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Plasma and ovarian tissue sphingolipids profiling in patients with advanced ovarian cancer

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HIGHLIGHTS

- Evaluation of selected sphingolipids in patients with advanced ovarian cancer
- Plasma C16-Cer,C18:1-Cer,C18-Cer concentration elevated in study group vs. control
- Increase concentration of 5 ceramide, S1P in ovarian tissue vs. control group
- Some sphingolipids can be used as potential biomarkers of ovarian cancer

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ABSTRACT

Purpose. The role of lipids in carcinogenesis through induction of abnormal cell lines in the human body is currently undisputable. Based on the literature, bioactive sphingolipids play an essential role in the development and progression of cancer and are involved in the metastatic process. The aim of this study was to determine the concentration of selected sphingolipids in patients with advanced ovarian cancer (AOC, FIGO III/IV, high grade ovarian cancer).

Methods. Seventy-four patients with ovarian cancer were enrolled. Plasma concentrations of C16-Cer, C18:1-Cer and C18-Cer were assessed by LC/MS/MS. The content of tissue sphingolipids was measured using a UHPLC/MS/MS.

Results. Plasma concentration of 3 ceramides: C16-Cer, C18:1-Cer and C18-Cer was significantly elevated in women with advanced ovarian cancer compared to control group ($P = 0.031$; 0.022; 0.020; respectively). There were increases in concentration of 5 ceramides: C16-Cer, C18:1-Cer, C18-Cer, C24:1-Cer, C24-Cer ($P = 0.025$; 0.049; 0.032; 0.005; 0.013, respectively) and S1P ($P = 0.004$) in ovarian tissue of women with advanced ovarian cancer compared to healthy individuals. Importantly, significantly higher risk of ovarian cancer when the plasma concentration of C16-Cer > 311.88 ng/100 μl (AUC: 0.76, $P = 0.0261$); C18:1-Cer > 4.75 ng/100 μl (AUC: 0.77, $P = 0.0160$) and C18-Cer > 100.76 ng/100 μl (AUC:0.77, $P = 0.0136$) was noticed.

Conclusions. Bioactive sphingolipids play an essential role in the development and progression of cancer and they also take part in the process of metastasizing. This study suggests that some sphingolipids can be used as potential biomarkers of advanced ovarian cancer and that they can play an important role in the pathogenesis of this disease.

1. Introduction

Ovarian cancer is the fifth most common cancer in European women. Around the world, more than 200,000 women are estimated to develop ovarian cancer every year, and about 100,000 die of the disease [1].

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Aggressive biology of ovarian cancer is strictly correlated with its histology. It is worth underlining that heterogeneity of observed ovarian cancer subtypes determining as far there are no screening tests for early diagnosis of this type of neoplasm and ovarian cancer is still routinely diagnosed in the advanced stages of that disease (manly FIGO stage III, IV) [2,3].

The role of lipids in carcinogenesis through induction of abnormal cell lines in the human body is currently undisputable. Based on the literature, bioactive sphingolipids play an essential role in the development and progression of cancer and are involved in the metastatic process [4,5]. Ceramides are some of the most important elements in the metabolism of sphingolipids. They can be either synthesized de novo from serine and palmitoyl-CoA in a reaction catalyzed by serine palmitoyltransferase (SPT) or produced through enzymatic hydrolysis of sphingomyelin by sphingomyelinase. Apart from ceramides, biologically active sphingolipids include: dihydroceramide, sphingosine-1-phsophate (S1P), sphingosine and sphinganine. Based on recent research, ceramides have pro-apoptotic properties and constitute an important antineoplastic factor (lipid tumor suppressors). S1P acts antagonistically to ceramides, as it induces cell transformation, cancer cell proliferation, cell survival and blocks the anti-apoptotic mechanisms of ceramides. Moreover, an increase in the expression of sphingosine kinase (SPHK1), an enzyme that transforms sphingosine to S1P, causes accumulation of S1P in the cell, promoting carcinogenesis and tumor formation [6–8].

Despite the important role of sphingolipids in cancer biology, their metabolism in different malignant tumors is poorly investigated. Some studies showed marked differences in ceramide content between tumor and respective healthy tissue [9–11]. Interestingly, the level of this sphingolipid was either decreased or increased, suggesting that alterations in ceramide metabolism in cancer tissue may depend on a type of tumor [6,7,10,12]. However, these studies focused only on selected sphingolipid species, and to date there are no complex data on ceramide metabolism in cancer tissue. In addition, sphingolipid metabolism in ovarian cancer has been still poorly investigated. Therefore, measurement of the sphingolipids could lead to better understanding of the influence of ovarian cancer biology and possibly provide new biomarker(s) for non-invasive testing.

