Original article

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DISTRIBUTION OF SERUM PHOSPHOLIPID FRACTIONS IN PATIENTS WITH DIABETES TYPE 2 AND DYSLIPIDEMIA

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The aim of the present paper was to investigate the serum concentration of total phospholipids and distribution of the phospholipid classes in patients with diabetes mellitus type 2 and dyslipidemia. We analyzed the serum phospholipids in 28 newly diagnosed patients with diabetes type 2, of which 12 men and 16 postmenopausal women aged 43-70 years. Sex- and age-matched control group was formed from 27 apparently healthy subjects. The results showed a significantly higher level of total phospholipids in the patient group when compared with healthy subjects ($p<0.001$). The relative concentration of phosphatidil-choline was significantly higher, and percentages of all other phospholipid classes were significantly lower in the patient group. The obtained results suggest an altered metabolic control of phospholipids in patients with diabetes type 2 with dyslipidemia. *Acta Medica Medianae 2012;51(3):13-17.*

Key words: *diabetes mellitus type 2, dyslipidemia, phospholipids, phospholipid classes*

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Introduction

Type 2 diabetes mellitus (DM) is the most prevalent metabolic disorder characterized by hyperglycemia resulting from relative deficiency of insulin production, a decreased insulin action or both (1). The number of patients with DM type 2 is constantly increasing worldwide. This disease is the fifth leading cause of mortality in the world, the fourth cause of mortality and the fifth cause of morbidity in Serbia (2). It has been recently estimated that there were around 400.000 patients with DM type 2 in Serbia, which was the prevalence of 5.4% (2).

In addition to abnormal glycoregulation, the metabolism of fats and proteins is also altered in DM. Hence, dyslipidemia is one of the major complications in type 2 DM (3). Lipoproteins play a particularly important role in the transport and metabolism of lipids, and the basic components of lipoproteins are phospholipids (PL). PLs are the major structural components of biological membranes that influence a number of cellular functions (4,5). Alterations in membrane PL composition have an impact on physiological status of the membrane, its fluidity and permeability, activity of membrane-bound enzymes and proteins involved in the transport systems, cell growth, viability, proliferation and apoptosis (1,6). Furthermore, PL serve as a pool of poly-unsaturated fatty acids which are precursors of biologically active eicosanoids: prostaglandines, leucotrienes, tromboxanes and lipoxins (7).

Due to a correlation with a number of functional and pathologic disorders, the alterations in concentration and distribution of serum PL are biomarkers of different diseases. It has been shown that obesity, Alzheimer disease and some types of cancer have been associated with an abnormal metabolism of PL (8-12). Our previous studies have shown significantly lower levels of serum PL, total cholesterol and HDL cholesterol in patients with prostate cancer (13) and non-Hodgkin lymphoma (14) compared with healthy individuals. Furthermore, the concentration of PL, as well as the levels of cholesterol and triglycerides (TG), are important predictors of cardiovascular events (15). This is especially important in patients with DM type 2, who have an increased risk of cardiovascular diseases (16). Several studies have also reported an association between DM type 2 and altered metabolism of fatty acids in PL (3,17-19). In spite of these facts, the data on concentration of PL and distribution of PL fractions in patients with DM type 2 and dyslipidemia are sparse.

Aim

The aim of our study was to determine the concentration of serum total PL and distribution of PL fractions in patients with DM type 2 and dyslipidemia.

Patients and methods

Patients

This study was conducted in the Center of Research Excellence in Nutrition and Metabolism, Institute for Medical Research in Belgrade. Twenty–eight patients with newly diagnosed DM type 2 (12 men and 16 postmenopausal women aged 43-70 years) entered the study and composed the DM group. All patients in the DM group had glycemia >7.0mmol/L and dys-lipidemia: 10 patients had increased level of total cholesterol (>5.2 mmol/L), 6 patients had increased TG level (>1.7 mmol/L), and 12 patients had combined hyperlipidemia. Women included in the study were postmenopausal for at least 12 months. Subjects over 70 years of age, patients with another endocrine, liver or kidney disease, cancer, uncontrolled hypertension (≥140/90) or previous myocardial infarction were excluded from the study. Persons with serum glucose >10 mmol/L, total cholesterol >7.8 mmol/L or TG >4.5 mmol/L were also excluded. None of the partici-pants used insulin, lipid lowering drugs, β-blockers, diuretics, anticoagulant therapy or hormone substitution. The control group was composed of 27 healthy subjects of both sexes, aged 40-63 years.

