Study of the lipidemic profile of diabetic patients. Negative correlation of cholesterol levels of diabetes type I patients with serum amylase concentration

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Abstract

Diabetes Mellitus type I (DM1) and II (DM2) share the common characteristic of high blood glucose concentration and the health complications resulting from uncontrolled hyperglycemia such as hyperlipidemia, cardiovascular problems, stroke, ketoacidosis, kidney failure and blindness but have different etiology. DM1 is practically an autoimmune disease. Genetic susceptibility together with environmental factors leads to disease development. The main characteristics of Diabetes type II (DM2) is insulin resistance in muscle and liver cells accompanied by loss of β-cell function. However, adipose tissue, gastro-intestinal tract, pancreatic a-cell activity, may be involved in disease development. In parallel to the impairment of endocrine pancreatic function, a reduction in exocrine function has also been observed in all types of Diabetes Mellitus. A decrease in amylase and lipase activity has been mentioned by many authors, although cases with elevated amylase have been referred. Most recently a trend for positive correlation between HDL cholesterol and amylase in Diabetes type II patients was shown. In the present study *we evaluated* the lipidemic profile and related factors such as cortisol, total serum antioxidant capacity (TAC) and amylase in patients suffering from diabetes type I and II. The relationship between different parameters was examined. Blood serum from 20 DM1 patients and 45 DM2 patients was used. Serum from 50 healthy individuals was used as control. Total cholesterol and triglycerides were measured using an enzymatic colorimetric method. Serum cortisol, auto-antibodies and anti-Neu5Gc antibodies were measured using immunoenzyme assays and TAC measurement was made using the ABTS method. Mean total cholesterol was 245.5mg/dL in Diabetes I patients and was significantly elevated compared to healthy individuals as well as Diabetes II patients (168.71±76.0mg/dL). The observed difference was statistically significant (P=0.0004). On the contrary, triglyceride values were within normal range in both cases (123.7±63.2mg/dL in DM1 and 168.1±76.0mg/dL in DM2 patients). Cortisol levels were elevated in both cases with higher values observed in Diabetes type I (280.5±162.9ng/mL in DM1 and 248.5±100.1ng/mL in DM2), while total antioxidant capacity was significantly reduced compared to healthy individuals, 1.470mM, with lower values observed in Diabetes type I (0.680±0.116mM in DM1 and 0.849±0.126mM in DM2). Amylase determination revealed a mean amylase value, 81.7U/ml, within normal range and a negative correlation between cholesterol levels and amylase (r=-0.770) in DM1 patients. No correlation was observed between the determined values or the presence of autoantibodies and antibodies against Neu5Gc in the samples. In conlusion, the lipidemic profile and overall atherogenic and cardiovascular risk factors were worse in Diabetes I compared to Diabetes II patients. Most interestingly, cholesterol levels exhibited a negative correlation with serum amylase values. Since, amylase is not known to be involved in lipid metabolism, cholesterol levels and serum amylase activity may have a common modulator related to Diabetes development.

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Introduction

Diabetes Mellitus type I (DM1) and II (DM2) share the common characteristic of high blood glucose concentration and the health complications resulting from uncontrolled hyperglycemia such as hyperlipidemia, cardiovascular problems, stroke, ketoacidosis, kidney failure and blindness but have different etiology [1].

DM1 is practically an autoimmune disease [1]. Genetic susceptibility together with environmental factors leads to disease development. The insulin gene and certain alleles of genes such as HLA, CTL4, PTPN22 and IL2RA related to immune response and involved in many autoimmune diseases are related to genetic susceptibility [1]. Auto-antibodies against insulin (IA) and islet cell components (ICA), glutamate decarboxylase (GAD-A) and protein tyrosine phosphatase (IA2-A) are detected in most patients [1]. Gradual loss of β-cell function leading to impaired or no insulin secretion is the main event characterizing the disease. Certain viruses such as enteroviruses, rotaviruses, intestinal bacterial composition and dietetic products such as cow's milk and gluten have been correlated with disease development [1]. Consumption of red meat containing the Neu5Gc sialic acid may be one more environmental factor according to our research [2].