2. Material and methods

The investigation conforms with the principles outlined in the Declaration of Helsinki and was approved by the Ethical Committee for Human Studies of the Medical University of Bialystok, Poland (recruitment between January 2015 till June 2016). All patients gave their informed consent prior to their inclusion in the study. The initial study included two groups of women: patients with suspicious of advanced ovarian cancer (AOC) (n = 165) and women with normal ovarian morphology confirmed by transvaginal sonography evaluation (control group, n = 149). The patients suspected to have an advanced ovarian cancer were examined in the Department of Gynecology and Gynecologic Oncology, Medical University of Bialystok and pre-treatment diagnosis were evaluated based on present adnexal masses with spread metastasis into pelvis and abdomen confirmed by CT scan, ascites, elevated level of Ca-125. 10 ml of peripheral blood (antecubital vein) was collected for EDTA probes from each patient 24 h before surgery procedures. The blood was centrifuged in 15 min, plasma subsequently separated and frozen at −80 °C temperature.

Radical bulky surgery procedures according to gynecologic oncology guidelines (hysterectomy, bisalpingo-oophorectomy, total omentectomy, appendectomy, others) were performed in the group of patients clinically suspected of ovarian cancer. All applied procedures were standardized and repeatable. Neoplastic samples were collected by University Tissue Biobanking Team (one pathologist, one technician; according to Standard Operating Procedures for Biobanking - SOP) from an ovarian surface not deeper than 1 cm, just after removal of the uterus and ovaries. Dissected tissues were immediately placed into liquid nitrogen and time from surgical closing last artery vasculating ovarian tumor to tissue collecting was not longer than 2 min. Normal ovarian tissues were obtained during operations because of fibroids or other non-oncology procedures. Hysterectomy was performed and ovarian samples were collected and handled as described above. Only women with histologically diagnosed advanced ovarian cancer serous type, the International Federation of Gynecology and Obstetrics (FIGO) Classification - stage III/IV, high-grade (2014 WHO Classification) were included in the study. We sequentially excluded from the group of 165 women: 48 borderline tumors, 43 of non-epithelial histology; and from the control group: 68 patients with some abnormalities in ovarian tissue diagnosed during histology evaluation. Final groups counted: advanced ovarian cancer: n = 74 patients, control group: n = 81 women.

2.1. The content of plasma sphingolipids

The level of sphingolipids in plasma was analyzed by LC/MS/MS approach as previously described by Blachnio-Zabielska et al. with minor modification [13]. Briefly, to each plasma sample (100 μl) were added 50 μl of the internal standard solution (17C–sphingosine and 17C-S1P, and C17-Cer Avanti polar lipids) as well as 1.5 ml of an extraction mixture (isopropanol: water: ethyl acetate, 35:5:60; v:v:v). The following sphingolipids were quantified: SPH (sphingosine), S1P (sphingosine-1-phosphate), SPA (sphinganine), ceramide C14:0-Cer (ceramides containing myristic acid), C16:0-Cer (ceramides containing palmitic acid), C18:1-Cer (ceramides containing oleic acid), C18:0-Cer (ceramides containing stearic acid), C20:0-Cer (ceramides containing arachidic acid), C22:0-Cer (ceramide containing behenic acid), C24:1-Cer (ceramides containing nervonic acid) and C24:0-Cer (ceramides containing lignoceric acid). The mixture was vortexed, sonicated and then centrifuged for 10 min at 4000 rpm (Sorvall Legend RT). The supernatant was transferred to a new tube and pellet was re-extracted. After centrifugation supernatants were combined and evaporated under nitrogen. The dried sample was reconstituted in 100 μl of LC Solvent A (2 mM ammonium formate, 0.15% formic acid in methanol) for LC/MS/MS analysis. Quantitative measurement was made using triple quadrupole mass spectrometer (Agilent 6460) in positive mode using multiple reaction monitoring (MRM). The chromatographic separation was performed on Agilent 1290 Infinity Ultra High Performance Liquid Chromatography (UHPLC). The analytical column was a reverse-phase Zorbax SB-C8 column 2.1 × 150 mm, 1.8 μm. Chromatographic separation was conducted in binary gradient using 2 mM ammonium formate, 0.15% formic acid in methanol as Solvent A and 1.5 mM ammonium formate, 0.1% formic acid in water as Solvent B at the flow rate of 0.4 ml/min. All sphingolipids were quantified against standard concentration curve. The flow was diverted to waste for the first and the last 4 min to prevent eluting impurities from entering the mass spectrometer.

2.2. The content of tissue sphingolipids

The content of sphingolipids was measured using a UHPLC/MS/MS (an ultra-high performance liquid chromatography coupled with triple quadrupole mass spectrometry) approach according to Blachnio-Zabielska et al. [13]. Briefly, the tissue samples (20 mg) were homogenized in a solution composed of 0.25 M sucrose, 25 mM KCl, 50 mM Tris and 0.5 mM EDTA, pH 7.4. Immediately afterwards, 50 μl of the internal standard solution (17C–sphingosine and 17C–S1P, and C17-Cer Avanti polar lipids) as well as 1.5 ml of an extraction mixture (isopropanol: water: ethyl acetate, 35:5:60; v:v:v) were added to each homogenate. The mixture was vortexed, sonicated and then centrifuged for 10 min at 4000 rpm (Sorvall Legend RT). The supernatant was transferred to a new tube and pellet was re-extracted. After centrifugation supernatants were combined and evaporated under nitrogen. The dried sample was reconstituted in 100 μl of LC Solvent A (2 mM

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