All study participants signed an informed consent and the whole study was approved by the Ethics Committee of our institution and conducted according to the principles of the Declaration of Helsinki.

Analytical methods

Blood samples were taken in the morning after a 12-hour fast. Glucose, cholesterol and triglyceride concentrations were measured in serum using the automated enzymatic methods with glucose oxidase, cholesterol oxidase and glycerol oxidase, respectively (EliTech Diagnostic, Sees, France). Serum HDL cholesterol was determined by measuring cholesterol concentration in the supernatant, after precipitation of the other classes of lipoproteins with phospho-tungistic acid and magnesium chloride (20). LDL cholesterol was estimated using the Friedewald formula (21). The total PL concentration in serum was determined by the Zilversmit method (22). Serum lipids were extracted with chloroform–methanol mixture (2:1v/v) as we previously described (23). The PL fraction was isolated from the extracted lipids by one-dimensional thin-layer chromatography (TLC) in a neutral solvent system (petrol ether-diethyl ether-acetic acid; 87:12:1v/v) on Silica Gel GF plates (Merck, Darmstadt, Germany). The relative concentrations of PL fractions were determined according to the method of Marinetti et al. (24), and expressed as percentages of total PL. Four fractions of PL were identified in serum: lyso-phosphatidylcholine (LPC), sphingomyelin (SM), phosphatidylcholine (PC) and phosphatidy-lethano-lamine (PE).

Statistical analysis

All the results were expressed as the mean ± SD. Normality was tested using the Shapiro-Wilk test. Since all variables showed normal distribution, the Student t-test for the comparisons between two groups was applied. The differences were considered significant at $p \leq 0.05$.

Results

The average serum concentrations of glucose and lipid parameters in both study groups are presented in Table 1. The patients in the DM group had significantly higher levels of total cholesterol, LDL-cholesterol and TG ($p<0.001$), and significantly lower level of HDL-cholesterol ($p<0.01$) when compared with the control group.

Table 1. Serum glucose and lipid status in the study participants

Serum concentration (mmol/l)	DM group (n=28)	Control group (n=27)
Glucose	8.43±1.01	4.24±0.36***
TG	2.79±1.03	0.61±0.29***
TC	6.29±0.88	4.43±0.42***
HDL	1.28±0.16	1.44±0.09**
LDL	3.66±0.68	2.70±0.26***

TG- triglycerides, TC-total cholesterol
HDL – HDL cholesterol, LDL – LDL cholesterol
**p≤0.01, ***p≤0.001

Table 2. Concentration of total phospholipids in serum and distribution of phospholipid fractions

	DM group (n=28)	Control group (n=27)
PL (mmol/l)	3.13±0.93	2.37±0.46 ***
PC/SM	2.95±0.43	1.88±0.32***
PC (%)	59.82±3.74	45.25±4.21***
SM (%)	20.58±2.74	24.02±2.15***
PE (%)	8.87±2.18	13.70±2.01***
LPC (%)	11.31±2.17	16.99±2.22***

PL- total serum phospholipids; SM – sphingomyelin
PC - phosphatidilcholine, PE – phosphatidil-ethanolamin
LPC – lysolecithin. ***p≤0.001

Significantly higher concentration of total PL was also found in the DM group than in the control group (Table 2). The ratio between phosphatidylcholine and sphingomyelin (PC/SM) in the sera was significantly higher in patients with DM type 2 than in the healthy subjects ($p<0.001$). Distribution of PL fractions was also altered in patients with DM type 2 and dyslipidemia. The percent of phosphatidil-choline

was higher in the diabetic patients than in the control group, while all other PL (SM, PE and LPC) were significantly reduced in the DM group when compared with the healthy subjects ($p<0.001$).