The main characteristics of Diabetes type II (DM2) are insulin resistance in muscle and liver cells accompanied by loss of β-cell function [3]. However, adipose tissue, gastro-intestinal tract, pancreatic a-cell activity, kidney and brain function maybe involved

in disease development [4]. The dipper etiology beyond this is a combination of genetic susceptibility, life style and environmental factors [5]. More than ten genes have been found to be related with susceptibility to DM2 [6]. Obesity and lack of physical activity are the two life style/environmental factors triggering DM2 [6]. The initially appearing insulin resistance, lipotoxicity, glucotoxicity, amyloid deposition or incretin axis abnormalities may be responsible for gradual β-cell failure [6]. Although, the presence of auto-antibodies has been associated with Diabetes type I, auto-antibodies are also detected in many patients diagnosed with Diabetes type II who belong in a separate category of Late Auto-immune Diabetes of Adulthood (LADA) patients [7]. On the other hand, insulin resistance is also present in some patients with Diabetes type I, as well.

In parallel to the impairment in endocrine pancreatic function, a reduction in exocrine function has also been observed in all types of Diabetes Mellitus [8]. A decrease in amylase and lipase activity has been mentioned by many authors [9], although cases with elevated amylase have been referred. Most recently, a trend for positive correlation between HDL cholesterol and amylase in Diabetes type II patients was shown by a research team [9]. To our knowledge, no study correlating cholesterol levels and amylase in Diabetes type I patients has been referred.

Increased levels of cortisol, the main stress hormone, may come as a result of biological stress induced by diabetes but can also induce hyperglycemia as well as hyperlipidemia [10-14]. Moreover, increased cortisol levels have been associated with oxidative stress by many scientists [15]. Oxidative stress is also known to be induced by hyperglycemia itself [16] and is usually observed in Diabetes mellitus patients [17]. Moreover, an axis of auto-immunity [18]-chronic inflammation [19]-free radical production may contribute to antioxidant depletion in patients suffering from Diabetes I or II. Increased hyperlipidemia combined with oxidative stress is among the main factors inducing atheromatosis, diabetic agiopathy, thrombotic incidents and cardiovascular problems which belong to commonly observed diabetic complications [20].

In the present study we evaluated the lipidemic profile and related factors such as cortisol, total serum antioxidant capacity (TAC) and amylase in patients suffering from diabetes type I and II. The relationship between different parameters was examined.

Materials and methods

Blood serum from 20 DM1 patients with mean age 43±16 years old (18-71) and 45 DM2 patients with mean age 64.7±11.9 years old (38-81) was used. All of them were patients regularly visiting the Diabetes Centre of Hippocration General Hospital of Thessaloniki, they were volunteers and were informed about the project. A questionnaire concerning the patients' history was kept. Pancreatitis or any other pathologic disorder concerning pancreatic function was not diagnosed in any of the patients. Blood collection was done between 8.00 and 9.00 a.m. and the serum was kept at -20^{o}C until the measurement. Serum from 50 healthy individuals was used as control.

Total cholesterol (TC) and triglycerides (TG) were measured using the enzymatic colorimetric kit of Spinreact. Serum cortisol was measured using the immunoenzyme assay kit of HUMAN. TAC was measured by the ABTS method, using the Antioxidant Assay Kit of Cayman. The determination of anti-insulin, anti-GAD and anti-IR antibodies was done using ELISA kits of Uscn Life Science Inc. The determination of anti-IA2 antibodies was done using an ELISA kit of Medipan GMBH/Berlin. For the determination of anti-Neu5Gc antibodies, 96 well, flat bottom, high binding microtiter plates were used. The wells were coated with Neu5Gc antigen purchased by Sigma. HRP conjugated Goat anti-human IgG or IgM or IgA (AbDserocet) were used as secondary antibody. Amylase was measured using 2-chloro-p-nitrophenyl-α-D-maltotrioside (CNPG3) as substrate. Statistical analysis was performed using student's t-test. For the estimation of correlation between parameters, a linear regression fit using the method of Reduction to Major Axis was applied.

