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EVALUATION OF CHANGES IN SERUM LEVELS OF FATTY ACID IN NEWLY DIAGNOSED TYPE 2 DIABETIC PATIENTS

ABSTRACT

In this study, the changes in levels of fatty acids betweennewly diagnosed type 2 diabetic patients and the control group were evealuated. In this study, 16:0, 16:1, 18:0 and 22:6 fatty acids levels of Type 2 diabetic group were found not to be statistically significant. The 18:2 fatty acid levels of Type 2 diabetic patients were lower than the control group and it was not found to be statistically significant. The 18:1 and 18:3 fatty acid levels of Type 2 diabetic patients were found to be statistically significantly lower ($p<0.05$) compared to the control group, the 20:3 and 20:4 fatty acid levels were found to be statistically significantly higher ($p<0.001$) compared to the control group.

Keywords: Newly Diagnosed Type 2 Diabetic Patients, Serum, Fatty acids, Gas Chromatography, Diabetic

YENİ TANI KONMUŞ TİP 2 DİABETİK HASTALARDA SERUM YAĞ ASİTLERİ DÜZEYLERİNDEKİ DEĞİŞİMLERİN DEĞERLENDİRİLMESİ

ÖZET

Bu çalışmada yeni tanı konmuş tip 2 diabetik hastalar ile kontrol grubu arasında yağ asitleri düzeylerindeki değişimleri değerlendirilmiştir. Yapılan çalışmada, Tip 2 diabetik grubunun 16:0, 16:1, 18:0 ve 22:6 yağ asitleri düzeyleri istatiksel olarak anlamsız bulunmuştur. Tip 2 diabetik hasta grubunun 18:2 yağ asitleri düzeyleri kontrol gurubuna daha göre düşük çıkmış ve istatiksel olarak anlamsız bulunmuştur. Diabetik hasta grubunun 18:1 ve 18:3 yağ asidi düzeyleri ise kontrol grubuna göre istatiksel olarak ($p<0.05$) anlamlı düşük bulunmuş, 20:3 ve 20:4 yağ asidi düzeyleri ise kontrol grubuna göre istatiksel olarak ($p<0.001$) anlamlı yüksek bulunmuştur.

Anahtar Kelimeler: Yeni Tanı Konmuş Tip 2 Diabetik Hastalar, Serum, Yağ Asitleri, GC, Diyabetik

1. INTRODUCTION (GİRİŞ)

Diabetes mellitus (DM) is an endocrine and metabolic disease characterized by carbohydrate, fat and protein metabolism disorders and hyperglycemia and resulting due to absolute or relative deficiency of insulin secretion by the pancreas or IR [1]. Diabetic subject have abnormal fat and lipoprotein metabolizma that includes altered fatty acid compososition of plasma and tissues, enhanced release of fatty acids, increaased production and reduced clearance of very-low-density lipoproteins and cholesterol, high triglycerides, and abnormalities of glucose metabolism in muscle [31]. Because their fat metabolism is unlike that of nondiabetics, one cannot extrapolate the findings of w3 effects in healty people or nondiabetic heart disease patients to diabetics [32].

DM, which may cause death with its acute and chronic complications, is known since ancient times and even today continues to be a major health problem [2]. Type 2 diabetes mellitus is one of the most frequently encountered metabolic disorder and is seen by 5-10% in the population of most of the developed countries. Diabetes is one of the major factors in the formation of undesirable situations such as blindness, kidney failure and lower limb amputation. More importantly, the cardiovascular disease risk is higher by 3-5 times in patients with type 2 diabetes [3].

Insulin resistance is a key parameter for type 2 diabetes pathogenesis. Insulin resistance may be defined as the deterioration of the biological response to insulin which is given exogenously or secreted endogenously. The deterioration in insulin-stimulated glucose transport and metabolism both in adipose tissue and skeletal muscle results in the lack of a suppression of glucose production in the liver [4]. High serum fatty acids found in the circulation of patients with diabetes leads to a resistance to insulin action in the liver. Increased fat oxidationmay impair glucose uptake and glycogen synthesis (lipotoxicity) [5].

Lipotoxicity is one of the events located in the pathophysiology of type 2 diabetes as well as glucotoxicity. Normally glucose by stimulating glycolysis and the Krebs cycle, and the free fatty acids by increasing acyl CoA estersaffect insulin secretion. However, remaining of plasma glucose and free fatty acids levels high for a long time causes a decrease in insulin secretion [6 and 7].

It has been shown in a study that fish oils containing polyunsaturated fatty acids decreased the rate of 20:4/20:5 by reducing the arachidonic acid (20:4) level and eicosapentaenoic acid (20:5) in platelets and thus they may reduce the risk of thrombosis [8, 9 and 10]. In this study, the serum fatty acid composition of newly diagnosed diabetic patients and the control group were examined departing from the literature and it was aimed to investigate the effects of fatty acids in diabetic patients.

2. RESEARCH SIGNIFICANCE (ÇALIŞMANIN ÖNEMİ)

In this study, the aim of the analysis of serum fatty acids of diabetic patientsemphasizes the importance of the relationship between diabetes and fatty acids even in newly diagnosed patients. We hope that this study will provide guidance to related studies with regulation of diabetes.