Discussion

In this study we quantified the serum phospholipids in patients with DM type 2 and dyslipidemia and in healthy subjects and established significant alterations in systemic PL metabolism in patients with DM. The level of total PL in the sera was significantly higher in diabetic patients than in healthy participants. Elevated levels of serum PL in patients with coronary heart disease than in healthy subjects were reported in 1951 by Gertler et al. (15). In spite of their crucial structural and physiological significance, the role of PL in atherosclerosis has not been well characterized so far. It has been established that the content of PL, and particularly SM, accumulated in human atheroma (25). This increase of PL originates partly from an increased synthesis of PL in arterial walls, and partly from the entrance of PL from circulation to the walls. PL accumulates with cholesterol and cholesterol-esters in arterial plaques (26). It has been proposed that the presence of PL rich in saturated fatty acids in arterial lesions indicated an early stage of atherogenesis.

Our study of the distribution of PL fractions showed that PC was the most common fraction in both groups. However, in the DM group, the relative concentration of PC was almost 60%, while in the control group it was around 45%. This distribution also influenced the other fractions, which were significantly lower in the patient group than in the healthy subjects. Distribution of PL fractions is often altered in diseases, and many functional disturbances may be related to changes in PL distribution (25). Studies on patients suffering from different types of cancer have reported lower levels of total PL when compared with healthy individuals, while the distribution of PL fractions showed higher PC and lower all the other fractions, similar to the diabetic patients in our study (13,27-29). One of the possible explanations of the disturbed PL fractions in patients with DM type 2 and dyslipidemia is decreased activity of the phospholipase A2 (PLA2). The PLA2 activity, which catalyzes hydrolysis of PL, is altered in diabetes, but these changes are different depending on the tissue (30). For example, PLA2 activity in plasma and liver was found to be reduced, whereas PLA2 activity in skeletal muscle and platelets was found to be increased by diabetes (31). However, after the insulin treatment, the PLA2 activity in plasma increased, and decreased in platelets and skeletal muscle (30). Given that patients in our study were not treated with insulin, reduced serum PLA2 activity probably caused an elevated percentage of PC and reduced LPC in the patient group. Sphingomyelin is also important in pathogenesis of type 2 DM. It has been shown that the content of SM in adipocyte and erythrocyte membranes directly correlated with the insulin level (32). In addition to altered glycoregulation, dyslipidemia in our patients could contribute to changed distribution of PL fractions. Engelmann et al. (33) found significantly higher level of PC and reduced percentage of SM in plasma and erythrocytes in patients with hyperlipidemia, which is in line with our results. The PC/SM ratio is an important indicator of membrane fluidity and a parameter which can affect many physiological functions of the membrane (34). Considering all these reasons, determination of total serum phospholipids as well as the distribution of PL fractions, can be important in patients with type 2 DM and dyslipidemia.

Conclusion

The obtained results suggest that patients with DM type 2 and dyslipidemia have an increased level of serum PL and altered distribution of PL fractions. The precise mechanisms underlying these alterations are yet to be delineated, and could contribute to improvement of prevention and/or the therapy of diabetes type 2 and dyslipidemia.

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RASPODELA FRAKCIJA FOSFOLIPIDA SERUMA U TIPU 2 DIJABETESA SA UDRUŽENOM DISLIPIDEMIJOM

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Cilj našeg rada bio je da se odredi koncentracija ukupnih fosfolipida i procentualna zastupljenost fosfolipidnih frakcija u serumu bolesnika sa dijabetesom tip 2 i udruženom dislipidemijom. U studiju je uključeno 28 bolesnika sa novootkrivenim dijabetesom tip 2, od kojih je 12 muškaraca i 16 žena u postmenopauzalnom periodu, starosti 43-70 godina. Kontrolnu grupu činilo je 27 zdravih osoba iste starosti. Rezultati istraživanja su pokazali statistički značajno viši nivo ukupnih fosfolipida u grupi bolesnika u poređenju sa zdravim osobama ($p<0.001$). Procentualna zastupljenost fosfolipidnih frakcija pokazala je statistički značajno veći udeo fosfatidil-holina i statistički značajno nižu učestalost svih ostalih frakcija fosfolipida u grupi obolelih. Dobijeni rezultati ukazuju na poremećenu kontrolu metabolizma fosfolipida kod obolelih od dijabetesa tip 2 sa udruženom dislipidemijom. *Acta Medica Medianae 2012;51(3):13-17.*

Ključne reči: *dijabetes melitus tip 2, dislipidemija, fosfolipidi, frakcije fosfolipida*