Results

Different lipidemic profile was observed in patients with Diabetes type I and type II. The average total cholesterol was 245.5mg/dL in patients with Diabetes I (Fig. 1).

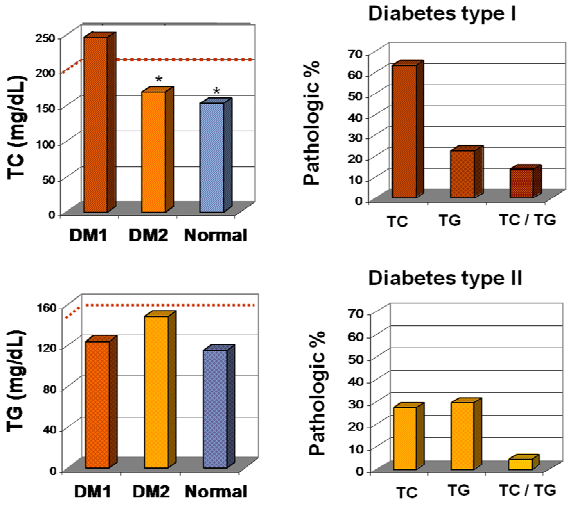

Figure 1. Average concentration of total cholesterol (TC) and triglycerides (TG) in normal individuals and patients with Diabetes type I (DM1) and type II (DM2). Percentage of individuals with pathologic values of cholesterol and triglycerides in the groups of DM1 and DM2 patients. Evaluation of existence of statistically significant difference from the DM1 group was performed using the Student's t-test. * stands for P=0.000. Dusted red line indicates the upper normal limit.

The concentration was higher than the upper normal limit and was significantly elevated compared to healthy individuals as well as to patients with Diabetes II (168.71±76.0mg/dL). The observed difference was statistically significant (P=0.000). In the Diabetes type I group, 63.6% of the patients had pathologic cholesterol levels and 22.7% had pathologic triglyceride levels, while 18.2% had both pathologic cholesterol and triglyceride levels. In the group of Diabetes type II, pathologic cholesterol concentration was observed in 28.6% of the patients and pathologic triglyceride levels were measured in 31% of the patients, while 4.6% of the patients had both pathologic cholesterol and triglyceride levels.

Cortisol levels were elevated in both cases with higher values observed in Diabetes type I (280.5±162.9ng/mL in DM1 and 248.5±100.1ng/mL in DM2). Average concentration was higher than the upper normal limit in DM1 group of patients (Fig. 2). Interestingly, 54.5% of the patients with Diabetes type I and 50% of the patients with Diabetes type II had pathologic cortisol levels. Total antioxidant capacity was significantly reduced compared to healthy individuals, 1.470mM, with lower values observed in Diabetes type I (0.680±0.116 mM in DM1 and 0.849±0.126 mM in DM2).

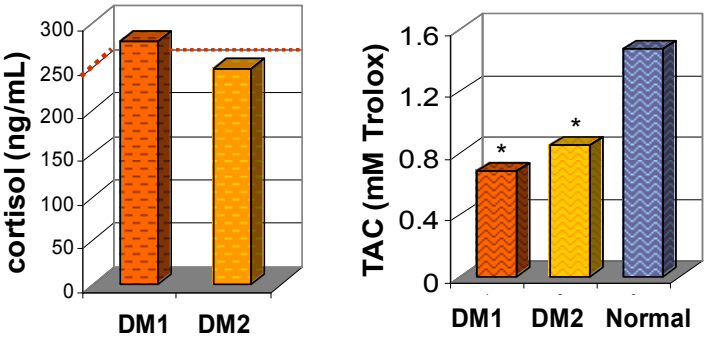

Figure 2. Cortisol and total antioxidant capacity concentration in patients with Diabetes type I (DM1) and II (DM2). Evaluation of existence of statistically significant difference from the normal individuals was performed using the Student's t-test. * stands for P=0.000. Dusted red line indicates the upper normal limit.