3. MATERIALS AND METHODS (MATERYAL VE METOD)

3.1. Selection of Patients and Controls (Hasta ve Kontrol Seçimi)

In this study, 23 patients admitted to the endocrinology department of Firat University Hospitalwithout any other disease but

newly diagnosed with type 2 diabetes and 20 healthy individuals were enrolled. It was preferred that the individual's formingthe patient and control groups had similar age and body mass index. Body mass index was calculated with the formula of (BMI)=kg/m^2. Waist circumference measurement was done with a tape within room clothets, on an empty stomach, standing and after a normal expiration. Insulin resistance (HOMA-IR) and beta-cell function (HOMA-β) were calculated using a mathematical process developed by Matthews et al [11].

HOMA-IR=[fasting insulin (μU/ml) x fasting glucose (mmol/l)]/ 22.5

HOMA-β= [20xfasting insulin (μU/ml)]/[fasting glucose (mmol/l)−3.5].

Samples taken from the patient and control groups were immediatelysubjected to the necessary processes in the biochemistry laboratory of Firat University Hospital.

3.1. Preparation of Samples (Örneklerin Hazırlanması)

Blood samples were taken into tubes containing K_3-EDTA and biochemistry tube without anticoagulant from the people in the patient and control groups following the 10-12 hour overnight fasting for this study. The blood was taken into biochemical tubes was immediately centrifuged at 4000 RCF (Relative Centrifugal Force) for 10 min.

3.2. Lipid Extraction and Preparation of Fatty Acid Methyl Esters (Lipid Ekstraksiyonu ve Yağlı Asit Metil Esterlerinin Hazırlanması)

Lipid extraction from serum samples was performed with the Hara and Radin method, which uses a 3:2 volume/volume (v/v) hexan-isopropanol mixture [12]. The fatty acids in lipids were converted to methyl ester derivatives, which are not polar and have volatile and stable structure, in order to perform a gas chromatographic analiysis. To prepare the methyl ester [13], the lipid extract in the hexan/ isopropranolol phase was transferred into 30 ml test tubes, which did not percolate. Five ml of 2% methanolic sulphuric acid was added and mixed well with a vortex. This mixture was left for methylation in the incubator at 50°C for 15 hours. The tubes were removed from the incubator, cooled to room temperature, and mixed well by adding 5 ml of 5% sodium chloride. Fatty acid methyl esters were extracted with 5 ml hexan, and the hexan phase was placed into the pipet, treated with 2% potassium bicarbonate (KHCO3) and kept for four hours to allow the phases to separate. The solution of the mixture that contained methyl esters was then subjected to evaporation at 45°C and under nitrogen flow, dissolved with 1 ml of hexan, placed into 2 ml autosampler covered vials, and analyzed with gas chromatography. Gas chromatographic analysis of fatty acid methyl esters after the fatty acids in the lipid extract were converted to methyl esters, they were analyzed with (Shimadzu GC 17 ver. 3) gas chromatography. For this analysis, a Machery-Nagel capillary column measuring 25μ, with a 0.25 μm inner diameter and Permabond 25 micron film thickness was used. During the analysis, the temperature of the column was kept at 120-20°C, the injection temperature was kept at 240°C, and the detector temperature was kept at 280°C. Nitrogen was used as the carrier gas. The mixture belonging to the standard fatty acid methyl esters was injected before the analysis of the fatty acid methyl esters of the samples, and the retention time of each fatty acid was determined. After this procedure, an analysis of the fatty acid methyl esters of the samples was performed. The results were determined as the percentage amount for each fatty acid among the fatty acids. Calculations were made using the GC solution 2.3 program.

3.3. Statistical Analysis (İstatistiksel Analiz)

Statistical analyzes in this study were performed using the SPSS 16 (SPSS, Inc, Chicago, III) software package. Data are expressed as mean7 SEM of the number (n) of experiments; A P-value of 0.05 was considered to be statistically significant. Fatty acid levels between the experimental groups were compared using a one-way ANOVA.

4. RESULTS (SONUÇLAR)

In this study 23 newly diagnosed type 2 diabetic patients and 20 healthy individuals were included. The mean ages of the control (47.7±7.40) and patient (50.9±9.30) groups and BMI ofthe control (28.8±4.20) and patient (30.5±4.90) groups were preferred to be close to each other (Table 1).

