concentration of total PL, PC and LPC in the A ($p < 0.05$) and VA group ($p < 0.01$) when compared with the indolent group at baseline (Fig. 1). In all 3 groups, level of total PLs and most of PL fractions showed an increasing trend during and after the end of chemotherapy, but these changes were nonsignificant. Thus, the significant differences between the groups A and I were lost after the 3 cycles of chemotherapy, and after completion of the therapy between the VA and I group. Furthermore, after receiving the complete therapy, only the patients with indolent NHL reached the level of all PL comparable to the healthy subjects.

Patients were also grouped according to clinical stage of NHL (CS I–IV). There were no significant differences between the groups at baseline, in spite of a decreasing trend of concentrations of all PLs from CS I to CS IV (Fig. 2). Interestingly, the treatment led to significant differences of all PLs between CS II and CS IV, as well as of total PL, LPC and SM between CS III and CS IV, while no differences were found between CS I and CS IV.

However, the most notably differences were found when the patients were divided according to the response to therapy in 3 groups: complete remission (CR), stable disease (SD) and progression (PG). As it can be seen in Fig. 3, the patients in CR and SD showed increasing trend in PL profiles in response to chemotherapy, while the PG group showed the opposite trend. Patients in the CR group showed a marked elevation of all parameters except PE, attaining the PL profiles statistically equivalent with those from healthy subjects. On the contrary, PG group displayed a gradual decrease of all PLs, except PE, at the end of chemotherapy. Calculated PC/LPC ratio,