Amylase determination revealed a mean amylase value, 81.7U/mL, within normal range and a negative correlation between cholesterol levels and amylase (r=-0.770, Fig. 3).

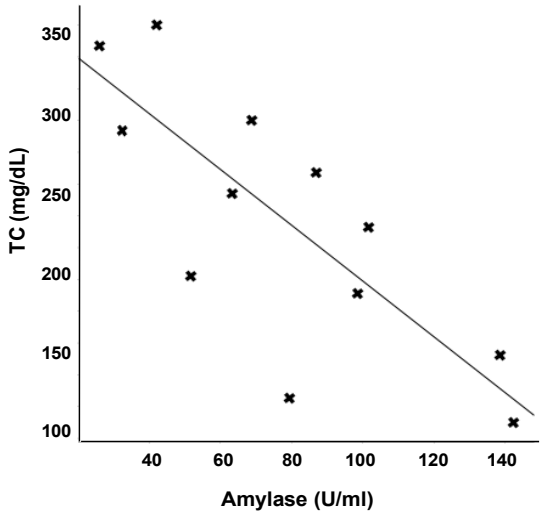

Figure 3. Correlation of Total Cholesterol with serum amylase activity in patients with Diabetes type I (r=-0.770).

Auto-antibodies and antibodies against the food derived antigen Neu5Gc were found in samples of both Diabetes type I and II. IgG, IgM or IgA antibodies against any of the determined antigens (Insulin, IA2, GAD and IR) were found in 50% of samples of Diabetes type I patients and 21.4% of Diabetes type II patients. In addition, IgG, IgM or IgA anti-Neu5Gc antibodies were found in 45.5% of Diabetes type I and 32.0% of Diabetes type II patients. A straight correlation between the presence of

these antibodies and any of the studied parameters was not observed.

Discussion

Hyperlipidemia is a common characteristic of patients with diabetes mellitus [21]. Combined with obesity, it may constitute one of the main causes of diabetes type II. However, insulin resistance and hyperglycemia may also lead to or enhance hyperlipidemia [6]. On the other hand, increased cortisol levels may induce hyperglycemia as well as hyperlipidemia [10-14] and confer in oxidative stress observed in diabetic and hyperlipidemic patients [15]. There are variations in lipidemic profile of diabetic patients reported in the literature, probably because of genetic, life style or age differences of the population [22-24]. In general, although many publications exist concerning lipid concentration in diabetes type II, fewer studies are referred to lipid profile of diabetes type I. A comparative study of lipid profile in patients with diabetes type I and II, also taking into account risk factors such as cortisol levels and antioxidant capacity has not come to our knowledge.

Our results indicate higher percentage of hypercholesterolemic patients and higher percentage of patients with combined hypercholesterolemia/hypertriglyceridemia in the group of Diabetes type I. Moreover, increased cortisol level and decreased total antioxidant capacity was observed in these patients. In general, the lipidemic profile and overall atherogenic and cardiovascular risk factors were worse in Diabetes I compared to Diabetes II patients studied by our team.

Auto-immunity has been related to inflammatory processes and free radical production [18]. So, increased auto-antibodies and antibodies against Neu5Gc, a red meat derived antigen with the ability to be incorporated in our cell membranes could be related with a worse antioxidant status and overall diabetic profile. Both, auto-antibodies and anti-Neu5Gc antibodies were mostly elevated in patients with Diabetes type I, were lower antioxidant capacity and worse lipidemic profile was observed. However, a straight correlation between antibodies and any of the studied parameters was not observed.

Interestingly, cholesterol levels exhibited a negative correlation with serum amylase values. It seems that low amylase concentration in diabetic patients, even when within normal rage may be associated with increased risk for atheromatosis and probably indicate a more extended effect of impaired insulin secretion and activity in pancreatic function. Since, amylase is not known to be involved in lipid metabolism, cholesterol levels and serum amylase activity may have a common modulator related to Diabetes development. Molecules involved in the islet - acinar axis [25], such as insulin itself or adrenomodulin which are affected in diabetes and are known to affect amylase activity could be candidates for this.

The authors declare that they have no conflicts of interest.

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