Table 1. Chemical composition (relative % peak area) of the fatty acids in the serum patients and control
(Tablo 1. Hasta ve kontrol serumunda yağ asitlerinin kimyasal bileşimleri (%))

Fatty Acids	Control (n: 20)	Type 2 DM (n: 23)	p Value
16:0	28.19 ± 0.52	29.15 ± 0.50	>0.05
16:1 n9	1.42 ± 0.60	1.30 ± 0.70	>0.05
18:0	10.71 ± 0.41	10.39 ± 0.29	>0.05
18:1 n9	19.33 ± 0.74	17.55 ± 0.46 a	<0.05
18:2 n6	26.04 ± 0.61	24.58 ± 0.64	>0.05
18:3 n3	1.51 ± 0.05	1.32 ± 0.06	>0.05
20:3 n3	1.61 ± 0.09	2.35 ± 0.12 b	<0.001
20:4	7.85 ± 0.49	10.49 ± 0.36 b	<0.001
22:6 n3	2.21 ± 0.10	2.99 ± 0.12	>0.05
ΣSatured	19.45 ± 0.46	19.77 ± 0.40	>0.05
ΣUnsatured	8.56 ± 0.38	8.65 ± 0.25	>0.05
Σ MUFA	10.37 ± 0.67	9.43 ± 0.58	>0.05
ΣPUFA	7.84 ± 0.27	7.87 ± 0.26	>0.05
Σ w3	3.86 ± 0.50	2.22 ± 0.10	>0.05
Σw6	19.95 ± 0.55	17.54 ± 0.50	>0.05

a:p<0.05, b:p<0.001, *: not detectable, Values are means ± SE

5. DISCUSSION (TARTIŞMA)

The prevalence of type 2 diabetes is increasing dramatically in the last 25 years. Today, the most effective way to protect against type 2 DM is prevention and treatment of obesity which is heading the list of modifiable risk factors for DM and prepares the ground for the emergence of many health problems due to its complications [14]. There is a strong relationship between obesity and insulin resistance in both diabetic and non-diabetic obese individuals [15]. Obesity represents the increase in fat tissue. A description of the insulin resistance associated with obesity is the release of factorsby adipose tissuewhich make some people insulin resistant more than the others [16, 17 and 18].

Free-fatty acids (FFA), TNF- and leptin are located among the most likely candidate factots which are related with the occurrence of insulin resistance with the increase in fat mass [19]. The foremost changing in obesityis accepted as triacylglycerol accumulation in adipocytes and the most obvious candidate is FFA concentrations which are increasing improperly. It has been shown that the increase in distribution of FFA in circulation may initiate insulin resistance [20].

Thelevels of FFA in the circulation which is released from adipocytes with lipolysis increase in obsesity [21]. Basal lipolysis

rate is increased by increasing fat mass, but the underlying mechanism is unknown. High FFA levels is expected to stimulate the liver and muscle insulin insensitivity by the glucose-fatty acid cycle [22]. Acetyl-CoA resulting in muscle with FFA oxidationleads to a decrease in glucose utilization by inhibiting pyruvate dehydrogenase. The increase in intracellular glucose arising as a result increase in intracellular glucose reduces the transmembrane concentration gradient which directs the entry of glucose into cells and causes a secondary reduction of glucose uptake. Acetyl-CoA accumulation in the liver inhibits pyruvate carboxylase and shows the effect on glucose metabolism by stimulating gluconeogenesis. Therefore increased FFA concentration leads to increased hepatic glucose production and decreased glucose uptake by muscle. Thus, it shows a tendency to increase the blood glucose concentration and effectively resists the effects of insulin. In addition, increased concentrations of FFA reduce more the concentration of insulin in the circulation by inhibiting the administration of insulin into the circulation by the liver.

FFA secreted directly into the portal circulation may especially be diabetogenic due to it is sent directly to the liver. This may explain the relationship which was reported to be between visceral fat deposition and insülin [21 and 23]. The hypothesis associated with fatty acid metabolism disorder among those for the complications of Diabetes Mellitus received considerable attention in recent years. It has been deterimiedn in a study conducted accordingly, 6 and 5 desaturation steps ofthe essential fatty acid metabolism were impaired in diabetes due to they were regulated by insülin [24, 25 and 26].

In another study, increased levels of linolenic acid and decreased levels of arachidonic acids and in addition, increased lipolysis-inducedoleic acidhave also been reported in diabetes [27, 28, 29 and 30]. As a result of study, the 16:0, 16:1, 18:0 and 22:6 fatty acids levelsof type 2 diabetic group were not found to be statistically significant. The 18:2 fatty acids levelsof type 2 diabetic group was found to be lower compared to the control group and it was not statistically significant. The 18:1 and 18:3 fatty acids levels of type 2 diabetic group were found to be statistically significantly lower compared to the control group ($p<0.05$). The 20:3 and 20:4 fatty acids levels were found to be statistically significantly higher compared to the control group ($p<0.001$).It has been revealed with this study that 20:4 fatty acid levels were higher in new diabetic patients. Tilvis has reported in his study that the arachidonic acid levels in erythrocytes of patients with diabetes were high and this is caused by not to use of arachidonic acid for the biosynthesis of prostanoids [30]. As a result, theaim of the analysis of serum fatty acids of diabetic patientsemphasizes the importance of the relationship between diabetes and fatty acids even in newly diagnosed patients. We hope that this study will provide guidance to related studies with regulation of diabetes.

NOTE (NOT)

For this study, Fırat University, Faculty of Medicine Ethics Committee dated from 26.07.2007, No.07 meeting and approval decision was taken No:1. Written for control patients (Informed Consent Form) and verbal consent was obtained. Patient and control samples taken from the group immediately Fırat University Hospital has undergone the necessary procedures in biochemistry labaoratuvar.

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